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# Methods

## 13.1 General Remarks

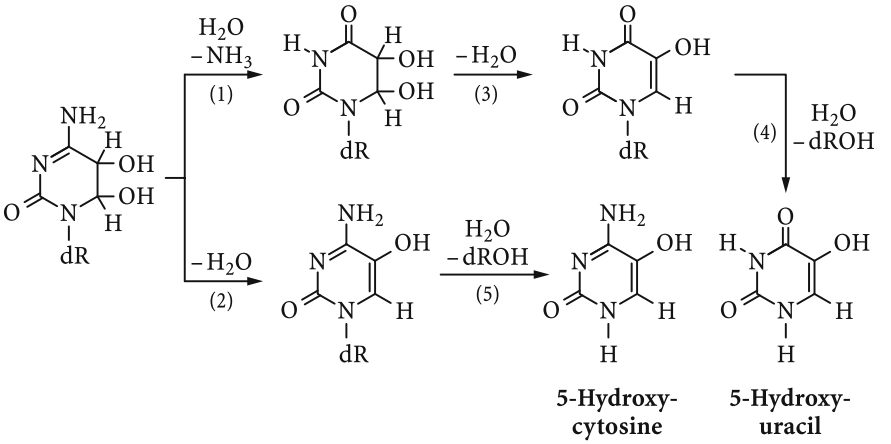
For the detection of free-radical-induced DNA damage and its repair, biochemical techniques are increasingly applied. It would exceed the allocated space to discuss these techniques here, and the reader is referred to the original literature. However, to assist biologists and biophysicists, some of the chemical techniques for measuring typical DNA lesions are discussed. Most of the kinetic data concerning the free-radical chemistry of DNA and its model systems has been obtained by pulse radiolysis. This technique is only available in a few laboratories worldwide. For this reason, it will be described in some detail here.

## 13.2 Detection of DNA Damage

In recent years, the sensitivity of the detection of free-radical-induced DNA damage has been considerably increased (for brief reviews, see Cadet and Weinfeld 1993; Cadet et al. 1997a, b, 1999b). There is often a problem with lesions that are refractory to an enzymatic excision (Dizdaroglu et al. 1978, and further examples are reported below), but it is expected that when enzymatic degradation techniques have been further developed, acid hydrolysis and its marked problems will no longer be required.

### 13.2.1 Excision of Damaged Bases by Acid Hydrolysis

Damaged DNA bases are usually still attached to the DNA backbone via a nucleosidic linkage. In order to release them for analysis, the nucleosidic linkage has to be hydrolyzed. Common procedures use 60–88% formic acid at 140 °C for 2 h. As a milder condition, 35% HF in pyridine at 37 °C for 2 h has been suggested (Douki et al. 1996) and, in particular, FAPY-A and FAPY-G withstand concomitant degradation much better (Douki et al. 1997). Nevertheless, all these conditions are very severe, and the product may be altered by this treatment. A case in point are the cytosine glycols which do not withstand this acid treatment and are released as 5OHCyt and 5OHUra [reactions (1)–(5)] (Dizdaroglu et al. 1986; Douki et al. 1996).




Although 35% HF in pyridine is considered to be milder than the formic acid treatment, HMUra is degraded under such conditions (Douki et al. 1996). Moreover, 60% formic acid is believed to be a stronger hydrolyzing agent as compared to 88% formic acid due to the higher degree of dissociation. Yet, the final yield of HMUra (in the plateau region after 2 h) is significantly lower in the case of the lower formic acid concentration (Douki et al. 1996). Thus, there is no ideal condition, and for each DNA lesion special conditions may have to be worked out to avoid major artifacts (Cadet et al. 1997a). Apparently, the discussion on potential artifacts continues (Dizdaroglu 1998) and experimental expertise acquired over the years in one laboratory is difficult to transfer to another one.

### 13.2.2 Excision by Enzymatic Hydrolysis

Glycosylases excise damaged nucleobases (for a review see Krokan and Standal 1997). They are grouped into 'simple glycosylases' that do not form DNA strand breaks and 'glycosylases/abasic site lyases' that concomitantly induce a stand break (Dodson and Lloyd 2002). This may have to be taken into account when using these enzymes for the detection of damaged sites (for the use of endonucleases for fingerprinting of DNA damage, see Epe and Hegler 1994).

As mentioned briefly above, the enzymatic excision of damaged nucleobases may cause some problems. A case in point is the action of nuclease P1. While a single 8-oxo-G lesion is excised as the damaged nucleoside, the clustered 8-oxo-G/Fo lesion is only obtained as modified dinucleotide (Maccubbin et al. 1992). Another example is the hydrolysis of dG*pC which severely inhibits the action of bovine spleen phosphodiesterase, while HMUrapA shows only very little inhibition (Maccubbin et al. 1991). Enzymatic hydrolysis of DNA is, in fact, the recommended method for the determination of HMUra (Teebor et al. 1984; Frenkel et al. 1985). It is recalled that mammalian cells cope with this DNA lesion with the help of a hydroxymethyluracil glycosylase (Hollstein et al. 1984).

When the Tg lesions is opened by ammonolysis, the resulting product (α-*R*-hydroxy-β-ureidoisobutyric acid) inhibits snake venom phosphodiesterase, λ exonuclease and the Klenow (exo$^-$) fragment (Matray et al. 1995; see also Greenberg and Matray 1997). It is, however, removed by *E. coli* Fpg and Nth proteins (Jurado et al. 1998).

A detailed study on the repair of the cA lesion is available (Brooks et al. 2000). The enzymatic excision of 8-oxo-A by Ogg1 from *S. cerevisiae* is only effective when this damage is paired with Cyt (but not Ade, Thy, Gua or Ura; Girard et al. 1998; for a review on the action of this enzyme in excising 8-oxo-G see Nishimura 2002). Substantial neighboring effects are also observed for the excision of other lesions such as 8-oxo-G or AP sites. Excision of 8-oxo-G by the *E. coli* Fpg protein is used as the first step for an improved detection of this lesion (Beckman et al. 2000; ESCODD 2003). There is a large variation in the yields of 8-oxo-G and concomitant discussions as to the best method for the detection of this DNA lesion. Attention has been drawn that incomplete digestion of the damaged DNA by the enzymatic cocktails typically used may be one of the reasons for such discrepancies, and an improved protocol has been suggested (Huang et al. 2001).

The development in this area of enzymatic action on the various damaged DNA sites continues to be very active. For this reason, only a very short account has been given as a kind of flavor for the reader to see in which direction research in this field seems to expand.

### 13.2.3 Detection of DNA Lesions by Gas Chromatography/Mass Spectrometry

Most of our present knowledge of free-radical-induced DNA lesions is based upon their identification and often also quantification by GC/MS. In order to convert the nucleobases and their free-radical-induced products into sufficiently volatile compounds the -NRH and -OH groups have to be trimethylsilylated. Carbohydrate-type products resulting from an alteration of the sugar moiety may be reduced with $NaBH_4$ after release or excision from DNA into the corresponding polyhydric alcohols (Beesk et al. 1979). Reduction with $NaBD_4$ incorporates a deuterium atom at the position of a carbonyl function (two deuterium atoms at a carboxyl group). The mass spectra of the trimethylsilylated polyhydric alcohols usually allows a firm assignment of their structure when the number of carbon atoms is known from the GC retention time (Dizdaroglu et al. 1974). A methoximation of the carbonyl function in combination with a trimethylsilylation of the hydroxyl groups also converts carbohydrate products into volatile compounds, and their mass spectra may provide additional information (Dizdaroglu et al. 1977). For the determination of the carbohydrate products, a polyhydric alcohol that is not formed under the given conditions can be used as internal standard. For the quantification of the base products, isotopically-labeled reference material which also undergoes the various prepurification steps (e.g., by semi-preparative HPLC) may be added (Bianchini et al. 1996; Douki et al. 1996; D'Ham et al. 1998). The determination of altered bases by GC/MS-SIM (SIM = single-ion monitoring), after trimethylsilylation, has become the standard method for

their determination (Dizdaroglu 1985, 1990, 1991, 1992; Dizdaroglu and Bergtold 1986; Fuciarelli et al. 1989; Dizdaroglu and Gajewski 1990; Jaruga et al. 2001).

