J. Mol. Evol. 15, 21–28 (1980)

Journal of Molecular Evolution

The Radiolysis of Tryptophan and Leucine with ^{32}P β-Radiation

Neal E. Blair and William A. Bonner

Department of Chemistry, Stanford University, Stanford, California 94305, USA

Summary. We have extended earlier experiments on the radiolysis of DL-tryptophan using ^{32}P β-radiation to longer reaction times, observing complete destruction of the tryptophan by secondary, non-radiolytic processes. We have also undertaken the irradiation of DL-leucine with ^{32}P β's at -196°, achieving radiolyses to the extents of ca. 20–30%, but observing no concomittant asymmetric bias. The implications of these observations are discussed with regard to the Vester-Ulbricht mechanism for the origin of optical activity.

Key words: β-radiolysis – Asymmetric radiolysis – Vester-Ulbricht Hypothesis – Origin of optical activity.

Introduction

The hypothesis of Vester (1957, 1959) and Ulbricht (1959, 1962) – that optically active organic compounds might have originated in nature as an ultimate consequence of parity violation (Lee and Yang, 1956) during β-decay of radioactive nuclides – has been the impetus for considerable experimentation in recent years. After the initial unsuccessful irradiation experiments of Vester and Ulbricht using a variety of organic substrates and β-emitters (Vester et al., 1959; Ulbricht and Vester, 1962), most subsequent investigators have utilized amino acids as irradiation substrates with which to search for the postulated stereoselective β-radiolysis. The β-radiation sources employed in these studies have included such nuclides as $^{90}Sr/^{90}Y$ (Garay, 1968), $^{90}Sr/^{90}Y$ Bremsstrahlen (Bonner, 1974; Bonner and Flores, 1975), ^{14}C (Bernstein et al, 1972; Bonner et al., 1978) and ^{32}P (Darge et al., 1976), as well as parallel- or antiparallel-spin longitudingally polarized electrons from a linear accelerator (Bonner et al., 1975; 1976/1977). The varying results of these experiments have recently been reviewed (Bonner et al., 1976/1977; Darge et al., 1976; Bonner and Lemmon, 1978a).

Offprint requests to: W.A. Bonner

0022–2844/80/0015/0021/$ 01.60

One of the more striking positive claims among the above studies is that of Darge et al. (1976), who exposed DL-tryptophan in frozen aqueous solution to the action of ^{32}P β-radiation for 12 weeks. They then reported that the tryptophan was 33% decomposed and that 'a preferential destruction of L-tryptophan has taken place, resulting in a relative enrichment of about 19% of the D-enantiomer'. The 33% gross degradation was estimated from ultraviolet absorption spectra, and the 19% optical enrichment by measurement of the optical activity (at 220 nm) of the crude, diluted reaction mixture. The observed optical rotation of this irradiated mixture was very small, however, only $+0.0007^{o}$ $\pm 0.0004^{o}$ (average of 16 observations). Since the claimed stereoselective effect was far larger than any previously reported and was based solely upon this minute optical rotation, and since the results had already been utilized by others in further theoretical calculations (Keszthelyi; 1977), we felt it desirable to confirm the experiments of Darge et al.

In this study (Bonner et al., 1979) we duplicated exactly the conditions of Darge et al. (1976) for the irradiation of DL-tryptophan with ^{32}P β-radiation, accompanying each experiment with a comparable non-radioactive control. After 85 days the residual irradiated tryptophan samples and controls were analyzed by g.c. (Bonner et al., 1974; Bonner, 1973) for percent degradation and enantiomeric composition. We found a slightly greater extent of gross degradation (43.5% average) than that reported by Darge et al. (1976) (33%), but found no evidence whatsoever for stereoselective radiolysis – that is, the irradiated residual tryptophan and the DL-tryptophan controls gave identical g.c. analyses. We concluded that the 19% enantiomeric enrichment reported by Darge et al. (1976) 'may have resulted from a systematic error in the very small polarimetric readings involved, or from an artifact present in the crude solutions examined'. In their more recent 'reply' to our work, Darge et al. (1979) argue that their optical rotation technique detected not only residual tryptophan but 'the sum of all potential chiral products' as well. If that is the case, the original calculation of 19% optical enrichment must be incorrect, since such a calculation can only be validly made on the basis of the precise enantiomeric composition of the residual irradiated tryptophan itself.

