Racemic D,L-asparagine causes enantiomeric excess of other coexisting racemic D,L-amino acids during recrystallization: a hypothesis accounting for the origin of L-amino acids in the biosphere.

doi: 10.1039/b409941a

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ChemComm
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Racemic D,L-asparagine causes enantiomeric excess of other coexisting racemic D,L-amino acids during recrystallization: a hypothesis accounting for the origin of L-amino acids in the biosphere

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Received (in Cambridge, UK) 1st July 2004, Accepted 10th August 2004
First published as an Advance Article on the web 7th September 2004

When recrystallizations were performed using a mixture of 12 D,L-amino acids (alanine, aspartic acid, arginine, glutamic acid, glutamine, histidine, leucine, methionine, serine, valine, phenylalanine, and tyrosine) with excess D,L-asparagine, all amino acids with the same configuration as asparagine were preferentially co-crystallized, indicating that it is the nature of a mixture of racemic amino acids to produce a spontaneous high enantiomeric excess.

It is well known that pure L-amino acids can cause spontaneous asymmetric synthesis and optical resolution.[1,2] However, their predominance over D-amino acids in the biosphere is a mystery. Despite much effort devoted to elucidating how and why L-amino acids were preferentially selected in the prebiotic earth,[3-5] no clear solution has been obtained. It has been suggested that L-amino acids originated from cometary and asteroidal materials,[6,7] with a maximal enantiomeric excess (ee) of 15.2%.[6] However, if it is the nature of a mixture of racemic amino acids to bring about a high ee, a terrestrial origin becomes realistic.

Since Pasteur's famous experiment,[8] recrystallization has been a common approach to achieving asymmetric resolution (although Pasteur did not show any ee of either isomer).[9,10] Recently, we reported that racemic D,L-asparagine (Asn) spontaneously yields crystals with ee values ranging from −59.7 to 88.9%, during recrystallization.[11] In addition, ee was induced in D,L-phenylalanine (Phe) and D,L-tryptophan (Trp) by cocrystallization with excess D,L-Asn.[11] Here, we report that D,L-Asn can cause enantioselective crystallization of other D,L-amino acids during simple recrystallization.

In this study, we determined ee by high-performance liquid chromatography (HPLC) on an instrument equipped with a column (Crownpak CR(+) manufactured by Daicel Chem. Ind. Ltd., Tokyo, Japan), which allowed us to perfect the separation of D- and L-amino acids. When the original racemic amino acids (Asn, alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), histidine (His), leucine (Leu), methionine (Met), Phe, serine (Ser), valine (Val), tyrosine (Tyr), and Trp) were analyzed, the ee of each isomer never exceeded 3%. This result is reasonable since a measured assay to assay experimental error of these chromatographic analyses was about 3%.

As a typical example, we dissolved D,L-Val (600 mg) and D,L-Asn monohydrate (2.1 g) in 10 ml of water at 100 °C, and subsequent recrystallization yielded crystals weighing 20–1230 mg. These were dissolved in water, and determination of ee was performed by chemical derivatization with *N*-acetylcysteine and *o*-phthalaldehyde, followed by HPLC with a fluorescence detector as described.[12] We carried out twelve independent experiments. In separate trials the ee for Asn crystallised from a racemic solution varied from −100 to +100%. In each trial the ee of co-crystallised amino acids was highly correlated with the value measured for Asn. The ee values of Asn (*x*-axis) and Val (*y*-axis) are shown in Fig. 1, with the regression line described by $y = 0.40x - 1.2$; $r = 0.99$. The amount of asymmetric induction in Val correlated almost linearly with that of Asn, and the ee of the influenced amino acid (Val) was less than that of the inducer (Asn). These results demonstrate that D,L-Asn greatly induces asymmetric selection of Val.