### 13.2.4 Determination of DNA Lesions by Liquid Chromatography

When the term 'liquid chromatography' is used these days, it usually refers to HPLC (for reviews see Zakaria and Brown 1981; Scoble and Brown 1983), but valuable separations can also be achieved by TLC (Cadet et al. 1983). Although detection in HPLC is mainly by UV spectrophotometry (e.g., Cadet et al. 1982), electrochemical detection is the method of choice in the case of the strongly reducing 8-oxo-G lesion (Floyd et al. 1986; Shigenaga et al. 1989, 1990; Berger et al. 1990; Mei et al. 2003). In a multi-laboratory test, this method proved to be much more reliable than GC/MS and HPLC/MS-MS methods (ESCODD 2003). The work-up of biological samples and the difficulties that one might encounter have been discussed (Claycamp and Ho 1993; Douki et al. 1997; Dany et al. 1999). This is of importance, since this compound is often used as a marker of oxidative DNA damage, for example, in assessing the role of dietary fats (Loft et al. 1998).

Electrochemical detection is also the method of choice for the reducing dCyd-derived products 5OHdCyd and 5OHdUrd that can be released enzymatically from oxidized DNA (Wagner et al. 2004). After water elimination, the Ug may also be determined by this technique.

The sugar lesions FUR and 5-MF (Joshi and Ganesh 1994), as well as DNA-adducts (Park et al. 1989) have also be detected with the help of HPLC.

For optical detection, the product to be determined must have a reasonable absorption coefficient. Saturation of the pyrimidine *C*(5)-*C*(6) bond destroys the chromophore, and for the determination of Tg yields excreted into the urine, this product was reduced with HI into Thy prior to an HPLC analysis (Cathcart et al. 1984).

The combination of HPLC and MS (cf. Frelon et al. 2000) is expected to become the method of choice in many cases. The state of the art has been reviewed (Cadet et al. 2002).

### 13.2.5 MALDI-TOF

Another very sensitive MS technique is MALDI-TOF. Its high-resolution mass spectra allowed, for example, the identification of the 8-oxo-G lesion by its +16-Da mass shift in a oligonucleotide heptamer at picomol level (Bartolini and Johnston 2000).

### 13.2.6 Capillary and Pulsed Field Gel Electrophoresis

Capillary gel electrophoresis (CGE) has the advantage of a short analysis time, high degree of automation and reproducibility combined with a good resolving power for dsDNA fragments. The mobility of a DNA fragment is given by equa-

tion (6), where $V$ is the steady-state velocity of the fragment, $k$ a factor related the net polyanion charge $Q$, $S$ the size of the fragment (also related to $Q$), $E$ the electrical field, $E_a$ the activation energy for the viscous flow, $R$ the universal gas constant and $T$ the absolute temperature.

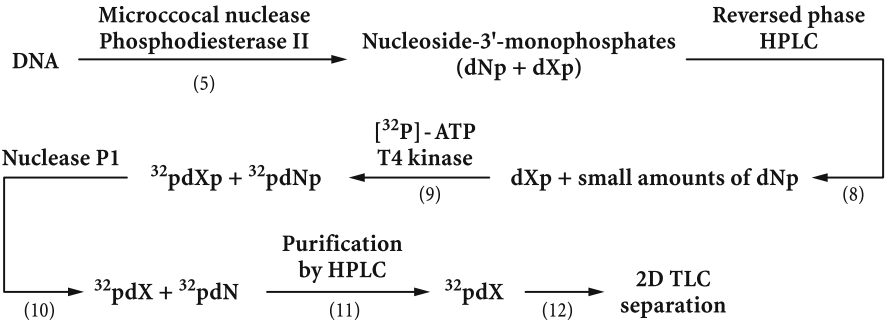
$$\ln \frac{1}{V} = \ln \frac{k}{ES} + \frac{E_a}{RT} \tag{6}$$

As an example for the study of DNA damage after irradiation using this technique may serve reference (Valenzuela et al. 2000).

In pulsed field gel electrophoresis (PFGE), intact DNA is treated with restriction enzymes to generate pieces small enough to resolve by electrophoresis in an agarose or acrylamide gel. With each reorientation of the electric field relative to the gel, small-sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind providing a separation from the smaller DNA (for a review see Anand and Southern 1990). Among others, PFGE seems to be the most sensitive technique for the determination of DSBs in cells (Rothkamm and Löbrich 2003).

### 13.2.7 $^{32}$P-Postlabeling

The $^{32}$P-postlabeling technique allows to improve the sensitivity of the detection of DNA damage (Cadet et al. 1998). The damaged DNA is enzymatically degraded into nucleotide-3-phosphates [reaction (7)]. The resulting mixture of unchanged nucleoside-3-phosphates (dNp) and damaged ones (dXP) are separated by HPLC [reaction (8)]. They are then labeled at the 5′-position with $^{32}$P [reaction (9)] and subsequently dephosphorylated at the 3′-position [reaction (10)]. This allows to proceed with a second purification and their identification by, for example, two-dimensional TLC [reactions (11) and (12)].




Factors that affect the determination of 8-oxo-G by this technique have been discussed in some detail (Möller et al. 1998). The determination of Tg by this technique (Hegi et al. 1989) is one of its most sensitive assays (Weinfeld and Soderlind 1991), many orders of magnitude higher than the earlier determination by HPLC (Frenkel et al. 1981). A $^{32}$P-postlabeling assay for the cA lesion which blocks gene

expression and is repaired by the nucleotide excision repair pathway (Brooks et al. 2000) has also been developed for the use in mammalian tissues (Randerath et al. 2001). Ade-*N*-1-oxide, a product that is formed when DNA is exposed to $H_2O_2$, has also been determined using this technique (Mouret et al. 1990).

### 13.2.8 Detection of Hydroperoxides

Hydrogen peroxide and organic hydroperoxides can be reduced by molybdate-activated iodide [Allen et al. 1952; reactions (13) and (14)]. The iodine atoms formed in these reactions combine to iodine which complexes with iodide [reactions (15) and (16)], and it is this $I_3^-$ complex which is measured ($\varepsilon$(3540 nm) = 25,000 $dm^3\ mol^{-1}\ cm^{-1}$)

$$ROOH + I^- \rightarrow RO^\bullet + I^\bullet + OH^- \quad (13)$$

$$RO^\bullet + I^- \rightarrow RO^- + I^\bullet \quad (14)$$

$$2\,I^\bullet \rightarrow I_2 \quad (15)$$

$$I_2 + I^- \rightarrow I_3^- \quad (16)$$

Hydrogen peroxide reacts considerably faster with this reagent ($t_{1/2}$ = 2.5 s) than organic hydroperoxides, and the differences in the rate of buildup not only allows to determine $H_2O_2$ and organic hydroperoxides side by side, but often the rate of reaction of a given hydroperoxide with this reagent can be used as a fingerprint for its presence (Dowideit and von Sonntag 1998; Flyunt et al. 2003). A certain disadvantage of this reagent is the fact that the iodine thus formed may react with other products (Al-Sheikhly et al. 1984). This problem can be overcome in certain cases by combining it with an HPLC separation (post-column derivatization mode). For a quantification of a hydroperoxidic product by this method the slowness of reaction of some hydroperoxides with this reagent may require the heating of the reaction coil, but in unfavorable cases the reaction may still remain incomplete. This may result in an underestimate of the hydroperoxide yield.

Hydroperoxides also react readily with $Fe^{2+}$ in acid media [reactions (17) and (18)]. The $Fe^{3+}$ formed can be complexed with xylenol orange. This complex has a strong absorption coefficient in the visible ($\varepsilon$(540 nm) = 26,800 $dm^3\ mol^{-1}\ cm^{-1}$). This method has been used with some advantage in the identification of the hydroperoxides formed upon $^\bullet$OH-attack on Thd by HPLC using the post-column derivatization technique (reagent: e.g. $2.3 \times 10^{-4}$ mol $dm^{-3}$ ammonium ferrous sulfate, $3.5 \times 10^{-2}$ mol $dm^{-3}$ sulfuric acid, $5.6 \times 10^{-4}$ mol $dm^{-3}$ xylenol orange; Wagner et al. 1990).

$$ROOH + Fe^{2+} \rightarrow RO^\bullet + OH^- + Fe^{3+} \quad (17)$$

$$RO^\bullet + Fe^{2+} \rightarrow RO^- + Fe^{3+} \quad (18)$$

As in the related Fricke actinometer, there is a given chance that more than two $Fe^{3+}$ are formed, and using the $Fe^{2+}$/xylenol orange system hydroperoxide yields may be overestimated as has been shown for the $Fe^{2+}$/$SCN^-$ system (Mihaljevic et al. 1996). The alkoxyl radical, besides being reduced by $Fe^{2+}$ [reaction (18)], may undergo rapid β-fragmentation (Chap. 7.2) and the alkyl radical thus formed adds $O_2$, yielding a peroxyl radical which can be reduced by $Fe^{2+}$ to the corresponding hydroperoxide. This sequence is a chain reaction, only interrupted by the reduction of the alkoxyl radical [reaction (18)]. In the Fricke actinometer $Cl^-$ can be added which converts $^{\bullet}OH$ into $Cl_2^{\bullet -}$. This radical reacts readily with $Fe^{2+}$ but does not continue the chain reaction by reacting with organic impurities. In the above assay, the addition of $Cl^-$ will not have a beneficial effect, because alkoxyl radicals do not oxidize $Cl^-$ in acid solutions.

