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Chiral amplification by polypeptides and its relevance to prebiotic catalysis

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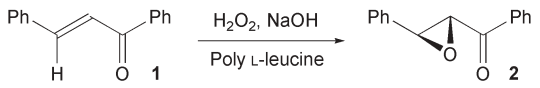
Polyleucine prepared from scalemic Leu-NCA monomers, shows high chiral amplification in the Juliá–Colonna epoxidation of chalcone.

The basic schema for the origin of life is generally accepted to consist of a sequence of four fundamental steps; namely the abiotic creation of amino acids and/or nucleic acids by a Urey type process, symmetry breaking/enantioselection for a single series of chiral building blocks, chiral amplification and finally organisation into self-sustaining systems.

There is no doubt that simple biomolecules can be formed by electric discharge through mixtures of gases, albeit that the exact conditions are still a matter for experimentation and debate. Many exotic theories have been advanced for symmetry breaking, such as the influence of parity violation or circularly polarised light.[1] However *ca.* 10% of all racemic materials which are capable of forming crystalline conglomerates (*i.e.* mixtures of individual crystals containing a single enantiomer) are amino acids or simple derivatives.[2] This is an ideal means for achieving high local concentrations of a single enantiomer of an amino acid, which can undergo condensation to give scalemic peptides. Herein we describe a new aspect of chiral amplification and primitive organisation involving a polypeptide.



Over twenty years ago, the seminal work of Juliá and Colonna showed that poly-(L)-alanine or poly-(L)-leucine could be employed to induce asymmetry into the Weitz–Scheffer epoxidation of α,β-unsaturated ketones. A considerable number of mixed peptide oligomers of defined structure have been prepared during the investigation of the mechanism of the Juliá–Colonna reaction. One remarkable aspect of these results, is that the enantioselection of the terminus for product formation appears to be largely independent of the rest of the chain. For example, a polyleucine catalyst consisting of five L-residues, five D-residues and ten L-residues (5 L/5 D/10 L), effected the efficient conversion of chalcone **1** into chalcone epoxide **2** of 89% ee *i.e.* with the same absolute configuration as would be obtained for an all L-catalyst.[3] This led us to prepare poly-leucine catalysts from scalemic leucine-*N*-carboxyanhydrides (Leu-NCAs) by methodology previously used for homochiral catalysts.[4]

The results were extraordinary (Table 1). Racemic monomer (entry 1) gave chalcone epoxide **2** (or *ent*-**2**) with negligible enantiomeric excess as expected and low conversion, whereas Leu-NCA monomers with a small enantiomeric excess produced chalcone epoxide efficiently with an enhanced enantiomeric excess (2.1 to 5.6 times higher; entries 2–6). This increase in enantiomeric excess is comparable with some of the best metal-centred chiral amplification systems.

The principles underlying the chiral amplification of scalemic catalysts are well established. In the simplest possible case, a heterodimer consisting of equal proportions of each enantiomer is catalytically inactive, whereas the small remaining amount of the major enantiomer is free to act as a catalyst, alone, as a homodimer, or as a higher-order aggregate, typically in association with a metal. Consequently all catalytic activity arises from the enantiomer which is present in excess; in principle 100% enantioselectivity is possible, albeit only a small proportion of the original scalemic material is acting as a catalyst. We rationalise our results in a similar way, by postulating a catalytic site consisting of homochiral amino acid residues.

If scalemic Leu-NCAs polymerise without stereo-discrimination[5] or discrimination by chain length, then the ratio of oligomers is described by a Bernoullian distribution, calculated from the enantiomeric ratio of the monomers. Suppose that we have a 60:40 mixture of L:D enantiomers, then the ratio of the dimers, LL, LD, DL and DD is 0.6^2, 0.6×0.4, 0.4×0.6 and 0.4^2 or expressed as percentages: 36, 24, 24, 16. If the homochiral dimers alone are catalytically active and they individually give products of 100% enantiomeric excess, then the ratio of products is 36/(36 + 16):16/52, which is an enantiomeric excess of 38.5%. This is nearly double the enantiomeric excess of the monomers (20%) and just over half of the material (52%) is acting as a catalyst.[6]

