

Demonstration of the parity-violating energy difference between enantiomers

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ABSTRACT Racemic mixtures of (+) and (–) sodium potassium tartrate, tris(1,2-ethanediamine)cobalt(III), and tris(1,2-ethanediamine)iridium(III) molecules were crystallized, and the optical activities of the resulting crystalline materials, dissolved in water, were carefully measured to study the influence of the parity-violating energy difference in the crystallization process. Although no effect was found in the case of tartrate, enantiomeric excess appeared in the crystallization of the cobalt and iridium complexes. These investigations, performed in our laboratory, demonstrated the contribution of the parity-violating neutral weak current to the forces acting in molecules.

The electromagnetic and weak interactions were unified by Weinberg, Salam, and Glashow in the concept of the electroweak interaction. This work is reviewed in simplified form in ref. 1. An important consequence of the unified theory of the electromagnetic and weak interaction is the existence of the weak neutral current that generates parity-violating interactions between electrons and electrons and between electrons and neutrons. Experiments involving elementary particles and atoms confirmed the theory. In enantiomeric molecules, the interaction induces a small difference between the energies of the different enantiomers. The theory was extended to calculations of the parity-violating energy difference (PVED) between the L- and D-amino acids, for example. The main impetus behind these calculations was the hope of explaining the origin of the homochirality of biomolecules, i.e., the phenomenon that living systems contain nearly completely L-amino acids and D-sugars (2). The asymmetry found in the weak neutral current incited the idea of the universal unity of the effects of parity violation in the inanimate and living world (for reviews see refs. 3–5). Other asymmetric forces also were considered as possible causes of homochirality. On the other hand, strong arguments exist that homochirality might have originated by spontaneous processes that appeared in the evolving system of molecules before life existed (6). The questions now are: was homochirality a result of spontaneous processes or of the action of a certain asymmetric physical agent and if the latter, was it the parity-violating weak interaction?

In the first approach to estimating PVED, Rein (7) calculated a difference of the order of 10^{-13} eV between the binding energies of L- and D-alanine. According to Gajzágó and Marx (8) an estimate of 10^{-13} eV is rather optimistic for the energy splitting between the mirror molecules. The same order of magnitude was found by Zel'dovich *et al.* (9). Later, in the calculations of Rein *et al.* (10) PVED $\approx 10^{-17}$ kT ($kT = 0.025$ eV at room temperature) was found for a model molecule (twisted ethylene), but the sign of the effect could not be determined. From the works of Mason, Tranter, and MacDermott (11–15), we know that the value of PVED is $\approx 1 \times 10^{-17}$

kT for the amino acids, the L-amino acids having the lower energy. New calculations demonstrated that the theoretical value of PVED is highly sensitive to the quality of methods applied. Bakasov *et al.* (16) and Lazeretti and Zanasi (17) have shown that the results may increase with 1–2 orders of magnitude. Even in this case PVED is very small.

Despite the smallness of PVED there have been suggestions that PVED is in fact the cause of the homochirality of biomolecules (18–20). Although the existence of a PVED between enantiomers is theoretically certain its experimental confirmation is still missing. Though spectroscopic methods, based on detailed calculations by Khriplovich (21–23) and later by Quack (24), have long been suggested for the direct measurement of the small energy difference between enantiomers, the presently available technical possibilities seem to exclude it. Therefore, at least to demonstrate the effect of PVED in enantiomers, indirect methods involving amplification come into view.

PVEDs usually are given in units of kT , because $\varepsilon = \text{PVED}/kT$ appears in the Arrhenius equation as a difference in activation enthalpies (25). It is easy to see that the ratio of reaction rates between L-L and D-D molecules is equal to ε . In polymerization or crystallization, the influence of this small ratio may increase in proportion to the number of monomers in the polymer or the number of molecules in the crystal according to Yamagata's linear model (26). The probability of obtaining a homochiral polymer of n monomers is a factor $n \times \varepsilon$ times larger for the enantiomer with the lower energy than that for the enantiomer with the higher energy. Critical comments point out that this model involves strong simplification of the problem (27, 28). This factor, for example, diminishes as the polymerization proceeds, finally, by using up all monomers from a racemic mixture, the numbers of L polymers and D polymers will be equal (27). The enantioselectivity, characterizing the difference between the probabilities of interactions in diastereoisomeric pairs of similar (DD and LL) and dissimilar (DL and LD) enantiomers also affects the amplification (28). Nevertheless, because ε is small, a large number of monomers (at least 10^{13} – 10^{14}) should polymerize, and the enantioselectivity should be $\approx 100\%$ to yield a measurable difference. The formation of such long polymers is dubious.

