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I.C.T.P., P.O. BOX 586, 34100 TRIESTE, ITALY, CABLE: CENTRATOM TRIESTE



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Experiments on the Origin of Homochirality of Biomolecules

L. KESZTHELYI
Institute of Biophysics
Biological Research Centre
Szeged
HUNGARY

Lecture notes to the 7th College on Biophysics:

Structure and function of Biopolymers: Experimental and Theoretical Techniques.

Experiments on the origin of homochirality of biomolecules.

L. KESZTHELYI

Institute of Biophysics, Biological Research Centre. Szeged, Hungary, H-6701

Introduction

In this lecture we will deal with the studies related to the weak interaction only. As it has been outlined in the previous lecture, this may be considered as a force which acts all over the Universe. This explains its importance. Table I contains a more or less complete list of the experiments performed earlier. In spite of the great efforts in this field really convincing, accepted results do not exist. Our program is to review the most recent experiments, and then discuss the possibilities to measure the parity-violating energy difference (PVED)

Recent experiments

Vester-Ulbricht processes

Separated L- and D-enantiomers were irradiated by spin polarized electrons and differences in responses were observed with different methods.

1) Conte and Pieralice irradiated D- and L-alanine with positrons and found that the number of γ -quanta from the annihilation radiation was larger in L- alanine than in D- alanine with $\approx 27\%$ (Conte and Pieralice, 1987). The energy of the Compton edge due to scattering of the annihilation radiation was found to be different in the enantiomers (Conte, 1989). Conte also reported differences in ESR spectra from the interaction of β^- -particles with D- and L-alanine (Conte, 1995).

2) Wang et al. (1993) observed differences in radiolysis behavior of D-and L-amino acid in primary stage and thermodynamic equilibrium state. The same group found differences in magnetic susceptibilities and an anomaly in specific heat in single crystals of D- and L-alanine and valine, respectively (Wang, et al. 1995a, b).

We may criticize these experiments from two points of view: i) both methods to measure the difference are very sensitive to differences in purity, crystal structure, *etc.* arising from the different production of them. ii) There is no information how was chosen the quantity of radioactive material used. A function between the size of the effect and the activity of the source should have been determined.

3) Both these problems were avoided in an earlier experiment of Kovács (1979). He found an excess of L-tartrate crystals in response to β -radiation crystalizing racemic mixture (Fig. 1a). The nucleation that may have been induced by this ionizing radiation is surely a non-linear process. We fitted the data with two Gaussian distributions centred at zero and at $35 \pm 3 \text{ deg cm}^2 \text{ decimol}^{-1}$. The area of the shifted peak is 15% of that of the unshifted peak. Its size depended on the quantity of radioactivity mixed in (Fig. 1b).

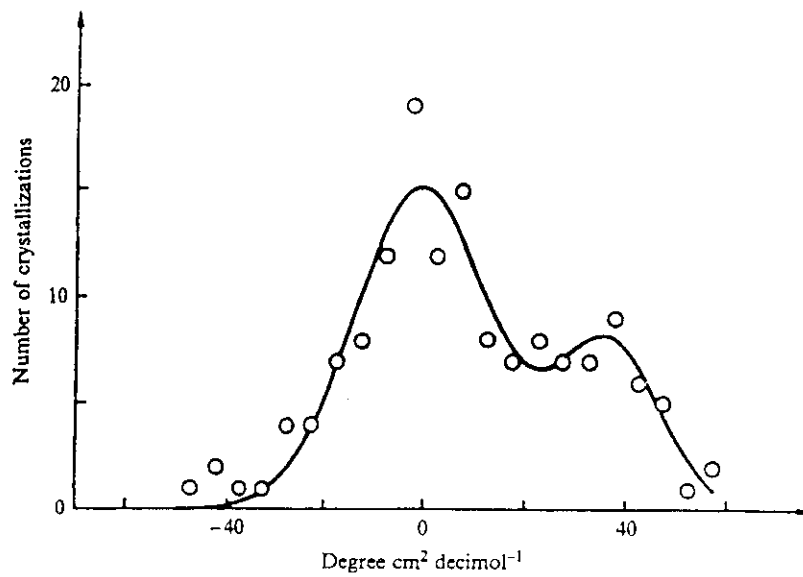


Fig. 1a. Distribution of the crystallizations of tartrate in presence of the β -radiation of ^{32}P . The data are fitted with two Gaussians.

