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# Entrapped Energy in Chiral Solutions: Quantification and Information Capacity

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A homogeneous solution of a chiral substance stores a residual chemical potential, related to its overall anisotropy. Therefore, by mixing solutions of opposite enantiomers, heat release may take place, corresponding to the mutual anisotropy annulment. In the following study we present proofs for this fundamental, yet unexplored, prediction by measuring the heat released upon mixing of aqueous solutions of D-proline with L-proline, as well as D-alanine with L-alanine, using isothermal titration calorimetry. Heat release in the range of 0.6−6 cal/mol was detected in these intermolecular racemizations at 30 °C. Its magnitude varied linearly with the apparent optical rotation, which complied with the possibility that the hydration envelope coating the chiral molecule is of a long-range condensed and asymmetrical configuration that can expand by integration with adjacent hydration envelopes. The ordered water in such hydration layers constitutes regions of "negative entropy", a basic medium for information storage. On the basis of our findings, a fundamental expression which combines entropy, information capacity, and thermal energy is proposed.

A homogeneous solution of a chiral substance is characterized by the magnitude and direction of rotation of a linearly polarized light. Such optical activity originates from randomly oriented chiral solute−solvent complexes which constitute an overall anisotropy. The lowered entropy, $-\Delta S$, induced by the asymmetrical organization of the solvent molecules in such a solution, implies that energy amounting to $T\Delta S$ has been invested in the system in the process of solubilization. It is therefore expected that intermolecular racemization by mixing solutions of chiral substances should release such stored energy as heat. Surprisingly, this basic possibility, which has far-reaching implications, was hitherto overlooked.

The putative energy stored in a chiral solution was tested in this study by mixing aqueous solutions of the chiral isomers of the amino acids proline and alanine. D- or L-proline are highly water soluble (up to ∼8 M at 25 °C) and are of exceptionally high specific optical activity ($|\alpha|^{D}_{25} = 81.2°$) and therefore provided good candidates for this study. D- and L-alanine are less soluble in water (up to 1.8 M at 25 °C) with a moderate specific optical activity, $|\alpha|^{D}_{25} = 13.6°$, hence served here mostly as reference systems. Stock solutions of 2 M of D- and L-proline in double-distilled water were prepared by weighing and confirmed by optical rotation measurements. The solutions were then applied for the detection of heat release upon mixing, $Q$, by isothermal titration calorimetry (ITC).

The heat of dilution of 1 M D-, L-, or DL-proline was first determined by ITC, following five sequential injections of 10 $\mu$L into 1.442 mL of water at 30 °C. The results, presented in Figure 1, corresponded to average and standard deviation of $0.78 \pm 0.04$, $0.78 \pm 0.02$, and $0.77 \pm 0.13$ mcal/mol for D-, L-, and DL-proline, respectively. Within our experimental range of measurements these values were considered identical, which provided the necessary common baseline for our subsequent ITC measurements.

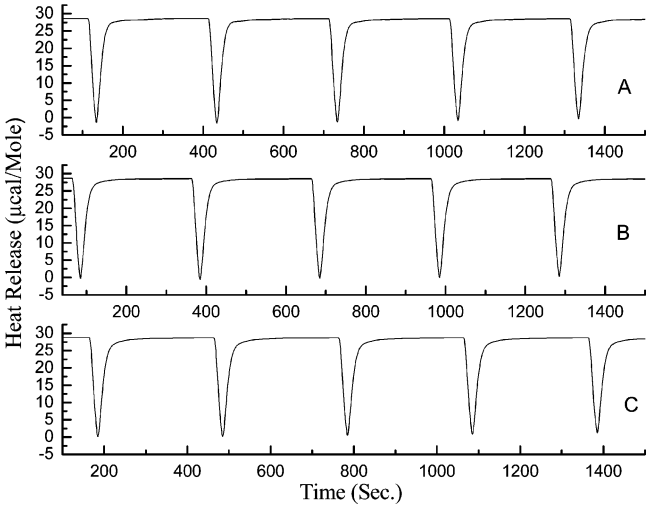


**Figure 1.** Heat of dilution of 1 M D- (curve A), L- (curve B), or DL- (curve C) proline in water, reflected in isothermic titration calorimetry profiles at 30 °C, ensuing five consecutive injections of 10 $\mu$L into 1.442 mL of water.