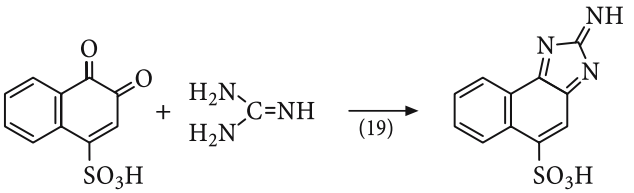
### 13.2.9
### Malonaldehyde, Base Propenals and Related Compounds

Malonaldehyde and related compounds react readily with 2-thiobarbituric acid (TBA, typically 2 g $dm^{-3}$, the resulting pH of the solution is thus around 2, at 70 °C for several minutes, depending on the reactivity of the compound to be analyzed) and turns yielding a pink color (in the case of malonaldehyde ε(532 nm) = 150,000 $dm^3$ $mol^{-1}$ $cm^{-1}$). The rate of reaction of the base propenals is faster than that of malonaldehyde (Rashid et al. 1999), i.e., they do not hydrolyze prior to their reaction with TBA. The reaction of the 5′-aldehydes derived from Pur deoxynucleosides react more slowly (Langfinger and von Sonntag 1985; von Sonntag 1994), and the color tends to fade. Other lesions damaged sugar moiety also give a positive TBA response, for example, 3′-oxo-dThd (Rashid et al. 1999) and unknown DNA-bound damaged sites (Krushinskaya and Shalnov 1967; Krushinskaya 1983; Rashid et al. 1999). The fact that there are definitely different products formed in the TBA reaction, although they all give rise to the same kind of absorption raises the question, whether the same absorption coefficient as has been determined for malonaldehyde can also be used for the other TBA-reactive compounds. With dGuo-5′-aldehyde, this is clearly not the case (Langfinger and von Sonntag 1985).

To avoid some of the ambiguities of the common TBA assay described above, malonaldehyde may be converted with methylhydrazine under very mild conditions into 1-methylpyrazol. The latter is extracted and determined by GC (Matsufuji and Shibamoto 2004).

### 13.2.10
### Guanidine Derivatives

Some Gua lesions are characterized by a guanidine residue that can be detected spectrophotometrically using 1,2-naphthoquinone-4-suphonate as the reagent (Kobayashi et al. 1987). The resulting product is shown in reaction (19).




### 13.2.11 Immuno Assays

Immuno assays have been developed for a variety of DNA lesion such as UV-damage (McConlogue et al. 1982; Eggset et al. 1987), Tg (Rajagopalan et al. 1984; Le et al. 1998) cA (Fuciarelli et al. 1985, 1987), Ade-$N^1$-oxide (Signorini et al. 1998), carcinogen-modified DNA (Müller et al. 1982), hydroxylation of guanine (Kasai and Nishimura 1986), 8-oxo-G (Yin et al. 1995), HMdUra (Lewis et al. 1978), DNA base damage (Lewis and Ward 1978) and X-ray-induced damage (Waller et al. 1981).

A fluorescent 90-mer oligonucleotide in combination with laser-induced fluorescence and capillary electrophoresis has been used as a versatile probe for a detection of DNA damage (Carnelley et al. 2001).

### 13.2.12 Atomic Force Microscopy

Atomic force microscopy has been developed to a stage that DSBs and the length of the resulting fragments can be detected by this technique (Pang et al. 1996). In this context it is of interest that neutron irradiation leads to the formation of very small fragments (Pang et al. 1997).

### 13.2.13 Comet Assay

The comet assay (Ostling and Johanson 1984) makes use of the fact that the smaller DNA fractions that are formed when the molecular weight of the cellular DNA is reduced by free-radical-induced DSBs move faster in an applied electric field (after cell lysis) than the undamaged DNA. Staining the DNA yields a comet-like pattern. Purified repair enzymes, applied to DNA during the course of the comet assay procedure, can greatly enhance the sensitivity and specificity of the assay (Collins et al. 1997; Sauvaigo et al. 2002). This assay is now widely applied (cf. Pouget et al. 1999), and a combination with immunofluorescence detection techniques allows even the detection specific damage and its repair (Sauvaigo et al. 1998). For the application of the comet assay in assessing environmental hazards, see Ündeger et al. (1999), for a comparison of γ-irradiated and BLM-treated cells, see Östling and Johanson (1987).

**Table 13.1.** Enzymes used for the identification of clustered DNA base damage. (Sutherland et al. 2000)

| Enzyme | Class recognized | Lesion recognized |
|---|---|---|
| *E. coli* Fpg protein (formamidopyrimidine-DNA glycosylase) | Oxidized purines | FAPY-A, FAPY-G, 8-oxo-G, 8-oxo-A, some abasic sites, to a lesser extent other modified purines |
| *E. coli* Nth protein (endonuclease III) | Oxidized pyrimidines | Ring-saturated or fragmented Thy residues, e.g. $H_2$Thy, Tg, 5-hydroxy-5-methylhydantoine, urea, DNA damaged at Gua, some abasic sites |
| *E. coli* Nfo protein (endonuclease IV) | AP sites | Several AP sites including oxidized AP sites, urea |

### 13.2.14 Detection of SSBs and DSBs with the Help of Supercoiled Plasmids

Plasmids are small pieces of circular supercoiled dsDNA. A SSB causes the plasmid to relax into the open circular form, a DSB into the ds linear form. These there forms can be separated by chromatographic methods (e.g., Bresler et al. 1979). This assay is widely used for studying effects of various agents including the action of enzymes on damaged DNA.

### 13.2.15 Detection of Clustered Lesions

DNA damage induced by ionizing radiation leads single lesions also to the formation of clustered lesions such as two close-by damaged bases on opposite strands (Chap. 12). For their detection, DNA is treated with an endonuclease that induces a SSB at a damaged site. If there are two closely separated lesions on opposite strands, such treatment induces a DSB which can be detected on a non-denaturing gel (Sutherland et al. 2000). The enzymes that have been used and their targets are compiled in Table 13.1.

## 13.3 Pulse Radiolysis and Laser Flash Photolysis

The pulse radiolysis technique is close to the better known laser flash photolysis (for a monograph see Bensasson et al. 1983). There is one essential difference: in pulse radiolysis the energy is absorbed by the solvent, e.g., by water in DNA solu-

tions, while in laser flash photolysis the energy of the photons is absorbed by the solute, e.g., by DNA. Although at high enough photon energies such as 193 nm or upon biphotonic excitation ionization does occur yielding radical cations and electrons, i.e. DNA radicals are formed upon UV-excitation, the application of laser flash photolysis in the study of DNA free-radical chemistry has found few applications so far. This is, however, changing as more and more photolabile compounds tailored to produce specific radicals are studied. In both techniques, the detection systems are the same, and the evaluation of the data follow the same principles. Some basics of these techniques will be described now.

The key reactions of the radiolysis of water leading to the formation of $^{\bullet}OH$, $e_{aq}^-$ and $H^{\bullet}$ and the conversion of $e_{aq}^-$ into a further $^{\bullet}OH$ have been discussed in Chapter 2.2. Here, it is sufficient to recall that the spur reactions are over in ca. $10^{-8}$ s, and from thereon the distribution of the radical species is practically homogeneous.