In view of the above negative results in our attempts to substantiate the steroeselective β-radiolysis claimed by Darge et al. (1976), we have now undertaken additional ^{32}P-irradiation experiments, modified in ways which we hoped might provide a greater chance for success. These experiments are described and discussed below.

Experimental

Prolonged ^{32}P β-Irradiation of DL-Tryptophan.

Three 2-ml aliquots of a solution of DL-tryptophan (5.1 mg) in 1.8×10^{-3}N HCl (100 ml) were placed in culture tubes and each was treated with 5 mCi (200 μl) of aqueous $NaH_2{}^{32}PO_4$ solution (New England Nuclear). A control for each sample consisted of 2-ml of the above DL-tryptophan solution mixed with 200 μl of aqueous 0.042 M NaH_2PO_4. The tubes containing these 'aqueous' samples were sealed and stored at -25^{o} for 418 days. Two 'solvent-free' samples were prepared by mixing 2-ml of the above DL-tryptophan solution each with 5 mCi of $NaH_2{}^{32}PO_4$ solution, as above, then evaporating the water at 80^{o} in an oven. The dry residues, sealed in culture tubes, were kept at -25^{o} for 416 days. Analogous 'solvent-free' controls were prepared.

One each of the above irradiated 'aqueous' and 'solvent-free' samples was divided into two equal portions and one portion of each was treated with 20 μl of a 1.3 x 10^{-2}M aqueous solution of L-tryptophan, for g.c. estimation of percent degradation by the enantiomeric marker technique (Bonner, 1973). The four portions were then evaporated and each was converted to its N-heptafluorobutyryl isopropyl ester derivative for g.c. determination of enantiomeric composition or percent degradation as described previously (Bonner et al., 1974; 1979). No tryptophan was found by g.c. in any of the irradiated samples. Tryptophan was readily detected in the corresponding non-irradiated control samples using similar g.c. techniques.

To be sure that the abscence of tryptophan in the above irradiated samples was not due to some unanticipated derivitization failure, one of the above samples was purified by ion exchange prior to derivitization. Overall recovery of the tryptophan was monitored by adding labelled tryptophan prior to ion exchange, and counting the column effluent. 0.25 mCi (2.6 μg) of 2,3-^{3}H-L-tryptophan (New England Nuclear) in 0.25 ml 1:1 ethanol: water was diluted with 8.0 ml water. A 20 μl aliquot of this solution was added to one of the above 'solvent-free' irradiated samples dissolved in 1.0 ml water. A 0.5-ml portion of this mixture was charged onto a 3.5-ml column of AG 50-W-X8 resin (Biorad, H^+) with 2 drops of 12 N HCl, and the column was washed with 10 ml H_2O, then 10 ml 2N NH_4OH. The final 14 ml was collected, from which a 300 μl aliquot was removed and added to 20 ml of Packard Instagel cocktail for scintillation counting. Comparison with a control indicated the 95% of the ^{3}H-labelled tryptophan was recovered. The remainder of the sample was evaporated and derivatized as before for g.c. analysis. Again no tryptophan was found in this irradiated sample. The ^{3}H-labelled tryptophan tracer added was present in too small concentration for g.c. detection.

Finally, the absence of tryptophan in the irradiated samples was confirmed by thin layer chromatography (t.l.c.). A 200 μl aliquot of one of the above 'aqueous' irradiated samples was evaporated to ca. 1.5 μl and applied to a pre-coated silica gel t.l.c. plate (Pierce Chemical Co., MQ6F). A portion of one of the 'aqueous' controls as well as a tryptophan solution 'standard' were also spotted on the plate, which was then eluted with a 4:2:5 mixture of 1-butanol: acetic acid: water. The spots were visualized both by ultraviolet light and by Ehrlich's reagent (10% p-dimethylaminobenzaldehyde in 1:4 con. HCl: acetone). While the control and standard showed strong spots for tryptophan, no such spot was evident for the irradiated sample.