The titrating and the resident solutions were identical in concentration (0.4−1.0 M) in order to maintain constant the chemical potential throughout the titration. Figure 2 presents a set of ITC mixing profiles obtained for 1.0 M D- and L-proline. As clearly indicated, mixing of solutions of the same enantiomer (Figure 2, upper) was void of any apparent heat liberation, as expected, yielding a lucid control for experiments that follow. In contrast, titration of L-proline with D-proline (Figure 2, middle) and vice versa (Figure 2, lower) liberated a considerable heat. The magnitude of the released calories per mole depended strongly on the concentration of the resident solute (see the summary in Table 1), which indicated that the increase in the solution anisotropy with concentration of the chiral solute is actually cooperative and not additive. It suggests that intermo-

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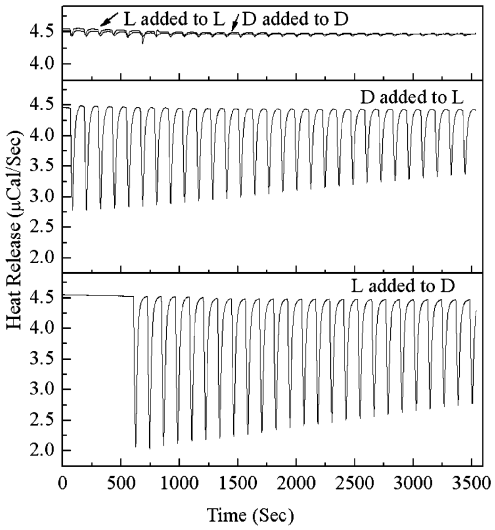


**Figure 2.** Isothermic titration calorimetry profiles at 30 °C obtained by mixing of 1 M L-proline and 1 M D-proline. Increments of 10 $\mu$L were injected to a reservoir of 1.443 mL at intervals of 100 s. The heat release was recorded as spikes of $\mu$cal/s: upper panel, L-proline added to L-proline and D-proline added to D-proline; middle panel, L-proline added to D-proline; lower panel, D-proline added to L-proline.

**TABLE 1: Changes upon Intermolecular Racemization of Proline and Alanine in Water at 30 °C: A Summary of ITC and Density Measurements**

| resident solute | concn (M) | liberated heat upon point racemization, $Q$, (cal·mol$^{-1}$) | $I_f RT$ (cal·mol$^{-1}$· optdeg$^{-1}$) | $I_f$ (optdeg$^{-1}$) | decreased density upon complete racemization $-\Delta\rho$ (mg·cm$^{-3}$) |
|---|---|---|---|---|---|
| L-proline | 2 | | | | 0.21 |
| | 1 | 4.6 | 4.3 | $7.1 \times 10^{-3}$ | 0.20 (0.0012)[a] |
| | 0.8 | 2.9 | 2.7 | $4.6 \times 10^{-3}$ | |
| | 0.6 | 1.0 | 2.0 | $3.3 \times 10^{-3}$ | 0.11 |
| | 0.4 | 0.6 | 1.3 | $2.2 \times 10^{-3}$ | |
| D-proline | 2 | | | | 0.19 |
| | 1 | 6.3 | 5.4 | $9.0 \times 10^{-3}$ | 0.18 (0.0016)[a] |
| | 0.6 | 1.2 | 2.4 | $4.0 \times 10^{-3}$ | 0.07 |
| L-alanine | 1 | 0.59 | 25 | $4.2 \times 10^{-2}$ | |
| D-alanine | 1 | 0.61 | 26 | $4.3 \times 10^{-2}$ | |

[a] $\Delta T$ was estimated from $Q$ under adiabatic conditions. The corresponding $\Delta\rho$ was then derived from the linear dependence of $\rho$ on temperature obtained from a handbook table (The Chemical Rubber Publishing Co. Cleveland, Ohio, 1988).

lecular associations between the anisotropic hydration units presumably build up to form a long-range anisotropic network. It is of interest that in the above ITC measurements the liberated heat from the D-proline reservoir systems was somewhat greater than those recorded in the parallel L-proline systems (see, for example, Figure 2), probably due to a difference in their hydration anisotropy.[1,2] Along the titration route, $Q$ decreased monotonously, approaching zero level upon complete intermolecular racemization. This pattern matched well with a simple linear relation between $Q$ and the absolute value of the residual optical activity, $|\alpha|$, or the excess concentration of the chiral compound, as shown in Figure 3.