There is hope of detecting PVED in crystallization because macroscopic crystals consist of a large number of molecules (first suggested in ref. 29, and later applied as described in ref. 13). If there is strong enantiomeric selection in crystallization from a racemic solution, a mixture of pure right and left crystallites will be separated. PVED could increase the number of crystallites of the enantiomer that has lower energy.

The extremely small value of ε probably rules out the possibility of observing PVED in the case of carbon-centred enantiomers. Its value, however, increases in proportion to the sixth power of the atomic number Z of the element in the

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Abbreviation: PVED parity-violating energy difference.

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asymmetry center (15). In our experiments enantiomers with Co ($Z = 27$) or Ir ($Z = 77$) at the asymmetry center were chosen for the crystallization procedures.

First, the crystallization experiment of sodium ammonium tartrate ($Z = 6$), performed earlier in our laboratory (30) was repeated with some modification. Later, detailed studies were carried out on Co and Ir complexes: tris(1,2-ethanediamine)cobalt(III) and tris(1,2-ethanediamine)iridium(III).

MATERIALS AND METHODS

The sodium ammonium tartrate was prepared as in ref. 30. The Co complexes, (+) and (−) tris(1,2-ethanediamine)cobalt(III), were synthesized according to ref. 31. The Ir complexes, (+) and (−) tris(1,2-ethanediamine)iridium(III), were a gift from Galsbøl and Rasmussen (University of Aarhus, Denmark) (32). Their optical and CD spectra were checked and found to agree with data in the literature.

The crystallization experiments were performed in the following way:

The (+) and (−) molecules were mixed to produce racemic solutions with CD values of zero within error.

After filtration against possible bacterial and other larger contamination (Millipore filter, $0.45 \mu\text{m}$), the racemic solutions were either distributed into many test tubes or kept in one Petri dish. Crystallizations were conducted at room temperature in a thoroughly closed desiccator, above P_2O_5 . After about 4 weeks, when about half of the dissolved molecules were precipitated as polycrystalline material, the crystalline phase was carefully separated from the supernatant and dried.

Aliquots of 5 mg (for Co) or 1 mg (for tartrate and Ir) of the crystalline material, taken from different test tubes or from different sites in the Petri dishes, were dissolved in 1 ml of water, and their CD signals were determined.

The CD signals were measured with a Jobin-Yvon (Longjumeau, France) CD-6 spectrometer, at the wavelength of the maximal CD signal of the enantiomers. To obtain the best possible accuracy and reproducibility, special methodology was developed. A long integration time (10 sec) was chosen, each sample was measured 100 times, and the average was calculated. Because the CD signals were close to the sensitivity limit of the CD spectrometer, data were taken alternately from the sample and from tri-distilled water.

The ranges of the determined CD values were divided into equal units, and the number of samples yielding data in the units was counted. The distributions of these numbers vs. the CD signals expressed in units related to the CD signals of the pure enantiomer were fitted with Gaussians (shift S_c and width W_c). The distributions for tri-distilled water (shift S_w and width W_w) characterized the accuracy of the CD spectrometer at the given wavelength. The values of shifts (S) and widths (W) for the complexes were determined with reference to those for the tri-distilled water. A set of data obtained in one of the four series of experiments in the case of Ir complexes is given, as an illustration, in Table 1.

Care was taken with the purity of the starting racemic solution (filtration). The used glassware (test tubes, Petri

Table 1. Parameters determined from the fits of Gaussian curves in Fig. 3

Scale	S_c	S_w	S	W_c	W_w	W
Ir complex	-39 ± 8	0.3 ± 5	-39 ± 9	191 ± 19	131 ± 11	140 ± 21

S_c , W_c and S_w , W_w are the shifts from zero and widths of distributions for the crystalline sample and for tri-distilled water, respectively. S and W are the main results of the experiments giving the shift and width caused by the crystalline material. The CD data are given in relation to those for the pure enantiomer: $\Delta\epsilon = 0.95 \text{ cm}^{-1}\cdot\text{M}^{-1}$ at the measuring wavelength of 270 nm (relative CD). Data are multiplied by 10^4 .

dishes, cuvettes) were washed with ethanol, then with tri-distilled water.