Short electron pulses are produced using Van de Graaff, LINAC or Febetron-type accelerators. Detection of products (intermediates) that grow in and decay as the cascade of reactions initiated by the pulse proceeds may be based on optical absorption, optical emission, electrical conductivity, EPR, CIDNP, polarography and light scattering. When pulse radiolysis is combined with a rapid-mixing device, the reactions of long-lived radicals with added substrates can also be studied (see below). Opaque solid DNA sample can also be investigated using the diffuse-reflectance technique (Wilkinson et al. 1984; O'Neill et al. 1989). Different types of pulse-generating and detection equipment have been described in the literature (Barker et al. 1970; Warman et al. 1980; Asmus and Janata 1982a,b; Janata 1982, 1992a,b; van Lith et al. 1983; Asmus 1984; Tabata et al. 1985; Patterson 1987; von Sonntag 1989; Janata et al. 1993; Bothe and Janata 1994; von Sonntag and Schuchmann 1994).

In a typical pulse radiolysis experiment, a submicrosecond pulse of high-energy electrons (2.5–10 MeV) passes through the cell containing the system to be investigated. Upon losing its energy, a 3-MeV electron (penetration depth in water ca. 10 mm) gives rise to as many as $1.8 \times 10^8$ radicals ($^{\bullet}OH$, $e_{aq}^-$ and $H^{\bullet}$) together with further charged particles ($H^+$ and $OH^-$) after completion of the spur reactions (Chap. 2.2). In pulse radiolysis, the number of primary radicals formed does not depend on the properties of the solute and its concentration. In laser flash photolysis, however, the substrate concentration and the absorption coefficient at the given excitation wavelength but also on the quantum yield of radical formation determine the efficiency of radical formation. The latter may often be the limiting factor for using this technique. Moreover in laser flash photolysis, detected intermediates are usually not only due to free radicals, but triplet states or non-radical products resulting from the reactions of the excited states may give rise to the observed effects as well.

Intermediates may be detected by UV-Vis spectroscopy. In a pulse radiolysis experiments, Čerenkov light is emitted as the high-energy electrons are slowed down. Hence, when the photon flux of the analyzing light ($I_0$) is low, emission signals are observed despite the fact that absorbing species are formed (in laser flash-photolysis this phenomenon is only observed if the quantum yield of fluorescence is high and the monitoring wavelength is set in this wavelength region).

The problem can be overcome by boosting for a few milliseconds the power of the lamp providing the analyzing light (with a 450-W xenon lamp about tenfold). Thus, the $I_0$ value of the analyzing light is now much higher than the intensity of the Čerenkov light (fluorescence) and signals reflect with sufficient accuracy the absorption properties of the intermediates formed during the pulse (laser flash). Recently, attention has been drawn to pitfalls by stray light (Czapski et al. 2005) and solute absobance (especially in laser flash photolysis; von Sonntag 1999). Data acquisition and storage are computerized which allows multiple-signal averaging. The dose in a pulse radiolysis experiment may be determined (Butler and Land 1996) by the thiocyanate dosimeter (Schuler et al. 1981), more recently revised (Buxton and Stuart 1995; $G(SCN)_2^{\bullet-} \times \varepsilon_{475\,nm} = (2.59 \pm 0.05) \times 10^{-4}\ m^2\ J^{-1}$).

In many free-radical reactions, neutral radicals give rise to charged species. For example, neutral peroxyl radicals may release $HO_2^\bullet/O_2^{\bullet-}$ ($pK_a(HO_2^\bullet) = 4.8$; Chap. 8.11). The equivalence conductance of $H^+$ and $OH^-$ is 315 and 175 $\Omega^{-1}$ mol$^{-1}$ cm$^{-1}$, respectively. Monoanions and monocations have values in the range of 45-60 $\Omega^{-1}$ mol$^{-1}$ cm$^{-1}$. When the neutralization is completed, the signal of the change of conductance produced by the charged species will be large and positive at pH below pH 7 [expression (20)], but smaller and negative above pH 7 [expression (21)]. The neutralization reaction must not push the pH out of the basic range. The lowest pH at which an experiment can be carried out in basic solution is hence approximately pH 9.

*Acid solution*:

$$\underline{\text{Pulse} \rightarrow H^+ + X^-} = +315 + 45\ \Omega^{-1}\ \underline{\text{mol}^{-1}\ \text{cm}^{-1}} \qquad (20)$$
$$\Delta\kappa = +360\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$

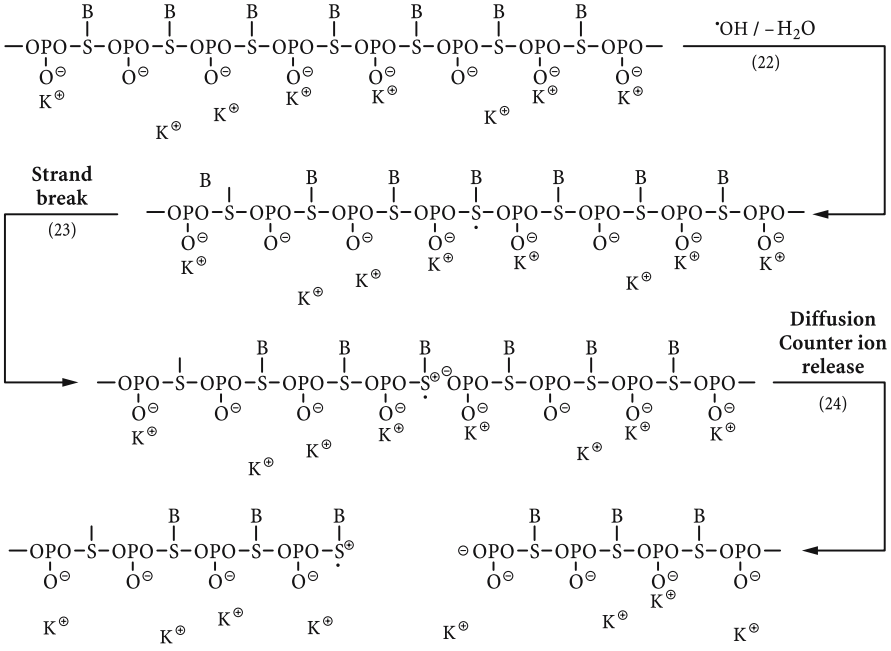
*Basic solution*:

$$\text{Pulse} \rightarrow H^+ + X^- = +315 + 45\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1} \qquad (21)$$
$$\text{Neutralization: } H^+ + OH^- \rightarrow H_2O = -315 - 170\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$
$$\Delta\kappa = -125\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$

The available set-ups are very sensitive, and a pH range between 2.5 and 12 is accessible. Obviously, the presence of buffers will have a considerable effect on the signal height, but in favorable cases a computer analysis may allow the quantification of the various contributions to the conductance signal even under such conditions (Das et al. 1987; Schuchmann et al. 1989).

For dosimetry, the reaction of the reaction of $^\bullet$OH with DMSO which yields methanesulfinic acid (92%; Veltwisch et al. 1980; Chap. 3.2) is usually used. This allows one to put the conductance signals on a quantitative basis (calculation of $G$ values), and the rates of reactions that are kinetically of first order can be determined for the time dependence of the signal evolution. DMSO dosimetry yields only a relative dose. For the determination of second-order rate constants, however, the exact dose must be known, and this can be determined by the 'zero conductivity change dosimetry' or 'neutralization kinetics dosimetry' (Schuchmann et al. 1991).

The application of the conductance changes has been of considerable importance in assessing the kinetics of free-radical-induced chain scission of charged polymers (Chap. 9.4). A polymeric electrolyte exerts a strong electric field in the vicinity of the polymer chain. This cause counterions to "condense" onto the polymer. When in single-stranded DNA, for example, •OH attacks the sugar moiety at, let us say at C(4′) [reaction (22), cf. Chap. 12.4], the number of condensed counterions does not change, nor at the very moment when the strand breaks upon the heterolytic cleavage of the phosphate bond [reaction (23)]. However, when the two ends diffuse apart, condensed counterions (*e.g.*, $K^+$) ions are released [reaction (24)]. This causes a conductance increase. Insofar as this process does not involve the formation of $H^+$, the conductance change signal remains positive even in an alkaline environment, since a neutralization reaction does not intervene. As long as the rate of the diffusive process (24) is fast compared to the preceding reactions (22) and (23), the observed kinetics are those of strand breakage [reaction (23); the •OH reaction (22) is never rate-determining]. In all the systems investigated thus far, this requirement has always been met.