We estimate that the detection limit for tryptophan in the above g.c. experiments was ca. 0.1. μg. In the t.l.c. experiments the control employed contained ca. 2.3 μg tryptophan.

^{32}P β-Irradiation of D-, L- and DL-Leucine

Two 100 μl aliquots of a solution of DL-leucine (143.2 mg; Aldrich) in 0.24N HCl (100 ml) were placed in ampoules along with 5 mCi (200 μl) of an aqueous solution of $NAH_2{}^{32}PO_4$ (New England Nuclear). The solutions were evaporated to dryness under vacuum and the ampoules were flame-sealed. Identically prepared samples containing L-leucine (0.143 mg; Aldrich) or D-leucine (0.140 mg; Sigma) along with 5 mCi of $NaH_2{}^{32}PO_4$ were likewise placed in sealed ampoules. Corresponding controls were made similarly for all samples by merely substituting a 200 μl aliquot of a solution of

$NaH_2PO_4 \cdot H_2O$ (0.5519 g/100 ml; J.T. Baker) for the $NaH_2{}^{32}PO_4$. All of the sealed ampoules containing the irradiation samples and the controls were suspended by wires in a Dewar flask filled with liquid nitrogen, then kept at this temperature for a period of 90 days, after which the ampoules were retrieved and their seals broken while still cold. Each sample was immediately (within 2 min) treated with 2.00 ml of a 1:1 ethanol: 6N HCl solution, and the resulting solution was quantitatively divided into two equal portions. Each portion was treated with 200 μl of aqueous sodium metabisulfite solution (92.2 mg/100 ml), and one portion from each sample was treated with 100 μl of an aqueous solution of L- (or D-) leucine (149.5 mg/100 ml) in order to permit g.c. determination of percent degradation by the enantiomeric marker technique. Each solution was then warmed and evaporated to dryness in a nitrogen stream, and the residues were treated with 1 ml of 4N HCl in 2-propanol and heated at 130° for 1.5 h (sealed tube). The solutions were again evaporated to dryness and the residues treated with 1 ml of dichloromethane and 50 μl trifluoroacetic anhydride. After 20 min the volatiles were again evaporated and the N-TFA-leucine isopropyl ester residues were dissolved in 10 μl dichloromethane for g.c. analysis.

In a second series of experiments involving higher β-irradiation doses, four 100 μl aliquots of a solution of DL-leucine (143.7 mg) in 0.24 N HCl (100 ml) were pipetted into ampoules each containing 12.5 mCi (500 μl) of aqueous $NaH_2{}^{32}PO_4$ solution. Controls were prepared with the sample quantity (0.144 mg) of DL-leucine mixed with 7.1 μl of an aqueous $NaH_2PO_4 \cdot H_2O$ solution (1 mg/100 ml). All samples were treated exactly as in the previous irradiation experiment, except that the samples were kept at liquid nitrogen temperature for 124-125 days. Only one-quarter of each sample was used for the percent degradation determination, D-leucine being used as the enantiomeric marker.

Gas Chromatographic Analyses.

The above irradiated and control samples, after conversion to their N-TFA-leucine isopropyl esters, were analyzed as previously described (Bonner et al., 1974) using 46 m x 0.5 mm (i.d.) stainless steel capillary g.c. columns coated with either N-docosanoyl-L-valine *tert*-butylamide phase (Charles et al., 1975) or the enantiomeric D-valine phase (Bonner and Blair, 1979), installed in a Hewlett-Packard 5700A gas chromatograph linked with a Hewlett-Packard 3380A digital electronic integrator recorder. At 110° (isothermal) and a nitrogen flow rate of 10 ml/min baseline resolution of the enantiomeric leucine derivatives was achieved with the L-isomer eluting in 6.3 min. and the D-isomer in 8.6 min, employing the above D-valine phase. All samples were run in replicate (5–8 times), with the control samples interspersed 'back to back' with the irradiated samples. In the second series of experiments involving 12.5 mCi ^{32}P-irradiations, all samples and controls were run on both the D-valine and L-valine phase g.c. columns to eliminate possible experimental artifacts. The results from the g.c. analyses are shown in Tables 1 and 2.