The observed heat release described above could have, in principle, originated from the enthalpy associated with the formation of a putative complex between D- and L-proline. To rule out this possibility, we determined the saturation point of aqueous solutions of D-, L-, and DL-proline at 25 °C. For all three solutions the determined saturation concentration was 8.0 $\pm$ 0.1 M (25 °C). The identical heat of dilution of D-, L-, and DL-proline, presented in Figure 1, clearly supports this observa-

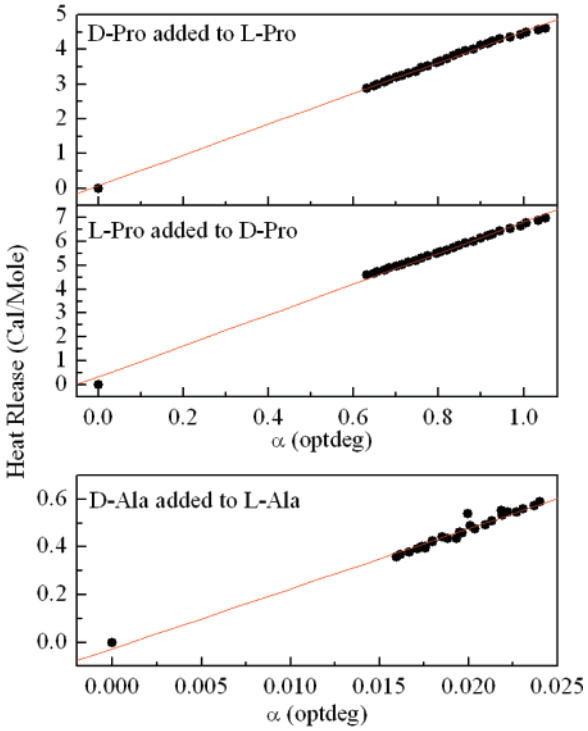


**Figure 3.** Change in specific heat release of intermolecular racemization with the level of absolute optical rotation, $|\alpha|$, derived from ITC recordings at 30 °C: upper panel, 1 M L-proline added to 1 M D-proline; middle panel, 1 M D-proline added to L-proline; lower panel, 1 M D-alanine added to 1 M L-alanine. The statistical significance of linearity in all three panels was $R > 0.98$.

tion. The possibility of formation of a complex in the DL-proline racemate should have been associated with a considerably lower saturation point and different heat of dilution. Further exclusion of this possibility was derived from a series of static proton NMR determinations of 2 M solutions of D-, L-, and DL-proline in $D_2O-H_2O$. Their corresponding spectra were identical (see Figure S1 in the Supporting Information). Additional support for the lack of interaction between these enantiomers is implied from a recent thermodynamic study on mixtures of D- and L-proline.[3] Therefore, the trivial possibility of a racemic complex formation in aqueous solutions of proline could be ruled out, in particular for the concentrations of the ITC experiments which were much below saturation.

With respect to possible effects induced by the presence of putative impurities, analogous ITC experiments were conducted with proline solutions which were contaminated with 1% glycine (i.e., 0.14 M). The resulted ITC profiles were virtually identical to those obtained with the pure solutions (not shown), thus indicating that in our experiments the effect of marginal amounts of impurities could be neglected.

Analogous experiments were performed with the enantiomers of alanine, the simplest chiral amino acid. The saturation points at 25 °C of D-, L-, and DL-alanine in water were confirmed to be 1.8 M for all three solutions. ITC determinations were performed with D- and L-alanine at the concentration range of 0.6−1.0 M. In this system, $Q$, corresponding to intermolecular racemization, was lower by about 1 order of magnitude than that obtained for the analogous proline system. Therefore, detailed ITC profiles were recorded only for 1 M alanine solutions. Linear correlations between $Q$ and $|\alpha|$, similar to those presented in Figure 3 for proline, were observed in the alanine systems as well (see Figure 3). The ITC results, including the

slopes obtained in the $Q$ versus $|\alpha|$ presentations, are summarized in Table 1.

