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On the Immunological Detection of X-Ray Induced DNA Damage*

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Summary. Denatured calf-thymus DNA was X-irradiated, coupled to methylated bovine serum albumin and injected into rabbits to study the possible formation of specific antibodies. The serological activity was tested by a modified Farr-test, a micro complement binding reaction and by caesium chloride isopycnic ultracentrifugation. It was found with all assays that the immunological reaction was mainly due to unspecific DNA-binding and only a very small amount to radiation products. It seems, therefore, that this approach is not suitable for the analytical investigation of DNA damage produced by ionizing radiation.

Introduction

The binding of specific antibodies to molecules is one of the most sensitive assay methods in biochemistry. It can even be used if the exact chemical nature of the determinant is not known. Although antibodies are normally formed against proteins, they can also be induced by nucleic acid components if they are coupled to methylated bovine serum albumin [9].

This method has been used for the assay of UV-induced pyrimidine dimers [1, 3, 5, 7, 9, 11, 12]. Attempts to use the same approach for DNA damage induced by ionizing radiation are less well documented. Only Hotz [4] reported the formation of a specific antibody. Lewis et al. [6] showed that a certain radioproduct in DNA, 5-Hydroxymethyluridine, can be detected by a serological method.

It was the aim of the present study to explore the possibilities of an immunological assay for the detection of X-ray induced DNA damage.

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Material and Methods

Antigens

Calf thymus DNA (Serva, Heidelberg) was used for injection and the complement binding assay. ^{3}H-labelled DNA which was necessary for the Farr-test and the CsCl-gradient centrifugation was prepared from E. coli (15 T^{-}) according to Marmur [8] with slight modifications [13]. Further purification was achieved by ultracentrifugation in a CsCl-gradient for 68 h at 35,000 rpm at 20° C (Heraeus-Christ centrifuge Omega II, swinging bucket rotor 52/Ti). DNA was denatured by heating to 95° C for 15 min and subsequent chilling in ice.

Experiments with UV-irradiated DNA showed that there is no difference between calf-thymus and E. coli DNA in terms of antigenic behaviour.

Irradiation

The X-ray source was a Philips tube operated at 80 kV and 30 mA (dose rate 440 Gray/min). All samples were exposed under well aerated conditions in open Petri-dishes. Calf-thymus DNA for immunization received a dose of 4,400 Gray irradiated in standard saline citrate (0.15 M NaCl, 0.015 M Na-citrate) (SSC) buffer (pH 7) at a concentration of 1 mg/ml. For the complement binding assay calf thymus DNA was dissolved in 1/10 SSC-buffer (pH 7) at a concentration of 125 µg/ml and irradiated with 440 Gray. ^{3}H-labelled E.coli DNA was exposed at a concentration of either 2 µg/ml or 10 µg/ml (CsCl-centrifugation) in 0.1 borate buffer (pH 7.8) or 0.05 borate buffer (pH 8) to 5–132 Gray or 54 Gray, respectively.

Preparation of Antisera

For immunization 1 mg denatured irradiated DNA was coupled to an equal volume (1 ml) of 1% methylated bovine serum albumin, and 2 ml Freund's complete adjuvans (Behring-Werke, Marburg) were added to this solution. About 1 ml in 100 µl portions of the mixture was intracutaneously injected into rabbits of about 2.5 kg. This procedure was repeated after about two weeks. A forthnight later the animals received four booster injections with 5 days intervals. After a further ten days the rabbits were killed and the blood collected. It was kept for one hour at room temperature and then in the refrigerator overnight. The particulate fractions were spun down with 3,000 g, the supernatant serum removed and stored at −80° C until further use. Before the assay the serum was incubated at 56° C for 30 min (45 min with complement binding assay) to inactivate complement.

Assays

Three methods were employed to investigate the binding between X-irradiated DNA and antisera obtained from immunized rabbits:

1. the Farr-test [2] (modified);
2. the micro-complement binding reaction [14];
3. isopycnic ultracentrifugation in a CsCl-gradient [4].
The procedures as described in the references were essentially followed. In all instances denatured irradiated DNA served as antigen.

Results

In the Farr-assay we found a rather high binding to unirradiated DNA and only a small increase with radiation dose. The curve passes a maximum and decreases below control values with higher doses (dose-range: 13–130 Gy). To assess the behaviour with small exposures in more detail unspecific binding was suppressed by adding excess unirradiated calf thymus DNA. The results of such an experiment are shown in Fig. 1. Control values are now low, but the increase with dose is very small and hardly significant. The application is further hampered because of the equivocal relationship so that a certain amount of binding cannot be related to DNA damage quantitatively. The reason for the observed behaviour is not clear. One possibility would be a saturation effect with more base damage but this should depend on antibody concentration. Experiments with increased serum concentration, however, gave essentially the same results so that this alternative has to be ruled out (data not shown). It may be that with increasing dose the DNA is structurally so severely damaged that binding cannot take place or that the activity bound decreases because the DNA-molecules are broken down to smaller sizes.