We also carried out similar recrystallization experiments using other D,L-amino acids and an excess of the inducer, D,L-Asn. A Crownpak CR(+) was used to determine the ee values of crystals obtained from recrystallizations that utilized Phe, Trp and Tyr as the D,L-amino acid, whilst chemical derivatization and HPLC[12] were used for the other amino acids, except Asp where ee values were measured using fluorescent chiral benzoxadiazole,[13] and Met, where Marfey's reagent[14] was used. Chemical derivatization yielded diastereomers of the starting D,L-amino acid. The fluorescence or absorbance peak area of the products derived from the D-isomer usually differed from that derived from the L-isomer. Therefore, the ratio of peak areas of these products was calibrated using authentic racemic amino acids.

Similar results to D,L-Val were obtained for Arg ($r = 0.99$), Asp ($r = 0.99$), Gln ($r = 0.96$), His ($r = 0.98$), Leu ($r = 0.99$), Met ($r = 0.97$), Phe ($r = 0.98$),[11] Ser ($r = 1.0$), Trp ($r = 0.98$),[11] and Tyr ($r = 0.79$), and the ee of the induced amino acids correlated almost linearly with that of Asn (Fig. 1). Thus, D,L-Asn greatly induced optical resolution of several racemic amino acids, though D,L-Ala and D,L-Glu did not afford a significant ee by similar treatment. These results demonstrate that D,L-Asn can cause an enantioselective crystallization of co-existing racemic amino acids. It was incidental whether the enrichment took place in L- or D-Asn, however, once the selection was made, the co-existing amino acid with the same configuration at the α-carbon was preferentially involved.

Finally, we dissolved a mixture of 2.0 g of D,L-Asn monohydrate,

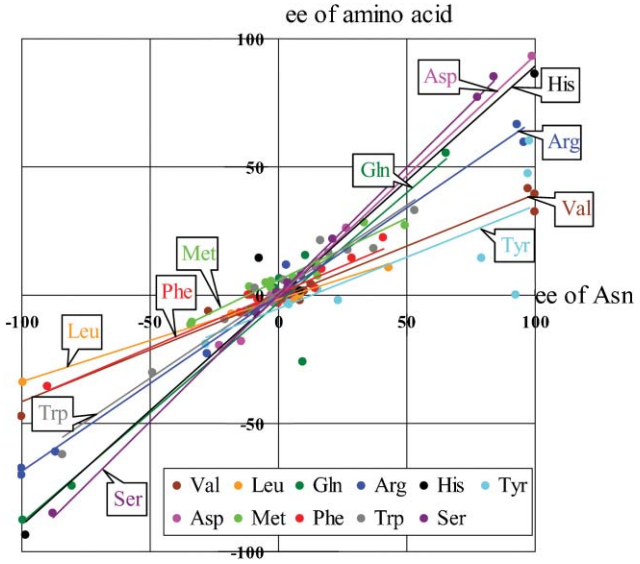

Fig. 1 Plot of the ee values of Asn and the other amino acids contained in crystals obtained by co-crystallization of excess D,L-Asn and individual D,L-amino acids. Excess D,L-Asn was recrystallized in the presence of each D,L-amino acid. The resulting crystals were dissolved in water, and the ee value of each amino acid was analyzed and plotted, as described in the text. The ee values of Asn (*x*-axis) and the other amino acid (*y*-axis) for each sample are shown.

DOI: 10.1039/b409941a

Table 1 The ee values (%) of amino acids contained in crystals obtained by recrystallization from a mixture of excess D,L-Asn with 12 D,L-amino acids

Trial	Asn	Ala	Arg	Asp	Gln	Glu	His	Leu	Met	Phe	Ser	Tyr	Val
1	0.23	−6.6	−2.4	1.2	2.3	−3.9	0.2	0.4	−9.8	−2.5	8.0	−5.5	−8.2
2	37.9	30.8	20.4	40.1	37.5	26.5	18.9	3.6	26.0	14.6	48.4	6.7	−0.4
3	33.1	43.0	35.2	48.5	52.2	41.8	18.8	8.0	40.0	22.5	56.6	14.5	5.0
4	79.4	91.0	82.6	100	94.9	92.0	70.0	42.4	40.6	71.2	100	ND[a]	41.8
5	62.9	52.8	39.2	59.7	50.6	61.3	26.1	11.2	54.9	41.4	67.4	36.1	22.8
6	−94.6	−87.1	−43.0	−100	−72.4	−77.4	−66.9	−13.3	−62.0	−39.9	−90.1	−30.1	−6.3

[a] ND: not detected.