Results and Discussion

Our original ^{32}P irradiation of DL-tryptophan involved an 85-day exposure (5.94 half-lives; ^{32}P 98.4% transmuted) and resulted in an average tryptophan decomposition of 43.5%. (Bonner et al., 1979). We have now duplicated this experiment using, however,

a 418 day exposure period. Analgous processing of the residue failed to reveal the presence of any residual tryptophan, using either g.c. or t.l.c. criteria. Since in our original 85-day experiments over 50% of the tryptophan was still present after more than 98% of the radioactivity had decayed, it is clear that subsequent chemical reactions (other than primary radiolysis) must have been responsible for the complete destruction of the tryptophan during the longer reaction period. Such secondary (presumably symmetrical) degradative reactions must also have competed during our original 85-day irradiations, and would clearly result in products obscuring those from stereoselective β-radiolysis. Our irradiation experiments were accordingly re-designed as follows to minimize the occurance of such competing secondary reactions. In the first place we employed DL-leucine as a target, since we had found that e.e.'s of leucine enantiomer mixtures could be measured by g.c. with considerably greater accuracy and precision than e.e.'s of tryptophan mixtures (Bonner et al., 1979). Secondly, the irradiations were conducted under anhydrous conditions to eliminate the initial radiolytic formation of HO radicals and hydrated electrons from water (Garrison, 1972) and the subsequent secondary reactions initiated by them. Thirdly, irradiations were conducted at -196° to slow down both the rate of secondary reactions and the migration of intermediate reaction species. Finally, after completion of the irradiations, the residues were immediately (within 2 min) treated with ethanol/HCl to quench free radicals (Bayly and Evans, 1967), and with sodium metabisulfite to reduce oxidizing species. The results of our initial leucine irradiations using 5 mCi ^{32}P are shown in Table 1.

Table 1 indicates that with ca. 18–23% gross degradation there was no stereoselective radiolysis of either enantiomer in the DL-leucine experiments, the enantiomeric

Table 1. 90-day irradiations of leucine with 5 mCi ^{32}P

No.	1	2	3	4
Leucine substrate	DL	DL	D	L
G.c. phase[a]	D	D	D	L
Sample analysis				
%D(S.D.)[b]	49.8(0.4)	50.0(0.1)	99.5(0.1)	0.5(0.1)
%L(S.D.)[b]	50.2(0.4)	50.0(0.1)	0.5(0.1)	99.5(0.1)
Control analysis				
%D(S.D.)[b]	49.6(0.4)	50.1(0.1)	99.1(0.0)	0.3(0.0)
%L(S.D.)[b]	50.4(0.4)	49.9(0.1)	0.9(0.0)	99.7(0.0)
E.e., %[c](S.D.)	0.4(0.8)[d]	0.2(0.2)[e]	–	–
Racemization, %[f](S.D.)	–	–	0.0	0.2(0.1)
Degradation, %(S.D.)[g]	23.2(0.1)	17.9(1.2)	31.5(0.2)	25.6(0.2)
Molecules dec./β (S.D.)[h]	465(3)	361(24)	621(3)	514(3)