Detection by laser light-scattering (LLS) is another means of determining changes in the molecular weight of polymers. This method is, of course, not restricted to charged polymers. LLS detection can be carried out at the 90° angle mode (Schnabel 1986 and references cited therein) but also at the low-angle mode (~ 7°, LALLS). The advantage of LLS is that the setup is simpler, and a better signal-to-noise ratio is obtained, but problems arising from form factors are minimized with LALLS. Intermolecular cross-linking gives rise to an increase chain scission to a decrease in signal intensity. Upon folding of a rod-like polymer the LSI

signal is also increased. Thus, in such a case, intramolecular cross-linking can also contribute to an increase in the LSI signal.

Although the signal-to-noise ratio in the case of the LALLS technique is much poorer than in the case of conductance method, one may have to use LALLS also in the case of charged polymers, whenever other sources of conductance changes are expected (e.g., release of $HO_2^{\bullet}/O_2^{\bullet -}$ in peroxyl radical reactions; Chap. 8.4).

The volume of activation which can provide very valuable information concerning the reaction mechanism is obtained by high-pressure pulse radiolysis (for a review in the area of transition metal ion chemistry, see van Eldik and Meyerstein 2000).

Single charged-particle beam irradiation of single cells has been developed to study various aspects of radiation biology such as the bystander effect. This subject exceeds the scope of this book, and only some reference to this technique is made here (Folkard et al. 1997a,b).

## 13.4 Gas Explosion and Rapid-Mixing Techniques

The gas explosion technique (Michael et al. 1973) allows one to change on the sub-millisecond time scale the environment of a target layer of plasmid DNA or cells by letting a burst of gases, e.g., $O_2$ or $H_2S$, enter the irradiation chamber at times before or after a short pulse of radiation has been given to the target. The gas burst is triggered by a solenoid valve, and the gas under pressure proceeds to the target at a typical speed of 70 m $s^{-1}$.

Another way of changing the environment or adding a reactant at a given time before or after a short pulse of radiation is the rapid mixing technique (e.g., Bielski and Richter 1977). In a stopped-flow set-up, long-lived radicals such as $O_2^{\bullet -}$ that have been generated from a solution contained in one syringe in the pulse-irradiated zone are reacted by mixing with a substrate contained in another syringe. Distortions of the kinetics are prevented by a hard stop. The typical rise time of such a set-up used to be 10 ms, but advanced techniques now provide a rise time of about 1 ms.

With some modifications, such a technique has been used to study the time scale of the effects of $O_2$ and sensitizers on cells in aqueous solution (Adams et al. 1968; Whillans 1982; Whillans and Hunt 1982). The rapid lysis technique works on the same principle. It permits cells to be lysed at a given time after a dose of radiation (Johansen and Boye 1975; Johansen et al. 1975; Sapora et al. 1975, 1977; Fox et al. 1976; Millar et al. 1980). This allows the study of repair kinetics at early times.

## References


Adams GE, Cooke MS, Michael BD (1968) Rapid mixing in radiobiology. Nature 219:1368–1369

Al-Sheikhly MI, Hissung A, Schuchmann H-P, Schuchmann MN, von Sonntag C, Garner A, Scholes G (1984) Radiolysis of dihydrouracil and dihydrothymine in aqueous solutions containing oxygen; first- and second-order reactions of the organic peroxyl radicals; the role of isopyrimidines as intermediates. J Chem Soc Perkin Trans 2 601–608

Allen AO, Hochanadel CJ, Ghormley JA, Davis TW (1952) Decomposition of water and aqueous solutions under mixed fast neutron and gamma radiation. J Phys Chem 56:575–586

Anand R, Southern EM (1990) Pulsed field gel electrophoresis. In: Rickwood D, Hames BD (eds) Gel electrophoresis of nucleic acids: a practical approach. IRL Press at Oxford University Press, New York, pp 101–123

Asmus K-D (1984) Pulse radiolysis methodology. Methods Enzymol 105:167–178

Asmus K-D, Janata E (1982a) Polarography monitoring techniques. In: Baxendale JH, Busi F (eds) The study of fast processes and transient species by electron pulse radiolysis. Reidel, Dordrecht, pp 115–128

Asmus K-D, Janata E (1982b) Conductivity monitoring techniques. In: Baxendale JH, Busi F (eds) The study of fast processes and transient species by electron pulse radiolysis. Reidel, Dordrecht, pp 91–113

Barker GC, Fowles P, Sammon DC, Stringer B (1970) Pulse radiolytic induced transient electrical conductance in liquid solutions, part 1. Technique and the radiolysis of water. Trans Faraday Soc 66:1498–1508

Bartolini WP, Johnston MV (2000) Characterizing DNA photo-oxidation reactions by high-resolution mass measurements with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 35:408–416

Beckman KB, Saljoughi S, Mashiyama ST, Ames BN (2000) A simpler, more robust method for the analysis of 8-oxoguanine in DNA. Free Rad Biol Med 29:357–367

Beesk F, Dizdaroglu M, Schulte-Frohlinde D, von Sonntag C (1979) Radiation-induced DNA strand breaks in deoxygenated aqueous solution. The formation of altered sugars as end groups. Int J Radiat Biol 36:565–576

Bensasson RV, Land EJ, Truscott TG (1983) Flash photolysis and pulse radiolysis. Contributions to the chemistry of biology and medicin. Pergamon Press, Oxford

Berger M, Anselmino C, Mouret J-F, Cadet J (1990) High performance liquid chromatography - electrochemical assay for monitoring the formation of 8-oxo-7,8-dihydroadenine and its related 2′deoxyribonucleoside. J Liquid Chromatogr 13:929–940

Bianchini F, Hall J, Donato F, Cadet J (1996) Monitoring urinary excretion of 5-hydroxymethyluracil for assessment of oxidative DNA damage and repair. Biomarkers 1:178–184

Bielski BHJ, Richter HW (1977) A study of the superoxide radical chemistry by stopped-flow radiolysis and radiation induced oxygen consumption. J Am Chem Soc 99:3019–3023

Bothe E, Janata E (1994) Instrumentation of kinetic spectroscopy – 13. a.c.-Conductivity measurements at different frequencies in kinetic experiments. Radiat Phys Chem 44:455–458

Bresler SE, Noskin LA, Kuzovleva NA, Noskina IG (1979) The nature of the damage to *Escherichia coli* DNA induced by γ-irradiation. Int J Radiat Biol 36:289–300

Brooks PJ, Wise DS, Berry DA, Kosmoski JV, Smerdon MJ, Somers RL, Mackie H, Spoonde AY, Ackerman EJ, Coleman K, Tarone RE, Robbins JH (2000) The oxidative DNA lesion 8,5′-(S)-cyclo-2′-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells. J Biol Chem 275:22355–22362

Butler J, Land EJ (1996) Pulse radiolysis. In: Punchard NA, Kelly FJ (eds) Free radicals, a practical approach. IRL Press at Oxford University Press, Oxford, pp 48–61

Buxton GV, Stuart CR (1995) Re-evaluation of the thiocyanate dosimeter for pulse radiolysis. J Chem Soc Faraday Trans 91:279–281

Cadet J, Weinfeld M (1993) Detecting DNA damage. Anal Chem 65:675A–662A

Cadet J, Berger M, Voituriez L (1982) Separation of radiation and photo-induced 5,6-dihydrothymine derivatives by reversed-phase high-performance liquid chromatography. J Chromatogr 238:488–494

Cadet J, Voituriez L, Berger M (1983) Separation of nucleic acid components and their radiation-induced degradation products on chemically bonded $C_{12}$ reversed-phase thin-layer plates. J Chromatogr 259:111–119

Cadet J, Douki T, Ravanat J-L (1997a) Artifacts associated with the measurement of oxidized DNA bases. Environ Health Perspect 105:1034–10392811–2816

Cadet J, Berger M, Douki T, Ravanat J-L (1997b) Oxidative damage to DNA: formation, measurement, and biological significance. Rev Physiol Biochem Pharmacol 131:1–87

Cadet J, Bianchini F, Girault I, Molko D, Polverelli M, Ravanat J-L, Sauvaigo S, Signorini N, Tuce Z (1998) Measurement of oxidative base damage to DNA: HPLC/$^{32}$P-postlabeling, imunological and non invasive assays. In: Aruoma OI, Halliwell B (eds) DNA and free radicals: techniques, mechanisms and applications. OICA International, London