To generalise this analysis, it is convenient to discuss the concepts in terms of the enantiomeric excess of *catalytic sites* recognising the fact that catalytic *molecules* may be diastereoisomers of each other. We will also assume that enantiomerically pure catalytic sites give products of 100% ee. Therefore the enantiomeric excess of the products of a given reaction is exactly the same as the enantiomeric excess of the catalytic sites. In general if the proportions of the two enantiomers of the monomers are L and D ($L + D = 1$) and the number of residues in the catalytic site is n, then the ee of the catalytic sites and their proportion in the mixture are as follows:

$$ee_n = (L^n - D^n)/(L^n + D^n)$$

$$\text{Catalyst}_n = L^n + D^n$$

For longer oligomers, the enantiomeric excess increases rapidly because it is proportional to a difference between powers, whereas

Table 1 Preparation and reactivity of scalemic poly-leucine oligomers

	Leu-NCA monomers		Chalcone epoxide **2** or *ent*-**2**		
Entry	Ratio	ee%	Conv.[a] (%)	ee[a] (%)	Predicted ee[b] (%)
1	1:1	0.0	0.73	1.0	0
2	1.1:1	4.8	39.0	26.5	22.5
3	1.2:1	9.1	51.0	45.3	41.0
4	1.5:1	20.0	72.5	73.5	73.7
6	2.5:1	42.9	95	92.0	94.1
7	1:0	100	100	96.0	96.0

[a] Errors: ee ±2%, conversion ±5%. All reactions were run with both excess D- and L-Leu-NCA and each entry is an average of 2–6 runs. [b] From Table 2, $n = 5$, except that chalcone epoxide **2** is formed with 96% ee by enantiomerically pure catalyst. The experimental and predicted ee are correlated by $y = 0.9582x + 3.475$, $R^2 = 0.9975$.

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Table 2 Predicted percentage enantiomeric excess and percentage active catalyst for unique catalytic sites containing *n* homochiral residues prepared from monomers of four enantiomeric purities

	Ratio, % ee of monomers							
	1.1:1, 4.76		1.2:1, 9.1		1.5:1, 20		2.5:1, 42.9	
n	ee[a]	Cat.[b]	ee	Cat.	ee	Cat.	ee	Cat.
1	4.76	100	9.1	100	20	100	42.9	100
2	9.5	50.1	18.0	50.4	38.5	52	72.4	59.2
3	14.2	25.2	26.7	25.6	54.3	28	87.9	38.8
4	18.8	12.7	34.9	13.1	67.0	15.5	95	26.7
5	**23.4**	**6.39**	**42.7**	**6.77**	**76.7**	**8.8**	**97.97**	**18.8**
6	27.8	3.23	49.8	3.53	83.9	5.1	99.18	13.3
7	32.2	1.64	56.4	1.84	88.9	2.96	99.67	9.5
8	36.4	0.83	62.3	0.966	92.5	1.75	99.86	6.78

[a] ee is the percentage enantiomeric excess of catalytic sites. [b] Cat. is the percentage of catalyst in the mixture of oligomers.

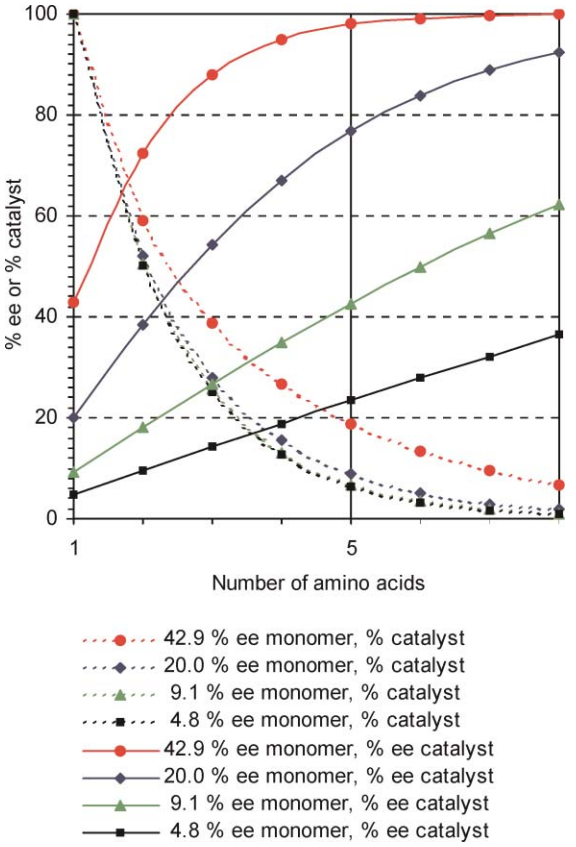

Fig. 1 Percentage catalyst and percentage enantiomeric excess of unique catalytic sites as a function of the number of monomer units prepared from monomers of four enantiomeric purities (*cf.* Table 2).[8]

the amount of catalyst decreases more slowly because it is a sum of powers.[7] Application of this analysis to longer oligomers and various enantiomeric ratios (those in Table 1) are shown in Table 2 and Fig. 1.