[a] N-docosanoyl-D-(or L-) valine *tert*-butylamide
[b] Standard deviation of 4–8 g.c. analyses
[c] Absolute value of %D - %L, corrected for control analysis
[d] D > L
[e] L > D
[f] Corrected for control analysis
[g] Average % degradation = 24.6 ± 1.24
[h] Average molecules decomposed per β = 490 ± 25

compositions of the irradiated samples and the controls agreeing within experimental error. In the separate irradiations of D- and L-leucine we note that little or nor radioracemization is observed in the undecomposed residue. This contrasts to the significant radioracemization which was found during room-temperature γ-radiolysis of solid-D- and L-leucine (Bonner and Lemmon, 1978 a and b), and is probably the consequence of the low temperature employed in the present β-irradiations. The rather poor agreement in the percent gross degradation among the four experiments in Table 1 appears to us to indicate the increased experimental error involved when the enantiomeric marker technique is scaled down to the use of only 0.1 mg or so of substrate. In any case the rather low gross radiolysis in these experiments (ca. 25% average) was thought a possible cause for our failure to detect stereoselectivity in the degradations. Accordingly, in the hope of achieving more extensive radiolysis, the experiments with DL-leucine were repeated using 2.5-times the initial quantity of ^{32}P as the β-source. Table 2 summarizes the results of four such replicate experiments in which 12.5 mCi ^{32}P were employed in each.

Table 2 indicates that again no stereoselective radiolysis was achieved. Any apparent e.e. from a given series of analyses with one g.c. phase was almost quantitatively reversed when the enantiomeric g.c. phase was employed. This suggests that a small, systematic bias may have attended the g.c. analyses, and emphasizes the importance of

Table 2. 124-day irradiations DL-leucine with 12.5 mCi ^{32}P

No.	1	2	3	4
G.c. phase	D[a]	D[a]	D[a]	D[a]
Sample analysis				
%D(S.D.)[c]	50.1(0.1)	50.3(0.3)	50.4(0.2)	49.7(0.1)
%L(S.D.)[c]	49.9(0.1)	49.7(0.3)	49.6(0.2)	50.3(0.1)
Control analysis				
%D(S.D.)[c]	50.0(0.1)	50.1(0.1)	50.1(0.1)	50.0(0.1)
%L(S.D.)[c]	50.0(0.1)	49.9(0.1)	49.9(0.1)	50.0(0.1)
E.e[d]., (S.D.)	0.2(0.2)[e]	0.4(0.4)[e]	0.6(0.3)[e]	0.6(0.2)[f]
G.c. phase	L[b]	L[b]	L[b]	L[b]
Sample analysis				
%D(S.D.)[c]	50.2(0.1)	–	50.0(0.0)	50.7(0.1)
%L(S.D.)[c]	49.8(0.1)	–	50.0(0.0)	49.3(0.1)
Control analysis				
%D(S.D.)[c]	50.3(0.1)	–	50.3(0.1)	50.2(0.1)
%L(S.D.)[c]	49.7(0.1)	–	49.7(0.1)	49.8(0.1)
E.e[d]., (S.D.)	0.2(0.2)[f]	–	0.6(0.1)[f]	1.0(0.2)[e]
Degradation, %(S.D.)[g]	22.2(0.2)	24.8(0.6)	29.8(0.3)	–
Molecules, dec./β(S.D.)[h]	177(2)	198(5)	238(2)	–

[a] N-docosanoyl-D-valine *tert*-butylamide
[b] N-docosanoyl-L-valine *tert*-butylamide
[c] Standard deviation of 4–8 g.c. analyses
[d] Absolute value of %D - %L, corrected for control analysis
[e] D > L
[f] L > D
[g] Average % degradation = 25.6 ± 0.7
[h] Average molecules decomposed per β = 204 ± 6

using enantiomeric g.c. phases (Bonner and Blair, 1979) in the g.c. evaluation of such small enantiomeric excesses.

The unexpected observation in Table 2 is that, despite the 2.5-fold radiation dose increase, the average percent degradation is the same as that seen in Table 1. This suggests that a saturation level of radiolysis intermediates may have built up and been 'frozen in' at -196°, and that this level of intermediates underwent comparable extents of secondary degradative reactions during the subsequent brief warm-up periods and processing times (ca. 2 min) in the two sets of experiments. Whatever the cause, our efforts to achieve more extensive radiolysis by using a 2.5-fold stronger ^{32}P radiation source were frustrated at liquid nitrogen temperatures.