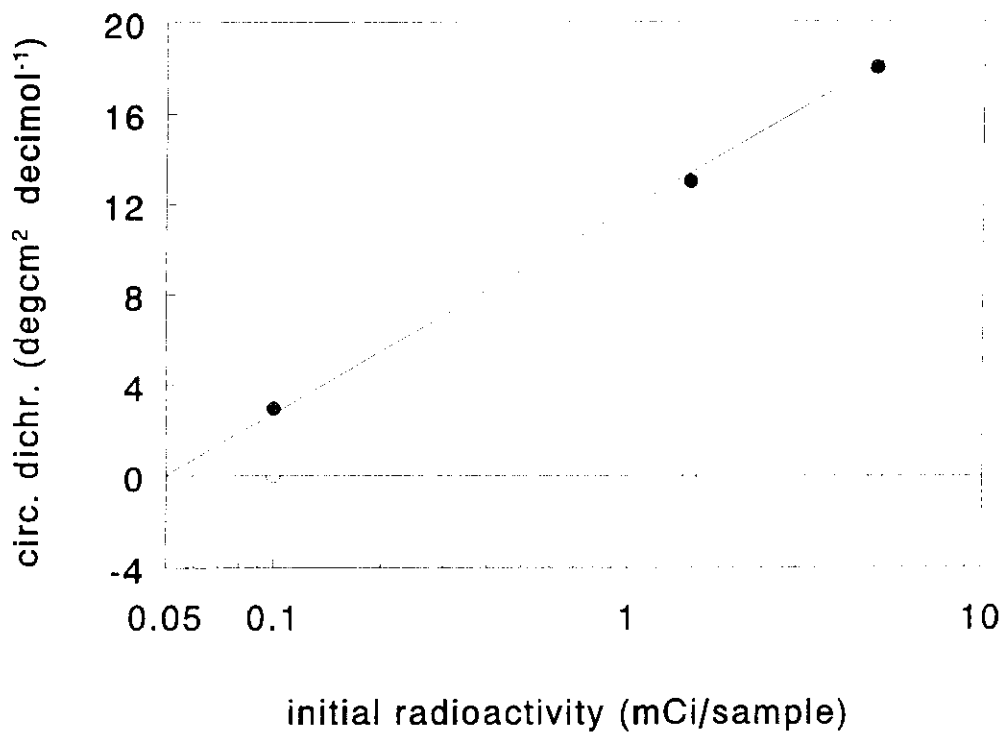


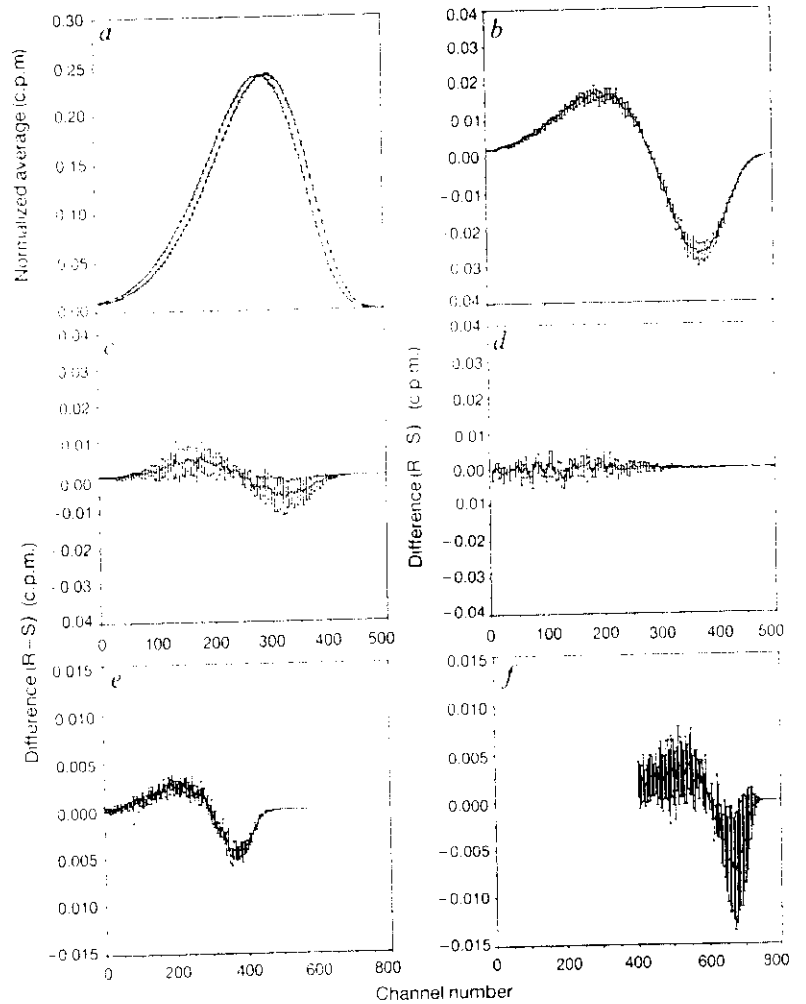
Fig. 1b. Average optical activity in dependence of the initial activity of ^{32}P β -radiation. \circ control, \bullet irradiated.