Comparison of the data in the tables and Fig. 1 shows that the observed enantiomeric excesses for the epoxidation of chalcone and those predicted for a catalytic site composed of five residues are identical within experimental error. A catalytic site composed of five homochiral units is in perfect agreement with studies of short oligomers bound to PEG[9] and our molecular model for the active site,[10] which requires five *terminal* homochiral residues. The vast majority of metal (M)/ligand (L) chiral amplification systems are ML_2 although recently an efficient ML_3 system has been reported.[11] In terms of this nomenclature, poly-leucine is acting as an L_5 catalyst and is thus the first metal-free chiral amplification system and the most complex based on number of components.

The application of Bernoullian analysis to the formation of scalemic peptides has been criticised for creating a "hopeless quagmire of undesired and inconsequential diastereoisomers".[12] Judged from the viewpoint of unique peptides acting as catalysts this statement is true. However based on a single catalytic site, the proportion of catalytically active material is independent of the degree of polymerisation because the probability of appending additional residues to a putative catalytic site is always 1. Therefore the enantiomeric excess only depends on the number of residues in the catalytic site for monomer of a given enantiomeric purity.

The formation of a homochiral catalytic site is a prerequisite for the efficient development of this form of enantioselective catalysis. Consider a putative catalytic site sequence LDLD and its enantiomer DLDL. Since both of these contain an identical number of each enantiomer of the residues, no matter what the relative proportion of the monomers, there will always be equal amounts of the two catalytic sites.

Any proposal about the origin of life must necessarily be speculative, however the results presented here provide an example whereby a modest enantiomeric excess of a monomeric unit is translated into oligomers expressing appreciable highly enantioselective catalytic activity.

Notes and references

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4 1,3-Diaminopropane (8.1 μl, 0.2 mmol) was added dropwise to L-Leu-NCA (0.8 g, 5.1 mmol) and D-Leu-NCA (1.2 g, 7.6 mmol) in THF (30 ml) which was stirred under a nitrogen atmosphere for 4 days. The white solid was filtered off, extracted with the following solvents (50 ml) for 30 min each: water, acetone–water (1:1), acetone–water (4:1), acetone (×2), ethyl acetate (×2) and diethyl ether (×2), and dried under high vacuum overnight (1.25 g, 87%).

5 If a peptide chain has an N-terminal L-residue, will it preferentially react with a L-NCA monomer or a D-NCA monomer? Most studies indicate that there is a weak preference for homocoupling: T. Hitz and P. L. Luisi, *Helv. Chim. Acta*, 2002, **85**, 3975; H. R. Kricheldorf and T. Mang, *Makromol. Chem.*, 1981, **182**, 3077.

6 A similar analysis of two-step *reactions* was made by K. Soai, H. Hori and M. Kawahara, *J. Chem. Soc., Chem Commun.*, 1992, 106, and (added after submission) for polymerisations: T. H. Hitz and P. L. Luisi, *Origins Life Evol. Biosphere*, 2004, **34**, 93.

7 This analysis is fundamentally the same as that proposed by Kagan: D. Guillaneux, S.-H. Zhao, O. Samuel, D. Rainford and H. B. Kagan, *J. Am. Chem. Soc.*, 1994, **116**, 9430, but we are proposing a new way to calculate the proportions of the homo- and hetero-chiral constituents (Kagan's α and β variables).

8 The permutation was implemented by converting decenary numbers to binary numbers in the range 0 to $2^{31} - 1$. The standard binary numbers were padded with zeros on the left and converted to 31 character strings, in which 0 represents the L-enantiomer and 1 the D-enantiomer. The individual examples were extracted (Right$) and analysed using standard string commands. A Microsoft® Visual Basic 3.0 program, in which chain length, catalytic site length and enantiomeric excess are definable variables is available from D. R. K. All other calculations were performed in Microsoft® Excel.

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10 Described in the previous paper in this issue.

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