THE RADIOLYSIS AND RACEMIZATION OF LEUCINE ON PROTON IRRADIATION

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Abstract. D- and L-Leucine have been subjected to 39–55 percent radiolysis using 0–11 MeV protons, both with the proton beam passing through the sample or being absorbed by it, and with quenching the sample immediately on completion of irradiation or after a 21-day interval. Racemization was small (1.1–1.7 percent) and comparable in all cases, suggesting that radioracemization and secondary degradative effects were not important factors in our recent unsuccessful attempts to induce optical activity in DL-leucine by partial radiolysis using 0–11 MeV longitudinally polarized protons.

Extending previous attempts by ourselves and others (Garay, 1968; Bernstein *et al.*, 1972; Bonner, 1974; Bonner and Flores, 1975; Bonner *et al.*, 1975; 1976/1977; 1978; 1979a, 1979b; Blair and Bonner, 1980; Darge *et al.*, 1976; 1979; Hodge *et al.*, 1979) to test the Vester–Ulbricht (V–U) hypothesis (Ulbricht and Vester, 1962) for the origin of optically active molecules in nature by the attempted asymmetric radiolysis of racemic amino acids using natural or artificially produced anti-parallel longitudinally polarized beta particles, we (Lemmon *et al.*, 1981) have recently subjected DL-leucine to partial radiolysis instead with longitudinally polarized protons of both parallel and anti-parallel spin. While reasons were advanced that polarized protons might be more effective than polarized electrons for engendering such asymmetric effects, we nevertheless were unable to detect any enantiomeric inequality in the undecomposed DL-leucine residues even when such radiolyses were conducted to the extent of 47–50 percent with protons of either spin polarity, either when they passed completely through the leucine samples or were stopped by them.

After our discovery (Bonner and Lemmon, 1978a) that ionizing radiation could cause the racemization of amino acids accompanying their radiolysis, we suggested that such radioracemization must inevitably diminish the effectiveness of the V–U mechanism for engendering optical activity by asymmetric radiolysis. In particular, it was pointed out (Bonner and Lemmon, 1978b) that if the rate of radioracemization were greater than the rate of asymmetric radiolysis, it is possible that no enantiomeric inequality might be produced in the undecomposed residue after partial radiolysis. It was later suggested (Bonner *et al.*, 1979a) that such effects might be responsible for the lack of asymmetric degradation noted by Hodge *et al.* (1979) during radiolysis of DL-leucine with longitudinally polarized electrons.

An additional factor potentially jeopardizing the effectiveness of the V–U mecha-

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nism is the possibility that secondary (presumably symmetrical) degradative reactions might accompany the primary asymmetric radiolysis and continue after the irradiation is terminated. Such effects have been observed during the ^{32}P-radiolysis of DL-tryptophan (Blair and Bonner, 1980), and to minimize these effects in our recent polarized proton irradiations of DL-leucine (Lemmon *et al.*, 1981), the irradiated samples were immediately quenched in hot 2-propanol saturated with HCl after they were removed from the proton beam.

In none of the attempted asymmetric radiolyses mentioned above, using either natural or artificially produced longitudinally polarized electrons, has a systematic attempt been made to assess the actual importance of competing radioracemization or of secondary degradative reactions in a particular experimental system, although it has been noted that negligible radioracemization accompanied the 25–30 percent gross radiolysis of solid D- and L-leucine with ^{32}P beta particles at $-196°$ (Blair and Bonner, 1980). Accordingly, further experiments seemed desirable to evaluate the potential importance of these factors in our recent unsuccessful attempts to induce optical activity in DL-leucine by irradiation with polarized protons (Lemmon *et al.*, 1981).