The integration of optical activity into the realm of thermodynamics, as could be inferred from the results presented above, may imply that heat release is also expected upon mixing of chemically unrelated chiral solutions of opposite optical rotation. However, despite bearing the same magnitude of optical rotation in opposite directions, the individual solutions, as such, could be of considerably different inherent chemical potential. In practice, the detected heat release in such mixing would, most likely, be dominated by the heat of dilution of each of the individual solutions. Furthermore, unlike the cases described above, where the chiral solutes and their hydration envelopes are of perfect mirror image, in the heterogeneous mixing they might be highly different, which can override the partial heat release due to neutralization of the optical activity. We, nevertheless, tested the ITC of heat release upon mixing of D-glucose ($\alpha_{25}^{D} = +52°$) with solutions of either L-proline, D-proline, or DL-proline, of the same concentration. The ITC profiles of these mixings were of only marginal difference (not shown), which corroborated with the above limitations.

Classical thermodynamics implies that in our mixing experiments, carried out under isothermal and isobaric conditions, the observed heat release should have been associated with a corresponding change in volume (or density). The expected change in volume could be evaluated by the predicted change in temperature, $\Delta T$, under adiabatic conditions, using the basic definition $\Delta T = Q/\mathrm{Cp}$, where Cp is the heat capacity of water. In our ITC experiments with proline, the estimated adiabatic $\Delta T$ was in the range of several millidegrees, which could account for only a marginal change in specific volume (see Table 1). In parallel, direct measurements of density of aqueous solutions of D- or L-proline in comparison to DL-proline were carried out at the concentration range of 0.6−2.0 M. The results, presented in Table 1, show that the solutions of both D- and L-proline are inherently of higher densities than those of DL-proline of the same concentration, however, at a magnitude much greater than that corresponding to the calculated values (see Table 1).

## Discussion

The enthalpy−entropy interplay in liquid mixing can be delineated in simple molecular terms. The enthalpy portion, $\Delta H$, in such processes originates from the difference in the net intermolecular binding energies before and after mixing. In most cases (e.g., alcohol−water mixing) it falls in the range of $\Delta H > 10$ kcal/mol, where a net change in one hydrogen bond amounts to approximately 5 kcal/mol. The change in entropy, $\Delta S$, corresponds to the net change in overall homogeneity (mostly increase) after mixing. The energy associated with the change in entropy, $T\Delta S$, is in most cases substantially lower than $\Delta H$ yet remaining in the range of kilocalories per mole. Mixing of solutions of different solutes in the same solvent (e.g., water), where no physical interaction between the solutes takes place, is in general accompanied by thermal energy lower than 1 kcal/mol, which predominantly corresponds to $T\Delta S$.[4] In simple isothermal dilutions, in particular at low concentration where the solution approaches "ideal", the associated thermal energy is much lower. For example, the liberated heat in our dilution experiments with 1 M D-, L-, or DL-proline (see Figure 1) was below 1 mcal/mol.

Calorimetric measurements in the millicalorie per mole range were up until recently neglected due to the absence of accurate instrumentation. In the last years, microcalorimetry techniques, in particular ITC[5,6] became available, which opened the way for measurements of processes associated with small heat changes in the range of microcalories to millicalories per mole. Processes of changes in hydration layer of solutes typically fall in this range.[4] High-sensitivity ITC is now one of the leading tools for determination of thermodynamic parameters in very low-energy processes, like the ones presented in this study.