The micro complement binding assay gave similar findings. A clear equivalent region could be found for X-irradiated as well as for unirradiated calf thymus DNA. If excess unirradiated DNA was added, however, no equivalence region was discovered which demonstrates that binding is unspecific.

The results with isopycnic centrifugation are shown in Fig. 2. There appears to be a specific binding since ^{3}H-labelled X-irradiated DNA is found in the protein-DNA band (Fig. 2a). However, excess unirradiated calf thymus DNA causes this peak to shift to the DNA band. Binding could also be found with unirradiated ^{3}H-DNA (Fig. 2b). To follow this more quantitatively graded

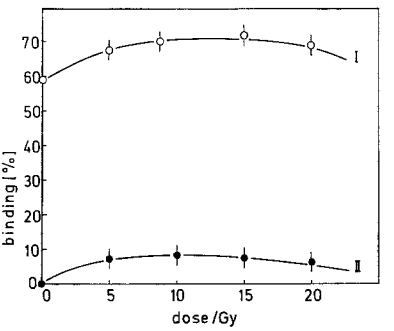

Fig. 1. Binding of (^{3}H) labelled denatured E. coli-DNA irradiated with different doses (5–20 Grays) to antiserum (50 fold diluted) without (○———○) and with (●———●) unirradiated denatured calf thymus DNA (5 µg/sample), assayed by $(NH_4)_2SO_4$-precipitation (Farr-test)

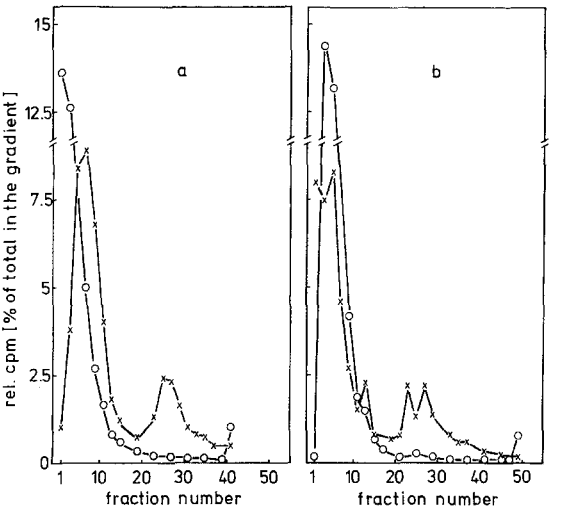

Fig. 2a and b. Density distribution of antigen – antibody complexes after centrifugation in a swinging bucket rotor (52/Ti, Heraeus Christ) for 20 h at 35,000 rpm at 10° C. Undiluted antiserum was incubated with (**a**) irradiated (^{3}H) E. coli DNA (×———×) and (**b**) unirradiated (^{3}H) E. coli DNA as control (×———×). To test specificity of the reactions 100 µg unirradiated calf thymus DNA was added to both samples (○———○). 1 ml of the reaction mixture [containing 1 µg (^{3}H) E. coli DNA] was overlaid on top of a 4 ml CsCl-borate buffer solution, pH 8.8 (ϱ = 1.6 g/cm^3), then centrifuged

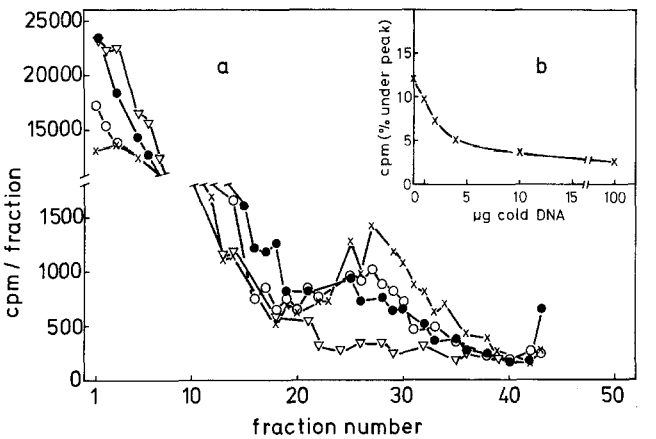

Fig. 3. (**a**) Density distribution of antigen – antibody complexes after centrifugation. Undiluted antiserum towards irradiated DNA incubated with 1 µg unirradiated (^{3}H) E. coli DNA and denatured unirradiated calf thymus DNA of different concentrations: 1 µg (× ——— ×), 2 µg (○———○), 4 µg (●———●), and 100 µg (△———△) per sample. (**b**) The decrease of (^{3}H) E. coli DNA under peak area (10–14 fractions, corresponding to the fraction number of the total gradient) with increasing concentrations of calf thymus DNA (cold DNA)

amounts of unirradiated unlabelled DNA were added to the samples with irradiated ^{3}H-DNA. Figure 3a shows that the peak height is progressively reduced. This is depicted quantitatively in Fig. 3b (insert).