Cadet J, D'Ham C, Douki T, Pouget J-P, Ravanat J-L, Sauvaigo S (1999a) Facts and artifacts in the measurement of oxidative base damage to DNA. Free Rad Res 29:541–550

Cadet J, Delatour T, Douki T, Gasparutto D, Pouget J-P, Ravanat J-L, Sauvaigo S (1999b) Hydroxyl radicals and DNA base damage. Mutat Res 424:9–21

Cadet J, Douki T, Frelon S, Sauvaigo S, Pouget J-P, Ravanat J-L (2002) Assessment of oxidative base damage to isolated and cellular DNA by HPLC-MS/MS measurement. Free Rad Biol Med 33:441–449

Carnelley TJ, Barker S, Wang H, Tan WG, Weinfeld M, Le XC (2001) Synthesis, characterization, and applications of a fluorescent probe of DNA damage. Chem Res Toxicol 14:1513–1522

Cathcart R, Schwiers E, Saul RL, Ames BN (1984) Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. Proc Natl Acad Sci USA 81:5633–5637

Claycamp HG, Ho K-K (1993) Background and radiation-induced 8-hydroxy-2'-deoxyguanosine in γ-irradiated *Escherichia coli*. Int J Radiat Biol 63:597–607

Collins AR, Dobson VL, Dusinska M, Kennedy G, Stetina R (1997) The comet assay: what can it really tell us? Mutat Res 375:183–193

Czapski G, Ozeri Y, Goldstein S (2005) Pitfalls and artifacts in measuring absorption spectra and kinetics: the effect of stray light in the UV and red regions. Radiat Phys Chem 72:229–234

Dany AL, Triantaphylidès C, Cadet J, Douki T (1999) Optimisation of *arabidopsis thaliana* DNA extraction for the analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine formation after gamma irradiation. J Chim Phys 96:152–161

Das S, Schuchmann MN, Schuchmann H-P, von Sonntag C (1987) The production of the superoxide radical anion by the OH radical-induced oxidation of trimethylamine in oxygenated aqueous solution. The kinetics of the hydrolysis of (hydroxymethyl)dimethylamine. Chem Ber 120:319–323

D'Ham C, Ravanat J-L, Cadet J (1998) Gas chromatography - mass spectrometry with high-performance liquid chromatography purification for monitoring the endonuclease III-mediated excision of 5-hydroxy-5,6-dihydrothymine and 5,6-dihydrothymine from γ-irradiated DNA. J Chromatogr B 710:67–74

Dizdaroglu M (1985) Application of capillary gas chromatography-mass spectrometry to chemical characterization of radiation-induced base damage of DNA: implications for assessing DNA repair processes. Anal Biochem 144:593–603

Dizdaroglu M (1990) Gas chromatography-mass spectrometry of free radical-induced products of pyrimidines and purines in DNA. Methods Enzymol 193:842–857

Dizdaroglu M (1991) Chemical determination of free radical-induced damage to DNA. Free Rad Biol Med 10:225–242

Dizdaroglu M (1992) Measurement of radiation-induced damage to DNA at the molecular level. Int J Radiat Biol 61:175–183

Dizdaroglu M (1998) Facts about the artifacts in the measurement of oxidative DNA base damage by gas chromatography-mass spectrometry. Free Rad Res 29:551–563

Dizdaroglu M, Bergtold DS (1986) Characterization of free radical-induced base damage in DNA at biologically relevant levels. Anal Biochem 156:182–188

Dizdaroglu M, Gajewski E (1990) Selected-ion mass spectrometry: assays of oxidative DNA damage. Meth Enzymol 530–544

Dizdaroglu M, Henneberg D, von Sonntag C (1974) The mass spectra of TMS-ethers of deuterated polyalcohols. A contribution to the structural investigation of sugars. Org Mass Spectrom 8:335–345

Dizdaroglu M, Henneberg D, von Sonntag C, Schuchmann MN (1977) Mass spectra of trimethylsilyl di-O-methyloximes of adosuloses and dialdoses. Org Mass Spectrom 12:772–776

Dizdaroglu M, Hermes W, Schulte-Frohlinde D, von Sonntag C (1978) Enzymatic digestion of DNA γ-irradiated in aqueous solution. Separation of the digest by ion-exchange chromatography. Int J Radiat Biol 33:563–569

Dizdaroglu M, Holwitt E, Hagan MP, Blakely WF (1986) Formation of cytosine glycol and 5,6-dihydroxycytosine in deoxyribonucleic acid on treatment with osmium tetroxide. Biochem J 235:531–536

Dodson ML, Lloyd RS (2002) Mechanistic comparisons among base excision repair glycosylases. Free Rad Biol Med 32:678–682

Douki T, Delatour T, Paganon F, Cadet J (1996) Measurement of oxidative damage at pyrimidine bases in γ-irradiated DNA. Chem Res Toxicol 9:1145–1151

Douki T, Martini R, Ravanat J-L, Turesky RJ, Cadet J (1997) Measurement of 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-oxo-7,8-dihydroguanine in isolated DNA exposed to gamma radiation in aqueous solution. Carcinogenesis 18:2385–2391

Dowideit P, von Sonntag C (1998) The reaction of ozone with ethene and its methyl- and chlorine-substituted derivatives in aqueous solution. Environ Sci Technol 32:1112–1119

Eggset G, Volden G, Krokan H (1987) Characterization of antibodies specific for UV-damaged DNA by ELISA. Photochem Photobiol 45:485–491

Epe B, Hegler J (1994) Oxidative DNA damage: endonulease fingerprinting. Methods Enzymol 234:122–131

ESCODD (2003) Measurement of DNA oxidation in human calls by chromatographic and enzymic methods. Free Rad Biol Med 34:1089–1099

Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC (1986) Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Rad Res Commun 1:163–172

Flyunt R, Leitzke A, von Sonntag C (2003) Characterisation and quantitative determination of (hydro)peroxides formed in the radiolysis of dioxygen-containing systems and upon ozonolysis. Radiat Phys Chem 67:469–473

Folkard M, Vojnovic B, Prise KM, Bowey AG, Locke RJ, Schettino G, Michael BD (1997a) A charged-particle microbeam. I. Development of an experimental system for targeting cells individually with counted particles. Int J Radiat Biol 72:375–385

Folkhard M, Vojnovic B, Hollis KJ, Bowey AG, Watts SJ, Schettino G, Prise KM, Michael BD (1997b) A charged-particle microbeam. II. A single-particle micro-collimation and detection system. Int J Radiat Biol 72:387–395

Fox RA, Fielden EM, Sapora O (1976) Yield of single-strand breaks in the DNA of *E. coli* 10 msec after irradiation. Int J Radiat Biol 29:391–394

Frelon S, Douki T, Ravanat J-L, Pouget J-P, Tornabene C, Cadet J (2000) High-performance liquid chromatography-tandem mass spectrometry measurement of radiation-induced base damage to isolated and cellular DNA. Chem Res Toxicol 13:1002–1010

Frenkel K, Goldstein MS, Teebor GW (1981) Identification of the *cis*-thymine glycol moiety in chemically oxidized and γ-irradiated deoxyribonucleic acid by high-pressure liquid chromatography. Biochemistry 20:7566–7571

Frenkel K, Cummings A, Solomon J, Cadet J, Steinberg JJ, Teebor GW (1985) Quantitative determination of the 5-(hydroxymethyl)uracil moiety in the DNA of γ-irradiated cells. Biochemistry 24:4527–4533

Fuciarelli AF, Miller GG, Raleigh JA (1985) An immunochemical probe for 8,5′-cycloadenosine-5′-monophosphate and its deoxy analog in irradiated nucleic acid. Radiat Res 104:272–283

Fuciarelli AF, Shum FY, Raleigh JA (1987) Intramolecular cyclization in irradiated nucleic acids: Correlation between high-performance liquid chromatography and an immunochemical assay for 8,5′-cycloadenosine in irradiated poly(A). Radiat Res 110:35–44

Fuciarelli AF, Wegher BJ, Gajewski E, Dizdaroglu M, Blakely WF (1989) Quantitative measurement of radiation-induced base products in DNA using gas chromatography-mass spectroscopy. Radiat Res 119:219–231