In our isothermal mixing experiments heat release of several calories per mole was detected by ITC upon mixing of aqueous solutions of D- and L-proline of the same concentration. This observation is somewhat unexpected and has never been observed before. The contribution of the heat of dilution of each of these solutes was more than 3 orders of magnitude lower, as shown in Figure 1, and could be therefore ignored. What could be then the source of the heat release of several calories per mole upon intermolecular racemization of aqueous proline? One trivial possibility is that a very weak interaction between D- and L-proline takes place and is beyond the detection by saturation point and NMR measurements which were carried out here and excluded this possibility. However, such interaction should have been reflected in a considerable difference between the heat of dilution of DL-proline and that of D- or L-proline. The second possibility, which is quite radical, is that the aqueous solutions of D-proline and L-proline, despite being identical in concentration, are not equal in their chemical potential. The only situation which can account for such a possibility is that the hydration layers around these enantiomers are somewhat different. In this respect, it has been recently suggested that the two spin isomers of water, ortho-water and para-water, have a selective preference for hydration of the D- and L-enantiomers of amino acids.[1,2,7] Yet, heat release due to isothermal scrambling of the presumed nonidentical hydration layers surrounding such enantiomers is expected to be similar in magnitude to the heat of dilution, i.e., in the range of millicalories per mole. In the following we outline a comprehensive interpretation which complies very well with the experimental findings. It is based on the fact that a homogeneous solution of a chiral compound stores a "negative entropy", $-\Delta S$, which corresponds to the degree of macroscopic anisotropy as expressed in units of optical rotation. Upon annulment of the stored negative entropy by intermolecular racemization heat is liberated in a magnitude of $T\Delta S$. Some interesting implications related to the correspondence between entropy and information capacity emerge in this interpretation.

The main feature of the heat release upon intermolecular racemization observed in this study was that the amount of calories per mole racemization was not constant, as could be expected, but declined linearly with the net optical rotation in the system. The mutual dependence between the released heat ($Q$), the increased specific volume ($1/\rho$), and the reduction in absolute optical rotation ($|\alpha|$), as summed up in Table 1, suggests that the solvent "envelopes" coating the chiral solute are of chiral configuration which is more condensed than the surrounding isotropic solvent. Furthermore, cooperativity in the solvent organization around each chiral center and between the scattered solute envelopes seems to prevail. This could be deduced from the linear dependence of $Q$ on $|\alpha|$, a parameter which correlates linearly with concentration, implying that $Q$ actually varies linearly with the square of concentration (see Table 1). The individual solvent molecules in these chiral envelopes are at a dynamic thermal exchange with the surrounding bulk solvent. Therefore, upon intermolecular racemization such envelopes gradually crumble with the ensuing heat release and volume expansion. At this stage no rigorous

information for delineating such structured solvent envelopes is at hand. Models for the hydration shells surrounding amino acids in water, based on analytical computations, have been proposed[8,9] but with no rigorous experimental verification. Accordingly, up to approximately 30 ordered water molecules surround one amino acid molecule at different vicinities. As for the chiral configuration of the hydration shell, the simplest model that may be suggested at this stage is either left- or right-handed helical twist, which in principle can be linked to adjacent hydration shells to form a macroscopic chiral network.

Quantification of the energy invested in the ordered chiral envelopes provides a unique direction for establishing a formal relation between the energy stored in the ordered solution and its apparent optical rotation, $|\alpha|$. This could be approached by applying the well-known Boltzmann equation where entropy and order are interrelated:

$$S_0 = R \ln W_0 \tag{1}$$

where $S_0$ is the maximal entropy of the system, $W_0$ is its maximal degree of disorder (i.e., complete homogeneity or isotropy), and $R$ is the universal gas constant. In a nonisotropic solution, the degree of disorder, $W$, is at a lower state and therefore acquires a "negative entropy", $-\Delta S$, of

$$\Delta S = S - S_0 = R \ln W/W_0 \tag{2}$$

This expression provides a route for introduction of a more complex physical measure, namely, information. The relations between information and entropy were discussed extensively in the literature.[10−13] Under a thermodynamic equilibrium, information is akin to negative entropy in that any creation of information necessitates energy investment. Presenting the degree of disorder, $W$, in a nonisotropic solution on the linear scale of information capacity, $I$, one gets[14]

$$-\Delta S = R \ln I/I_0 \tag{3}$$

where $I_0$ corresponds to the information capacity at the isotropic state. Any anisotropy in the system should correspond to $I > I_0$ and thus to negative entropy, as expressed in eq 3. The internal energy invested in the formation of the anisotropy in the system is, therefore,

$$-T\Delta S = RT \ln I/I_0 \tag{4}$$

The degree of information, $I$, could be alternatively presented on a scale related to the maximal information capacity of the system, $I_{max}$. Accordingly,

$$-\Delta S = R \ln I_{max}/(I_{max} - I) \tag{5}$$

With the use of a simple exponential approximation under conditions where $I_{max} \gg I$, eq 5 is reduced to the linear form

$$-\Delta S = RI/I_{max} \tag{6}$$

while the energies corresponding to eqs 5 and 6 are

$$-T\Delta S = RT \ln I_{max}/(I_{max} - I); \quad T\Delta S = RTI/I_{max} \tag{7}$$