The average number of molecules decomposed per β-particle in Tables 1 (490) and 2 (204) are of interest. While the comparative disagreement in the two values may be accounted for as suggested above, their generally small magnitude contrasts sharply to the decompositions per β noted during the room-temperature self-radiolysis of ^{14}C-labeled amino acids ($9-36 \times 10^3$; mean energy/β; ca. 50 KeV) (Bonner, Lemmon, Noyes, 1978) and during the room temperature bombardment of DL-leucine with longitudinally polarized 120 KeV linear accelerator electrons (ca. 3000) (Bonner et al., 1976/1977). It seems likely that these large differences may be due to the higher energy and lesser effectiveness of the ^{32}P β's (mean energy/β, ca. 570 KeV) and to the fact that at -196° secondary reaction paths for the degradation of leucine intermediates are curtailed.

The complete absence of asymmetric bias in our present and earlier (Bonner et al., 1979) radiolyses of aqueous tryptophan at -25° and our present radiolyses of water-free leucine at -196° using ^{32}P β-radiation and its accompanying Bremsstrahlen leave it an open question whether or not the Vester-Ulbricht β-decay/Bremsstrahlen mechanism for the origin of optical activity is a viable one. Since the question of a possible relationship between asymmetry at the elementary particle level and chirality at the molecular level is presently unanswered and is clearly important as regards the question of the origin of life, we would like to urge others to extend earlier experiments and to develop new ones having a bearing on the matter.

Acknowledgment. We are indebted to the National Aeronautics and Space Administration for a research grant (NGL-05-020-582) which supported the above investigation.

References

Bayly, R.J., Evans, E.A. (1967). J. Labeled Compounds, Vol. III, Suppl. 1, 349–379

Bernstein, W.J., Lemmon, R.M., Calvin, M. (1972). "Molecular Evolution, Prebiological and Biological", D.L. Rolfing, A.I. Oparin, ed., pp. 151–155, New York: Plenum

Bonner, W.A. (1973). J. Chromatographic Sci. 11, 101–104

Bonner, W.A. (1974). J. Mol. Evol. 4, 23–39

Bonner, W.A., Blair, N.E. (1979). J. Chromatogr. **169**, 153–159

Bonner, W.A., Blair, N.E., Flores, J.J. (1979). Nature **281**, 150

Bonner, W.A., Dort, M.A. Van, Flores, J.J. (1974). Anal. Chem. 46, 2104–2107

Bonner, W.A., Dort, M.A. Van, Yearian, M.R. (1975). Nature **258**, 419–421

Bonner, W.A., Dort, M.A. Van, Yearian, M.R., Zeman, H.D., Li, G.C. (1976/1977). Israel J. Chem. 15, 89–95

Bonner, W.A., Flores, J.J. (1975). Origins Life 6, 187–194
Bonner, W.A., Lemmon, R.M. (1978a) Biorg. Chem. 7, 175–187
Bonner, W.A., Lemmon, R.M. (1978b). J. Mol. Evol. 11, 95–99
Bonner, W.A., Lemmon, R.M., Noyes, H.P. (1978). J. Org. Chem. 43, 522–524
Charles, R., Beitler, U., Feibush, B., Gil-Av., E. (1975). J. Chromatogr. 112, 121–133
Darge, W., Laczko, I., Thiemann, W. (1976). Nature 261, 522–524
Darge, W., Laczko, I., Thiemann, W. (1979). Nature 281, 151
Garay, A.S. (1968). Nature 219, 338–340
Garrison, W.M. (1972). Radiat. Res. Rev. 3, 305–326
Keszthelyi, L. (1977). Origins Life 8, 299–340
Lee, T.D., Yang, C.M. (1956). Phys. Rev. 104, 254–258
Ulbricht, T.L.V. (1959). Q. Rev. Chem. Soc. 13, 48–60
Ulbricht, T.L.V., Vester, F. (1962). Tetrahedran 18, 629–637
Vester, F. (1957). Seminar, Yale University, Feb. 7
Vester, F., Ulbricht, T.L.V., Krauch, M. (1959). Naturwissenschaften 46, 68

Received September 12, 1979; Revised October 16, 1979