4) Campbell and Farago measured the transmission of spin-polarized 5 eV electrons through the left- and right- handed isomers of camphor vapour (Campbell & Farago, 1985, 1987). They found a relative difference near to 10^{-2} in the transmission of the electrons polarized to 28% through the isomers. This value is far too large. The authors, however noted that at this low energy a resonance effect at around 5 eV might appear.

Recently, two groups repeated this experiment and did not find an asymmetry larger than the experimental error $\approx 10^{-4}$ for camphor (Gay *et al.*, 1995; Kessler, 1995). They changed the energy of the polarized electrons in the range of the expected resonances (0.5 - 7 eV). Experiments were successful in finding asymmetries in electron scattering in the case of a compound of three camphor like molecules containing a heavy atom, ytterbium (Kessler, 1995).

4) Cerenkov radiation generated by β -particles from radioactive ^{32}P nuclei in R- and S-phenol butyric acid was measured by Garay and Ahlgren-Beckendorf (1990) and Garay *et al.* (1994). These experiments have two merits *i. e.* the enantiomers used were in liquid form and only high energy electrons could generate the light because the Cerenkov radiation appears in transparent materials if the velocity of the electrons v is larger than the velocity of light cn in the medium (here n is the refractive index). The data are reproduced in Fig. 1. The peak amplitude of the photomultiplier impulses due to Cerenkov light was smaller in R- than in S-liquids. The energy difference calculated from the amplitudes was ≈ 40 keV for the average energy of 650 keV of the β -particles from ^{32}P , *i. e.* a ratio of $\approx 6\%$. The difference may not originate from the Cerenkov radiation, because the energy loss emitted in the form of light is not more than 1 keV (Jelley, 1958). The solution of this intriguing problem could be the different absorption of the Cerenkov radiation itself in the optically active liquid or in the different sensitivity of the measuring system for the light coming from the two liquids.

FIG. 1 a. Cerenkov pulse-height spectrum of ^{32}P dissolved in R- or S-PBA. Differential spectra (b-f) are generated by subtraction of the count rate in each channel of S from those of R. Differential spectra of b. R- and S-PBA shown in a; c. external ^{32}P and d. Compton electrons from ^{137}Cs . Scintillation differential spectra for e. ^{35}S and f. ^{32}P . Scintillation solutes were PPO and POPOP. see ref. 12 for method. A smaller amplitude for these processes may reflect the multiple achiral energy transfer processes of the liquid scintillation measurement. The first part of the ^{32}P spectra was removed to minimize the Cerenkov effect recorded in low-energy channels and concomitant irradiation from β -particles from unavoidable ^{31}P contamination. The latter may contribute to the high standard deviation on the ^{32}P differential scintillation spectrum. Note the different scales on the ordinate of the differential spectra. All spectra represent the average of four PBA samples measured in random order; the instrument was calibrated before each measurement. Saturation of photomultiplier and overspill between channels were avoided by assuring low count rates; samples of $<200,000$ total c.p.m. were used. All spectra were taken in five-channel increments and normalized to the total count rate. Error bars represent the standard deviation for each point. This set of experiments was repeated eight times with different batches of enantiomers and ^{32}P , a total of 32 spectra. All measurements yielded consistent results.