Discussion

All three methods employed appeared to show that antibodies prepared from X-irradiated DNA bind to this "antigen". Close inspection revealed, however, that this was not specific for X-ray induced determinants but due to unspecific binding to DNA. An excess of unirradiated DNA always reduced the "specific" binding. It must be concluded therefore, that X-irradiated DNA cannot serve as an antigen – at least not with our immunization method; this is in contrast to UV-irradiated DNA (1,3,5; also our own results, to be published). It may be that X-ray induced changes in single-stranded DNA do not cause structural changes prominent enough to be recognized by the immune system. It may be argued that radiation induced degradation of the DNA used for the immunization prevents the formation of antibodies. This can be ruled out, however, since we always find a large amount of binding to unirradiated DNA which could not be detected with the blood of unimmunized animals. Our conclusions seem to contradict the report by Hotz [4]. He found binding of γ-irradiated ^{3}H-labelled DNA using the isopycnic centrifugation method which is in agreement with our results. Unspecific binding between serum from immunized animals and unirradiated DNA, however, was not checked; it was only shown that binding does not occur between DNA and serum from unimmunized animals which is in line with our experience. We have to conclude from our investigation that X-irradiated DNA is only a very weak specific antigenic determinant – if at all – and that therefore immunological methods are not applicable in the same way as it can be done after UV-exposure to investigate X-ray induced DNA damage in biological systems.

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References

1. Cornelis, J. J., Rommelaere, J., Urbain, J., Errera, M.: A sensitive method for measuring pyrimidine dimers in situ. Photochem. Photobiol. **26,** 241–246 (1977)
2. Farr, R. S.: A quantitative immunochemical measure of the primary interaction between J*BSA and antibody. J. Infect. Dis. 103–262 (1958)
3. Fink, A., Hotz, G.: Immunological reaction of UV-induced radiation damage in coliphage DNA. Z. Naturforsch. **32c,** 544–549 (1977)
4. Hotz, G.: Immunological recognition of specific antigenic determinants in UV- and Gamma-irradiated phage-DNA. Radiat. Environ. Biophys. **12,** 41–50 (1975)
5. Levine, L., Seaman, E., Hammerschlag, E., van Vunakis, H.: Antibodies to photoproducts of deoxyribonucleic acids irradiated with ultraviolet light. Science **153,** 1666–1667 (1966)

6. Lewis, H. L., Muhleman, D. R., Ward, J. F.: Serologic assays for γ-irradiated DNA base damage. Radiat. Res. **67,** 635 (1976)
7. Lucas, C. J.: Immunological demonstration of the disappearance of pyrimidine dimers from nuclei of cultured human cells. Exp. Cell Res. **74,** 480–486 (1972)
8. Marmur, J.: A procedure for the isolation of DNA from microorganisms. J. Mol. Biol. **3,** 208–219 (1961)
9. Natali, P. G., Tan, E. M.: Immunological detection of thymidine photoproduct formation in vivo. Radiat. Res. **46,** 506–518 (1971)
10. Plescia, O. J., Braun, W. Palczuk, N. C.: Production of antibodies to denatured deoxyribonucleic acid (DNA). Biochemistry **52,** 279–285 (1964)
11. Saenko A. S., Ilyncia, T. P., Podgorodnichenko, V. K., Porerenny, A. M.: Simple immunochemical methods for measuring DNA repair rate in UV-irradiated bacteria. Immunochemistry **13,** 779–781 (1976)
12. Seaman, E., van Vunakis, H., Levine, L.: Serologic estimation of thymine dimers in the deoxyribonucleic acid of bacterial and mammalian cells following irradiation with ultraviolet light and postirradiation repair. J. Biol. Chem. **247,** 5709–5715 (1972)
13. Vielkind, J., Schwab, M., Anders, F.: Fate of bacterial DNA injected into embryos of poeciliid fish. In: Genetics and mutagenesis of fish. Schröder, H. J. (ed.). Berlin, Heidelberg, New York: Springer-Verlag 1973
14. Wassermann, E., Levine, L.: Quantitative microcomplement fixation and its use in the study of antigenic structure by specific antigenic and antibody inhibition. J. Immunol. **87,** 290–295 (1960)

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