RESULTS AND DISCUSSION

The crystallization of sodium ammonium tartrate was carried out in one Petri dish, and crystalline samples taken from different sites were measured. Fig. 1 shows the measured distributions after crystallization of racemic sodium ammonium tartrate. Similarly to ref. 30 when the crystallizations were conducted in different test tubes, the fitted Gaussian curve was symmetric around the zero value, i.e. no significant shift was found.

Two types of crystallization procedures (in different test tubes or in one Petri dish, as in the case of tartrate) also were compared for the Co complexes, starting from the same racemic solution. Table 2 contains the parameters S and W calculated from the fitted Gaussian curves. Because the experiment taking samples from many locations of the crystallized material in one Petri dish is more simple than dealing with many test tubes, and the results of the two methods agreed within standard error, later crystallizations were performed in Petri dishes.

Fig. 2 shows the measured distributions in the case of the Co complex together with the distribution for tri-distilled water. The data recorded with the initial racemic solution coincide, within standard error, with the data for tri-distilled water. The distribution of the CD signals for the crystalline material is shifted from the zero value and is broadened relative to the distributions for the initial racemic solution (not shown) and for tri-distilled water. Values of the shift $S = -(2.3 \pm 0.5) \times 10^{-4}$ and width $W = (4.7 \pm 0.8) \times 10^{-4}$ for the crystalline material were calculated as outlined above (see the example for the Ir complex in Table 1).

Fig. 3 depicts the results in the case of the Ir complexes. It represents one of the four independent crystallizations (serial number 2 in Table 3). The distribution of the crystalline material is shifted in the same direction as for the Co complexes and is broadened. From the raw data in Fig. 3 $S = -(39 \pm 9) \times 10^{-4}$ and $W = (140 \pm 21) \times 10^{-4}$ are calculated. Table 3 lists the shifts determined in the four experiments, which differed in the proportion to the crystallized material. The shifts are larger when less material is crystallized, as theoretically expected (27).

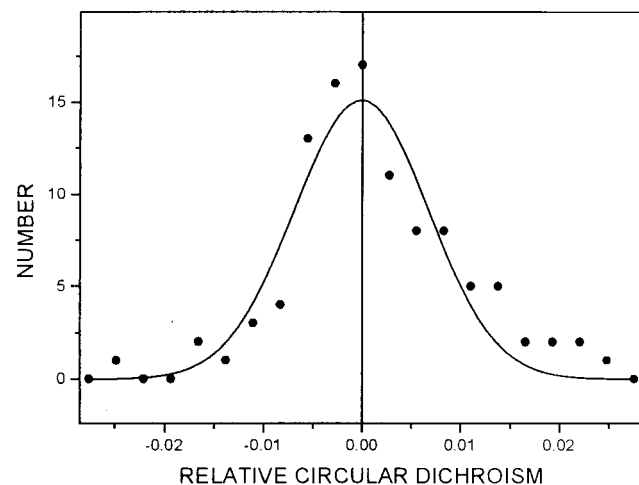


Fig. 1. Distribution of the CD data for sodium ammonium tartrate, crystallized in one Petri dish from racemic solution. Points represent measured values; line is fitted Gaussian. The CD data are given in relation to those for the pure enantiomer: $\Delta\epsilon = 2.2 \text{ cm}^{-1}\cdot\text{M}^{-1}$ at the measuring wavelength of 215 nm. The fitted values are: shift $S = -(0.9 \pm 6) \times 10^{-4}$, width $W = (136 \pm 12) \times 10^{-4}$.

Table 2. Relative shifts (S) and width (W) of the distribution of the CD data taken from Gaussian fits in crystallization of tris(1,2-ethanediamine)cobalt(III) complex depending on the type of the crystallization method

	Test tubes	Petri dish
Shift ($S \times 10^4$)	-3.30 ± 0.51	-2.67 ± 1.08
Width ($W \times 10^4$)	11.01 ± 1.05	6.67 ± 4.26

To avoid spurious conclusions, accepting that the shifts in the distributions are caused by PVED, different control experiments were performed. Two serious obstacles were considered.

Possible bacterial contamination could partially deplete one of the enantiomers. Therefore, as already mentioned, special care was taken with the purity of the starting racemic solution (filtration), and the tubes and dishes were carefully washed. To obtain further verification, samples from the racemic solutions were preserved for a year. Their CD signal did not change and biological contaminations (e.g., cell-like entities) were not found by microscopic observation.