Measurement of PVED

1) We suggested the Mössbauer effect, which has high energy resolution, for the measurement of PVED (Keszthelyi, 1995). Mössbauer nuclides have an excited state that emits γ -radiation, and the same γ -radiation can be absorbed by another nuclide of the same sort. In this "resonance" absorption, the energy resolution is extremely high. The electrons surrounding the nucleus influence this energy. We expect that the PVED appears directly and differently in the energy of the γ -radiation absorbed by the nuclides at the asymmetric centre of the enantiomers (this assumption needs theoretical confirmation).

The possibility depends on the ratio of the value of PVED and the natural width of the excited states Γ . Values of PVED were calculated simply by multiplying the PVED for carbon ($Z = 6$) by $(Z/6)^6$, while the width of the excited states Γ may be taken from (Vértes & Nagy, 1990). The ratios of PVED and Γ are very small. In spite of this we tried to measure the energy of the excited state for ^{191}Ir ($Z = 77$) in L- and D-Tris(1,2-ethanediamine) Iridium III complexes and this way establish an upper limit for PVED. (The complexes were prepared in Copenhagen by F. Galsbøl and B. S. Rasmussen; the measurements were made in Munich by F. Wagner.) The positions of the of absorption peaks in mm/s units are:

$$\text{L} = -1.80182 \pm 0.00745; \quad \text{D} = -1.80182 \pm 0.01616.$$

The upper limit of the energy difference calculated from the error is $4 \times 10^{-9} \text{ eV} = 3.6 \times 10^{-3} \times \text{PVED}$.

2) Bonner performed a careful study to crystallize an organic substance, benzodiazepinooxazole derivative. He found a spontaneous resolution of the enantiomers of this substance. It turned out, however, that the number of crystallization leading to the different enantiomers was the same (Bonner, 1994; 1995; Miller and Bonner, 1995).

CP and T violation

We have to mention another idea in connection with the problems of homochirality. Modern elementary particle physics has revealed that the combined operations, space inversion **P** and charge conjugation **C**, are violated too, in contrast with the initial ideas (for a simplified review see Barron, 1991). The transformation **PCT** is conserved according to the general principle. Thus, the time parity should not be conserved either. These notions prompted Garay to dwell on the possible role of the asymmetry of time in biological processes (Garay, 1993). He assumes that time passes differently in different enantiomers. Experimentally, he measured the time course of β -decay of ^{32}P and ^{22}Na in different enantiomers. He writes that "no quantitative conclusions can be drawn from the results".

Conclusions

In the light of the two contribution to this meeting, I reach the conclusion that *the chance origin of homochirality is more probable than the causal origin*. This does not mean, however, that research in the causal direction is superfluous. There are many reasons for this: new knowledge may accumulate on the basic nature of the chirality; the chiral interaction; the absolute asymmetric synthesis; the electroweak interaction; *etc.* Naturally, a verification of

the causality in the origin of homochirality is never excluded from any chiral interaction not considered so far.

We will now mention certain experiments that seem to be important for future work in this area:

- Miller-type experiments in strong external magnetic fields parallel to and antiparallel to the electric field.
- High-tech. Mössbauer experiments to measure the parity-violating energy difference directly.
- Crystallization experiments on molecules that have a high Z element at the asymmetric centre in order to find a shift in the enantiomeric distribution.
- Repetition of the crystallization of tartrate in the presence of β -radiation in another laboratory, preferentially with β^- - and β^+ -particles;
- Repetition of the measurement of Cerenkov radiation with improved technology and also a theoretical study of Cerenkov radiation for polarized electrons and chiral substances;
- The search for extraterrestrial homochirality.

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