We have conducted four irradiations of D- and L-leucine in the Lawrence Berkeley Laboratory 88-inch sector-focused cyclotron under conditions previously employed for irradiations of DL-leucine (Lemmon *et al.*, 1981). In the present experiments, however, the use of longitudinally polarized protons was unnecessary, and unpolarized protons of the same 0–11 MeV energy range were utilized. The leucine sample disks (129 mg) were irradiated as D- and L-pairs, again alternating each pair between variable and fixed aluminum absorbers in the proton beam, such that the beam passed through the first sample disk and was stopped by the second. Beam intensities were about 14 namps and irradiation times were 144–147 minutes, such that the measured integrated beam total was 120 microcoulombs. Since the energy loss in each sample was 5 MeV/H^+, each sample received a dose of *ca.* 5×10^8 rads (Lemmon *et al.*, 1981). After irradiation two of the samples were quenched immediately by dissolving in a hot 4.5 *M* solution of HCl in 2-propanol. Half of each solution was treated with the appropriate 'enantiomeric marker' of the original D- or L-leucine to determine percent degradation (Bonner, 1973), and the resulting mixtures were converted to N-trifluoroacetylleucine isopropyl ester derivatives for gas chromatographic (g.c.) analysis for enantiomeric composition. The g.c. analyses were conducted using columns loaded with the enantiomeric g.c. phases N-docosanoyl-D-valyl- (D-phase) or N-docosanoyl-L-valyl-*tert*-butylamide (L-phase), as previously described (Bonner and Blair, 1979). The remaining two samples were allowed to stand at room temperature for 21 days, then were dissolved, divided, and similarly converted to N-TFA isopropyl ester derivatives for g.c. analysis. Both D- and L-phase g.c. columns were used for the g.c. analyses of each sample in order to provide a 'symmetry check' and to disclose any systematic errors that might be inherent in the experiments or analyses (Bonner and Blair, 1979). The results of these experiments are shown in Table I.

At the outset Table I shows overall good agreement in both degradation and

TABLE I

Degradation and Racemization of D- and L-Leucine on Irradiation with 0–11 MeV Protons

Expt.	Leucine	Beam[b]	Days[c]	D-Phase Column					L-Phase Column				
				% D	% L	(±)[d]	% Deg	% Rac	% D	% L	(±)[d]	% Deg	% Rac
1	L	PT	0	0.87	99.13	0.05	44.1	1.7	0.85	99.15	0.11	44.9	1.7
2	L	A	21	0.82	99.18	0.03	38.8	1.6	0.76	99.24	0.06	39.4	1.5
3	D[a]	PT	21	99.47[e]	0.53[e]	0.01	54.9	1.1	99.20[e]	0.80[e]	0.13	54.8	1.6
4	D[a]	A	0	99.34[e]	0.66[e]	0.05	49.9	1.3	99.18[e]	0.82[e]	0.01	49.8	1.6

[a] Contained 0.36% L-Leu.
[b] A = Beam absorbed in sample; PT = beam passed through sample.
[c] 0 = Sample derivatized immediately after irradiation; 21 = sample derivatized after 21 days.
[d] Standard deviation of 3–4 analyses.
[e] Corrected for L-Leu content of original D-Leu.

racemization results for each experiment using either the D- or L-phase g.c. columns, indicating no systematic bias in the experiments. We also note that the amount of racemization of the leucine engendered by 5×10^8 rads irradiation with the proton beam is rather low (1.1–1.7 percent) compared to that observed during γ-radiolysis (Bonner and Lemmon, 1978b), and does not vary appreciably if the beam passes through the sample or is absorbed by it, or if the sample is derivatized immediately after irradiation or after 21 days. The L-leucine pair samples are consistantly about 10 percent less degraded than the corresponding D-leucine samples (Expt. 1 *vs* 3, and 2 *vs* 4), which is probably due to uncontrolled parameters in the proton-beam intensity. The degradation caused by the higher-energy protons passing through each first leucine sample in the beam, however, is consistantly 5–5.5 percent higher than that caused when the lower-energy protons are fully absorbed by the second sample in the beam (Expt. 1 *vs* 2, and 3 *vs* 4), suggesting that the average energy deposited in the first sample was about 5 percent higher. Finally, at least in the present type of experiment, the data in Table I make it appear improbable that our failure to observe asymmetric radiolysis of DL-leucine at 50 percent degradation with longitudinally polarized 0–11 MeV protons (Lemmon *et al.*, 1981) was attributable to competing radioracemization or to secondary (symmetrical) degradation effects.

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