Girard PM, D'Ham C, Cadet J, Boiteux S (1998) Opposite base-dependent excision of 7,8-dihydro-8-oxo-adenine by the Ogg1 protein of *Saccharomyces cerevisiae*. Carcinogenesis 19:1299–1305

Greenberg MM, Matray TJ (1997) Inhibition of Klenow fragment ($exo^-$) catalyzed DNA polymerization by (5*R*)-5,6-dihydro-5-hydroxythymidine and structural analogue 5,6-dihydro-5-methylthymidine. Biochemistry 36:14071–14079

Hegi ME, Sagelsdorff P, Lutz WK (1989) Detection by $^{32}$P-postlabeling of thymidine glycol in γ-irradiated DNA. Carcinogenesis 10:43–47

Hollstein MC, Brooks P, Linn S, Ames BN (1984) Hydroxymethyluracil DNA glycosylase in mammalian cells. Proc Natl Acad Sci USA 81:4003–4007

Huang X, Powell J, Mooney LA, Li C, Frenkel K (2001) Importance of complete DNA digestion in minimizing variability of 8-oxo-dG analyses. Free Rad Biol Med 31:1341–1351

Janata E (1982) Pulse radiolysis conductivity measurements in aqueous solutions with nanosecond time resolution. Radiat Phys Chem 19:17–21

Janata E (1992a) Instrumentation of kinetic spectroscopy. 9. Use of a computer for automatic performance of start-up procedures on a 4 MeV Van de Graaff electron accelerator. Radiat Phys Chem 40:217–223

Janata E (1992b) Instrumentation of kinetic spectroscopy. 10. A modular data acquisition system for laser flash photolysis and pulse radiolysis experiments. Radiat Phys Chem 40:437–443

Janata E, Lilie J, Martin M (1993) Instrumentation of kinetic spectroscopy. 11. An apparatus for AC-conductivity measurements in laser flash photolysis and pulse radiolysis experiments. Radiat Phys Chem 43:353–356

Jaruga P, Rodriguez H, Dizdaroglu M (2001) Measurement of 8-hydroxy-2′-deoxyadenosine in DNA by liquid chromatography/mass spectrometry. Free Rad Biol Med 31:336–344

Johansen I, Boye E (1975) Radiation-induced DNA strand breaks in *E. coli* measured within a fraction of a second. Nature 255:740–742

Johansen I, Brustad T, Rupp WD (1975) DNA strand break measured within 100 milliseconds of irradiation of *Escherichia coli* by 4MeV electrons. Proc Natl Acad Sci USA 72:167–171

Joshi RR, Ganesh KN (1994) Metallodesferals as a new class of DNA cleavers: Specificity, mechanism and targetting of DNA scission reactions. Proc Indian Acad Sci (Chem Sci ) 106:1089–1108

Jurado J, Saparbaev M, Matray TJ, Greenberg MM, Laval J (1998) The ring fragmentation product of thymidine C5-hydrate when present in DNA is repaired by *Escherichia coli* Fpg and Nth proteins. Biochemistry 37:7757–7763

Kasai H, Nishimura S (1986) Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated glucose and oxygen radical-forming. Environ Health Perspect 67:111–116

Kobayashi Y, Kubo H, Kinoshita T (1987) Fluorometric determination of guanidino compounds by new postcolumn derivatization system using reversed-phase ion-pair high-performance liquid chromatography. Anal Biochem 160:392–398

Krokan HE, Standal RSG (1997) DNA glycosylases in the base excision repair of DNA. Biochem J 325:1–16

Krushinskaya NP (1983) Radiation-induced chemical alterations in sugar moiety of DNA: Carbon-carbon bond rupture. In: Dobo J, Hedvig P, Schiller R (eds) Proceedings of the 5th Tihany symposium on radiation chemistry. Akedemiao Kiado, Budapest, pp 1061–1066

Krushinskaya NP, Shalnov MI (1967) Nature of breaks in the DNA chain upon irradiation of aqueous solutions. Radiobiology 7:36–45

Langfinger D, von Sonntag C (1985) γ-Radiolysis of 2′-deoxyguanosine. The structure of the malondialdehyde-like product. Z Naturforsch 40c:446–448

Le XC, Xing JZ, Lee J, Leadon SA, Weinfeld M (1998) Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. Science 280:1066–1069

Lewis HL, Ward JF (1978) Serologic assay of DNA base damage. In: Hanawalt PC, Friedberg EC, Fox CF (eds) DNA repair mechanisms. Academic Press, New York, pp 35–38

Lewis HL, Muhleman DR, Ward JF (1978) Serologic assay of DNA base damage. I. 5-hydroxymethyldeoxyuridine, a radiation product of thymidine. Radiat Res 75:305–316

Loft S, Thorling EB, Poulsen HE (1998) High fat diet induced oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in rats. Free Rad Res 29:595–600
Maccubbin A, Evans M, Paul CR, Budzinski EE, Przybyszewski J, Box HC (1991) Enzymatic excision of radiation-induced lesions from DNA model compounds. Radiat Res 126:21–26
Maccubbin AE, Evans MS, Budzinski EE, Wallace JC, Box HC (1992) Characterization of two radiation-induced lesions from DNA: Studies using nuclease P1. Int J Radiat Biol 61:729–736
Matray TJ, Haxton KJ, Greenberg MM (1995) The effects of the ring fragmentation product of thymidine C5-hydrate on phosphodiesterases and Klenow (exo$^-$) fragment. Nucleic Acids Res 23:4642–4648
Matsufuji H, Shibamoto T (2004) The role of EDTA in malonaldehyde formation from DNA oxidized by Fenton reagent systems. J Agric Food Chem 52:3136–3140
McConlogue LC, Ward JF, Lewis HL, Norman A (1982) Radioimmune assay of induction and removal of UV lesions in total and staphylococcal nuclease-resistant DNA of mammalian chromatin. Radiat Res 89:381–395
Mei N, Tamae K, Kunugita N, Hirano T, Kasai H (2003) Analysis of 8-hydroxydeoxguanosine 5′-monophosphate (8-OH-dGMP) as a reliable marker of cellular oxidative DNA damage after γ-irradiation. Environ Mol Mutagen 41:332–338
Michael BD, Adams GE, Hewitt HB, Jones WBG, Watts ME (1973) A posteffect of oxygen in irradiated bacteria: A submillisecond fast mixing study. Radiat Res 54:239–251
Mihaljevic B, Katusin-Razem B, Razem D (1996) The reevaluation of the ferric thiocyanate assay for lipid hydroperoxides with special considerations of the mechanistic aspects of the response. Free Rad Biol Med 21:53–63
Millar BC, Fielden EM, Steele JJ (1980) Effect of oxygen-radiosensitizer mixtures on the radiation response of Chinese hamster cells, line V-79-753B, in vitro. II. Determination of the initial yield of single-strand breaks in the cellular DNA using a rapid lysis technique. Radiat Res 83:57–65
Mouret JF, Odin F, Polverelli M, Cadet J (1990) $^{32}$P-postlabeling measurement of adenine-*N*-1-oxide in cellular DNA exposed to hydrogen peroxide. Chem Res Toxicol 3:102–110
Möller L, Hofer T, Zeisig M (1998) Methodological considerations and factors affecting 8-hydroxy-2′-deoxyguanosine analysis. Free Rad Res 29:511–524
Müller R, Adamkiewicz J, Rajewsky MF (1982) Immunological detection and quantification of carcinogen-modified DNA components. In: Armstrong B, Bartsch H (eds) Host factors in human carcinogenesis. IARC Scientific Publications, Lyon, pp 463–479
Nishimura S (2002) Involvement of mammalian OGG1(MMH) in excision of the 8-hydroxyguanine residue in DNA. Free Rad Biol Med 32:813–821
O'Neill P, Al-Kazwini AT, Land EJ, Fielden EM (1989) Diffuse reflectance pulse radiolysis of solid DNA: the effect of hydration. Int J Radiat Biol 55:531–537
Ostling O, Johanson KJ (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun 123:291–298
Östling O, Johanson KJ (1987) Bleomycin, in contrast to gamma irradiation, induces extreme variation of DNA strand breakage from cell to cell. Int J Radiat Biol 52:683–691
Pang D, Popescu G, Rodgers J, Berman BL, Dritschilo A (1996) Atomic force microscopy investigation of radiation-induced DNA double strand breaks. Scann Microsc 10:1105–1110
Pang D, Berman BL, Chasovskikh S, Rodgers JE, Dritschilo A (1997) Investigation of neutron-induced damage in DNA by atomic force microscopy: experimental evidence of clustered DNA lesions. Radiat Res 150:612–618
Park J-W, Cundy KC, Ames BN (1989) Detection of DNA adducts by high-performance liquid chromatography with electrochemical detection. Carcinogenesis 10:827–832
Patterson LK (1987) Instrumentation for measurement of transient behavior in radiation chemistry. In: Farhataziz, Rodgers MAJ (eds) Radiation chemistry. Principles and applications. Verlag Chemie, Weinheim, pp 65–96
Pouget J-P, Ravanat J-L, Douki T, Richard M-J, Cadet J (1999) Use of the comet assay to measure DNA damage in cells exposed to photosensitizers and gamma radiation. J Chim Phys 96:143–146
Rajagopalan R, Melamede RJ, Laspia MF, Erlanger BF, Wallace SS (1984) Properties of antibodies to thymine glycol, a product of the radiolysis of DNA. Radiat Res 97:499–510