Undetected chiral impurities, infecting the racemic mixture, may induce favorable nucleation of one of the enantiomers. They may originate from the prevalent homochiral biosphere. In an experiment, performed earlier in our laboratory, Kovács (33) studied the influence of different chiral compounds on the crystallization of sodium potassium tartrate and found that excess seeding appears above $\approx 0.1\%$ concentration of the added enantiomer. We considered that the best compound to promote forced nucleation is the compound itself. In some experiments, the solution contained 0.25% from the enantiomer that would shift crystallization in the positive direction opposite to the observed one. Measurements showed that the distribution of the crystalline phase was shifted in the negative direction even in these cases.

The shifts for the supernatants also were checked. The distribution of the CD signals always was shifted in the positive, i.e. opposite, direction to those of the crystalline phase.

These circumstances support that the shifts, determined in cases of Co and Ir complexes, are not the product of contam-

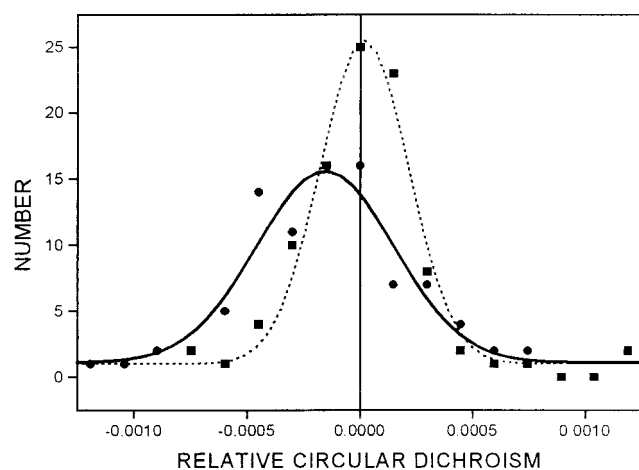


Fig. 2. Distribution of the CD data for the crystalline phase of the complex tris(1,2-ethanediamine)cobalt(III). Dashed line: tri-distilled water; solid line: precipitated crystalline material. The CD data are given in relation to those for the pure enantiomer: $\Delta\epsilon = 1.89 \text{ cm}^{-1}\cdot\text{M}^{-1}$ at the measuring wavelength of 490 nm. The fitted values are: shift and width for the crystalline material, $S_c = -(2.1 \pm 0.4) \times 10^{-4}$, $W_c = (6.1 \pm 0.8) \times 10^{-4}$; for tri-distilled water, $S_w = +(0.2 \pm 0.1) \times 10^{-4}$, $W_w = (3.9 \pm 0.3) \times 10^{-4}$, and from these the calculated values $S = -(2.3 \pm 0.5) \times 10^{-4}$, width $W = (4.7 \pm 0.8) \times 10^{-4}$. The negative shift corresponds to surplus of (-) enantiomer.

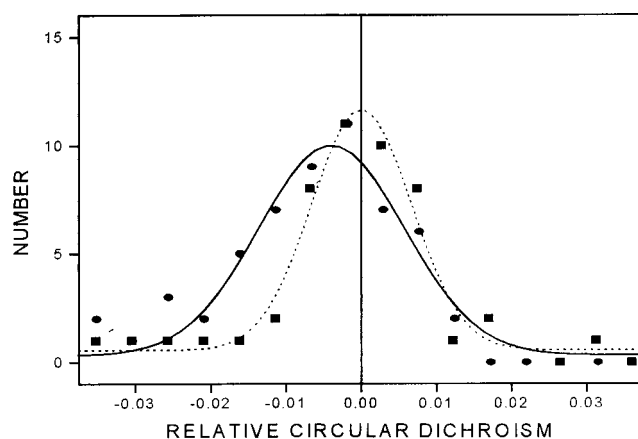


Fig. 3. Distribution of the CD data for the crystalline phase of the complex tris(1,2-ethanediamine)iridium(III). Points represent the measured values; lines are fitted Gaussians. Dotted line: tri-distilled water; solid line: precipitated crystalline material. The CD data are given in relation to those for the pure enantiomer: $\Delta\epsilon = 0.95 \text{ cm}^{-1}\cdot\text{M}^{-1}$ at the measuring wavelength of 270 nm. The fitted values are: shift for the crystalline material $S_c = -(39 \pm 8) \times 10^{-4}$, $W_c = (191 \pm 19) \times 10^{-4}$; for tri-distilled water $S_w = (0.3 \pm 5) \times 10^{-4}$, $W_w = (131 \pm 11) \times 10^{-4}$, and from these the calculated values $S = -(39 \pm 9) \times 10^{-4}$, width $W = (140 \pm 21) \times 10^{-4}$. The negative shift corresponds to surplus of the (+) enantiomer.

inations or errors in measurements and evaluations, but are real and caused by PVED.