Equations 6 and 7 remain futile unless combined with a measurable parameter which is associated with the induced order. As the macroscopic anisotropy in a solution of a chiral compound is expressed in units of optical rotation, $|\alpha|$, the latter could provide such a scale for $I$. At low concentrations (e.g., below 1 M) this scale is practically linear since $|\alpha|$ increases linearly with concentration. Under this scale the maximal capacity of information, $I_{max}$, denoted $|\alpha|_{max}$, corresponds to the limiting optical rotation of the system which can sustain information. Accordingly, eq 7 will turn into the simple correlations between optical activity and the corresponding energy storage, which are presented in eqs 8 and 9:

$$Q = -T\Delta S = RT \ln |\alpha|_{max}(|\alpha|_{max} - |\alpha|)^{-1} \tag{8}$$

For most practical cases $|\alpha| \ll |\alpha|_{max}$ which converts eq 8, by the exponential-to-linear approximation applied above, to

$$Q = -T\Delta S = I_f RT|\alpha| \tag{9}$$

$I_f$ is defined here as the "information coefficient" which translates the apparent $|\alpha|$ value, or its change, into the stored thermal energy, in units of $RT$. Formally, it corresponds to the reciprocal of $|\alpha|_{max}$. In our ITC experiments $I_f$ was derived from the slopes of the linear dependence of $Q$ on $|\alpha|$, as presented in Figure 3. This linear dependence was of high statistical significance ($R > 0.98$) in all experiments, which adds strong support for the validity of eq 9. The derived $I_f$ values presented in Table 1 show that this coefficient increases markedly with concentration, indicating a reciprocal marked decrease of $|\alpha|_{max}$ with concentration. This intriguing characteristic may reflect on the structural changes in the bulk solute−solvent network which follow changes in concentration as indicated in Figure 3. Besides concentration, other factors like temperature may also affect $I_f$, as part of the overall restructuring in the macroscopic chiral domain.

Further experimental support is needed for establishing the universal nature of eq 9 as a fundamental expression for the prediction of the amount of energy stored in a homogeneous solution of a chiral substance. The quantitative relations between chirality, information, and energy, inferred from this expression, could be of far-reaching implications which await verification.

## Experimental Section

L-Proline, D-proline, L-alanine, and D-alanine of highest purity available (all >99%) were purchased from Fluka. Degree of rotation of polarized light was measured at 25 °C with a JASCO digital polarimeter (model P-1010, $\lambda = 589$ nm, ±0.05° accuracy) using a cylindrical quartz cell of 6 mL and optical path of 50 mm. The specific optical rotations at 25 °C in water for D- or L-proline are $|\alpha|^{D}_{25} = 81.2°$ and $|\alpha|^{D}_{25} = 13.5°$ for D- or L-alanine.

Isothermal titration calorimetry measurements were performed with a VP-ITC calorimeter produced by MicroCal Inc. (Northampton, MA). The volume of the reservoir cell was 1.442 mL, thermostated at 30 °C. Injection volumes of 10 $\mu$L were delivered during 20 s, with time intervals of 100 s between injections. The ensuing heat release was displayed in units of $\mu$cal/s and then translated to cal/mol. Analysis and data fit procedures were performed with MicroCal Origin software.

Saturation points were determined with 10 M solutions of D-, L-, and DL-proline, prepared in boiling water that were allowed to gradually cool to 25 °C in sealed vessels and were kept there for 7 days. Then, supernatant aliquots of 1 mL were dried under vacuum. The residues were weighed, and the saturation concentrations were evaluated. An analogous procedure was applied for D-, L-, and DL-alanine with 4 M solutions. Density measurements were performed at 30 °C with a DMA-5000 density meter (Anton Paar).

**Supporting Information Available:** Proton NMR spectra of D-, L-, and DL-proline in $D_2O-H_2O$ solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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