50 mg of all racemic Ala, Asp, Arg, Glu, Gln, His, Leu, Met, Ser, and Val, and 25 mg of D,L-Phe and D,L-Tyr, in 10 ml of water, and performed recrystallization. After dissolving all crystals in an appropriate volume of water, we determined the ee of all amino acids by chemical derivatization and HPLC[12,13] (Table 1). In trial 1, a nearly racemic mixture was obtained. In trials 2–5, L-rich mixtures were obtained, and in trial 6, a D-rich mixture was obtained. These results show that co-existing amino acids with the same configuration as Asn were also preferentially co-crystallized in this experiment.

It is worthwhile to note that ee of Ala and Glu, which did not give ee by recrystallization with D,L-Asn alone, was induced from a mixture of these 13 racemic amino acids. It is surprising that the maximal ee was 100%, and these ee values are much higher than those observed in the recrystallization of each amino acid with D,L-Asn alone. These observations indicate that a mixture of racemic amino acids causes spontaneous and effective optical resolution by itself, even if asymmetric synthesis of a single amino acid does not occur without the aid of an optically active molecule.

In the recrystallization of D,L-Phe with excess D,L-Asn, the ee of each crystal indicated that the ee of Asn of either isomer was nearly 100% and that of Phe of the corresponding isomer was 60–93%.[11] These results indicated that L-Asn crystallized preferentially involving L-Phe probably due to thermodynamic stability compared to the combination of L-Asn and D-Phe and *vice versa*, and that asymmetric selection resulted from the relative content of crystals enriched with either Asn isomer.[11] In the present study a similar selection in the very early stages of the crystallisation process may also determine the ee of whole crystals.

Based on these results, we propose that enantio-selective crystallization of racemic amino acids induced by spontaneous resolution of a co-existing racemic molecule such as D,L-Asn, contributed to the selection of L-amino acids in the biosphere. Furthermore, the resulting ee is sufficiently high to account for the predominance of L-amino acids on the earth.

This work was supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Notes and references

1 L. Addadi, Z. Berkovitch-Yellin, I. Weissbuch, J. van Mil, L. J. W. Shimon, M. Lahav and L. Leiserowitz, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 466.
2 S. Pizzarello and A. L. Weber, *Science*, 2004, **303**, 1151.
3 S. Mason, *Chem. Soc. Rev.*, 1988, **17**, 347.
4 M. Avalos, R. Babiano, P. Cintas, J. L. Jimenez and J. C. Palacios, *Chem. Rev.*, 1998, **98**, 2391.
5 G. L. J. A. Rikken and E. Raupach, *Nature*, 2000, **405**, 932.
6 S. Pizzarello, M. Zolensky and K. A. Turk, *Geochim. Cosmochim. Acta*, 2003, **67**, 1589.
7 J. Bailey, A. Chrysostomou, J. H. Hough, T. M. Gledhill, A. McCall, S. Clark, F. Menard and M. Tamura, *Science*, 1998, **281**, 672.
8 L. Pasteur, *C. R. Acad. Sci. Paris*, 1848, **26**, 535.
9 A. Collet, M.-J. Brienne and J. Jacques, *Chem. Rev.*, 1980, **80**, 215.
10 D. K. Kondepudi, R. J. Kaufman and N. Singh, *Science*, 1990, **250**, 975.
11 S. Kojo and K. Tanaka, *Chem. Commun.*, 2001, 1980.
12 N. Nimura and T. Kinoshita, *J. Chromatogr.*, 1986, **352**, 169.
13 D. Jin, T. Miyahara, T. Oe and T. Toyo'oka, *Anal. Biochem.*, 1990, **269**, 124.
14 J. G. Adamson, T. Hoang, A. Crivici and G. A. Lajoie, *Anal. Biochem.*, 1992, **202**, 210.