The potential mechanism of PVED to generate shifts in crystallization already has been described (5, 29), but a short recapitulation of the idea will be presented. If there is enantiomeric selection in crystallization from a racemic solution, a mixture of pure (+) and (-) crystallites will grow. The optical activity of a sample from the crystalline material may deviate from zero, and, if a large number of crystallizations is carried out, a distribution will appear around a mean value. Such distributions, centered at zero optical activities, have been reported (30, 34, 35). This distribution is caused by the finite number of crystallites N in each sample. The different samples may contain either more (+) crystallites than (-) crystallites and vice versa. A Gaussian fitted to the distribution gives the width of the distribution W and the shift of its maximum from the zero value S . The width W related to the optical activity of the pure enantiomer is equal to the scatter in the number of crystallites \sqrt{N}/N in the sample and demonstrates that enantiomeric selection is prevalent. The average number of molecules in the individual crystallites is

$$n = \frac{P \times A}{N \times M}, \quad [1]$$

where P is the mass of the crystallized material in one sample, $A = 6 \times 10^{23}$ is the Avogadro number, and M is the molecular weight of the substance.

A shift from zero appears in the distribution if one of the enantiomers had an advantage in the process of crystallization. It is assumed that this advantage is proportional to ϵ . The

Table 3. Shifts (S) of the distribution of the relative CD data as a function of the ratio of the amount of crystallized to the amount of initial material (R , in %) for the complex tris(1,2-ethanediamine)iridium(III)

Serial number	R , %	$S \times 10^4$
1	14	-119 ± 18
2	52	-39 ± 9
3	75	-31 ± 12
4	55	-24 ± 18

Table 4. Collection and evaluation of the data obtained for the three complexes studied

Complex	$S \times 10^4$	N	n	ε	x
Tartrate	-0.92 ± 5.7	5.5×10^3	5.8×10^{14}	1×10^{-17}	ND
Co	-2.3 ± 0.5	4.6×10^6	1.9×10^{12}	8.3×10^{-14}	0.77 ± 0.02
Ir*	-119 ± 18	5.1×10^3	2.3×10^{14}	4.5×10^{-11}	0.59 ± 0.01

S calculated shift, N average number of crystallites in one sample, n average number of molecules in one crystallite, x critical exponent, ND not determined.

* S from the experiment serial number 1.

proportionality factor may be n , which would be correct only if the crystals were growing linearly and if the linear amplification caused by polymerization was correct. Consequently, proportionality factors different from n occur. The following equation may be used to estimate ε from a crystallization experiment:

$$\varepsilon = \frac{S}{n^x}, \quad [2]$$

where x characterizes the connections between the molecules in the crystals. It may have a value of 0.5 if the crystals grow on the surface or of 0.33 if growth takes place in the bulk, or other values, too, but it is surely smaller than 1, which is excluded by an analysis of the three above-mentioned crystallization experiments (5).

In Table 4, the values of the measured shifts are collected for all three complexes together with the average number of crystallites N in a sample and molecules n in a crystallite. The theoretically expected values of ε (using the Z^6 dependence and the results of the British group, refs. 11–15) and the values of the critical parameter x calculated from Eq. 2 also are included.

The recorded shifts grow with the atomic number Z . Shift was not found in the case of tartrate with carbon in the asymmetry center. The ratio of shifts for Ir and Co complexes is ≈ 50 , less than the expected 540. It is clear that the value of the shift depends on the crystallization process witnessed by the different critical parameters x . Nevertheless, we think that the tendency of PVED to grow with Z is clear.

In this connection, the work of Tranter (13) should be mentioned, who claimed that the 1% difference found in the number of L- and D-quartz crystals in nature, is a quantitative confirmation of the influence of PVED. There are two reasons to refute it:

The factor of amplification by crystallization cannot be equal to the number of molecules in a crystallite, as assumed in Tranter's work (13). This problem already has been pointed out in ref. 29. Moreover, it has clearly been shown that the existing experimental data strongly contradict Tranter's idea (ref. 5, Table 2).

Tranter's idea is "supported" with the 1% difference in the number of L- and D-crystallites of quartz deduced from data on 16,807 crystallites. This 1% is just the statistical error.

Our study appears to have provided an experimental demonstration of the participation of the parity-violating weak interaction in addition to the electromagnetic interaction in molecules. In spite of that we consider the PVED as the determining agent for the origin of the homochirality of biomolecules as an open question as discussed in refs. 5 and 6.

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