Randerath K, Zhou G-D, Sommers RL, Robbins JH, Brooks PJ (2001) A $^{32}$P-postlabelling assay for the oxidative DNA lesion 8,5′-cyclo-2′-deoxyadenosine in mammalian tissues. J Biol Chem 276:36051–36057

Rashid R, Langfinger D, Wagner R, Schuchmann H-P, von Sonntag C (1999) Bleomycin vs. OH-radical-induced malonaldehydic-product formation in DNA. Int J Radiat Biol 75:110–109

Rothkamm K, Löbrich M (2003) Evidence for the lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Nat Acad Sci USA 100:5057–5082

Sapora O, Fielden EM, Loverock PS (1975) The application of rapid lysis techniques in radiobiology. I. The effect of oxygen and radiosensitizers on DNA strand break production and repair in *E. coli* B/r. Radiat Res 64:431–442

Sapora O, Fielden EM, Loverock PS (1977) The application of rapid lysis techniques in radiobiology. II. The time course of the repair of DNA fixed damage and single-strand breaks in *Escherichia coli* mutants. Radiat Res 72:308–316

Sauvaigo S, Serres C, Signorini N, Emonet N, Richard M-J, Cadet J (1998) Use of the single-cell gel electrophoresis assay for the immonofluorescent detection of specific DNA damage. Anal Biochem 259:1–7

Sauvaigo S, Petec-Calin C, Caillat S, Odin F, Cadet J (2002) Comet assay couple to repair enzymes for the detection of oxidative damage to DNA induced by low doses of γ-radiation: use of YOYO-1, low-background slides, and optimized electrophoresis conditions. Anal Biochem 303:107-109

Schnabel W (1986) Pulse radiolysis studies concerning oxidative degradation processes in linear polymers. Radiat Phys Chem 28:303–313

Schuchmann H-P, Deeble DJ, Phillips GO, von Sonntag C (1991) Pulse radiolysis with conductometric detection: two approaches to absolute dosimetry. Radiat Phys Chem 37:157–160

Schuchmann MN, Schuchmann H-P, von Sonntag C (1989) The p$K_a$ value of the $^{\cdot}O_2CH_2CO_2H$ radical: the Taft $\sigma^*$ constant of the -$CH_2O_2^{\cdot}$ group. J Phys Chem 93:5320–5323

Schuler RH, Hartzell AL, Behar B (1981) Track effects in radiation chemistry. Concentration dependence for the scavenging of OH by ferrocyanide in $N_2O$-saturated solutions. J Phys Chem 85:192–199

Scoble HA, Brown PR (1983) Reversed-phase chromatography of nucleic acid fragments. High-Performance Liquid Chromatogr 3:1–47

Shigenaga MK, Gimeno CJ, Ames BN (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc Natl Acad Sci USA 86:9697–9701

Shigenaga MK, Park J-W, Cundy KC, Gimeno CJ, Ames BN (1990) In vivo oxidative DNA damage: measurement of 8-hydroxy-2′-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection. Methods Enzymol 186:521–530

Signorini N, Molko D, Cadet J (1998) Polyclonal antibodies to adenine $N^1$-oxide: characterization and use for the measurement of DNA damage. Chem Res Toxicol 11:1169–1175

Sutherland BM, Bennett PV, Sidorkina O, Laval J (2000) Clustered DNA damages induced in isolated DNA and human cells by low doses of ionizing radiation. Proc Natl Acad Sci USA 97:103

Tabata Y, Kobayashi H, Washio M, Tagawa S, Yoshida Y (1985) Pulse radiolysis with picosecond time resolution. Radiat Phys Chem 26:473–479

Teebor GW, Frenkel K, Goldstein MS (1984) Ionizing radiation and tritium transmutation both cause formation of 5-hydroxymethyl-2′-deoxyuridine in cellular DNA. Proc Natl Acad Sci USA 81:318–321

Ündeger Ü, Zorlu AF, Basaran N (1999) Use of the alkaline comet assay to monitor DNA damage in technicians exposed to low-dose radiation. J Occup Environ Med 41:693–698

Valenzuela MT, Núñez MI, Guerrero M, Villalobos M, de Almodóvar JMR (2000) Capillary electrophoresis of DNA damage after irradiation: apoptosis and necrosis. J Chromatogr A, 871:321–330

van Eldik R, Meyerstein D (2000) High-pressure radiolysis as a tool in the study of transition metal reaction mechanisms. Acc Chem Res 33:207–214

van Lith D, de Haas MP, Warman JM, Hummel A (1983) Highly mobile charge carriers in hydrated DNA and collagen formed by pulsed ionization. Biopolymers 22:807–810

Veltwisch D, Janata E, Asmus K-D (1980) Primary processes in the reactions of $OH^{\bullet}$ radicals with sulphoxides. J Chem Soc Perkin Trans 2 146–153

von Sonntag C (1989) Pulse radiolysis, a method of choice for the fast kineticist - past, present and future. Anonymous new trends and developments in radiation chemistry. International Atomic Energy Agency, Vienna, pp 13–21

von Sonntag C (1994) Topics in free-radical-mediated DNA damage: purines and damage amplification – superoxide reactions - bleomycin, the incomplete radiomimetic. Int J Radiat Biol 66:485–490

von Sonntag J (1999) The influence of solute absorbance in laser flash photolysis – actinometry in experiment and theory at non-vanishing absorbance. J Photochem Photobiol A: Chem 126:1–5

von Sonntag C, Schuchmann H-P (1994) Pulse radiolysis. Methods Enzymol 233:3–20

Wagner JR, Berger M, Cadet J, van Lier JE (1990) Analysis of thymidine hydroperoxides by post-column reaction high-performance liquid chromatography. J Chromatogr 504:191–196

Wagner JR, Hu C-C, Ames BN (2004) Endogeneous oxidative damage of deoxycytidine in DNA. Proc Nat Acad Sci USA 89:3380–3384

Waller H, Friess E, Kiefer J (1981) On the immunological detection of X-ray induced DNA damage. Radiat Environ Biophys 19:259–264

Warman JM, de Haas MP, Hummel A, van Lith D, Verberne JB, Loman H (1980) A pulse radiolysis conductivity study of frozen aqueous solutions of DNA. Int J Radiat Biol 38:459–459

Weinfeld M, Soderlind K-JM (1991) $^{32}$P-postlabeling detection of radiation-induced DNA-damage: Identification and estimation of thymine glycols and phosphoglycolate termini. Biochemistry 30:1091–1097

Whillans DW (1982) A rapid mixing system for radiobiological studies using mammalian cells. Radiat Res 90:109–125

Whillans DW, Hunt JW (1982) A rapid-mixing comparison of the mechanisms of radiosensitization by oxygen and misonidazole in CHO cells. Radiat Res 90:126–141

Wilkinson F, Willsher CJ, Warwick P, Land EJ, Rushton FAP (1984) Diffuse reflectance pulse radiolysis of opaque samples. Nature 311:40–42

Yin B, Whyatt RM, Perera FP, Randall MC, Cooper TB, Santella RM (1995) Determination of 8-hydroxyguanosine by an immunoaffinity chromatography-monoclonal antibody-base ELISA. Free Rad Biol Med 18:1023–1032

Zakaria M, Brown PR (1981) High-performance liquid column chromatography of nucleotides, nucleosides and bases. J Chromatogr 226:267–290