Polyamino acids as synthetic enzymes: mechanism, applications and relevance to prebiotic catalysis

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Polyamino acids, such as polyleucine, behave as synthetic enzymes in the asymmetric epoxidation of chalcone and other electron-deficient alkenes (the Julià–Colonna reaction). The influences of reaction conditions, of the molecular structure of the catalysts and of the scaling-up of the process on the enantioselectivity of the reaction have been determined. The kinetics and mechanism have been investigated using a soluble PEG-polyleucine conjugate, which behaves in a similar way to an enzyme, showing saturation kinetics for both chalcone and HOO^-. Enantioselective catalysis is achieved with peptides with as few as five residues and scalemic catalysts show high chiral amplification. Here, we discuss the relevance of these-enzyme like catalysts to prebiotic processes, such as the role of small peptides in the formation of optically active cyanohydrins.

Introduction

There are three important reasons for studying and using small polyamino acids such as polyleucine in organic syntheses: (i) they are efficient catalysts for promoting highly enantioselective epoxidations; (ii) they have interesting laboratory and, more importantly, large-scale applications; and (iii) they exhibit enzyme-like synthetic properties, which suggests they had a role in the prebiotic origin of life.

Asymmetric syntheses performed in the presence of synthetic or natural polypeptides are of increasing interest because they could be considered as simplified models of enzymatic reactions. Moreover, the epoxide functional group is one of the most useful synthons in organic synthesis. Therefore, the preparation of chiral oxiranes under catalytic conditions is still a challenging target in the area of stereoselective reactions.

We and others discovered more than 20 years ago [1–3] a highly enantioselective method for epoxidizing electron-deficient alkenes in a triphasic system (polyamino acid–aqueous phase–organic phase) (Figure 1). Since then, a

great deal of work has been done to investigate the structural requirements of the polyamino acids used as catalysts, to simplify the reaction system and to enlarge the scope of this synthetic methodology.

Here, we examine various aspects of polyamino acid catalyzed oxidations, such as small-scale syntheses and catalyst formulation, scaling up, mechanistic aspects and, last but not least, their relevance to prebiotic catalysis.

Small-scale syntheses and catalyst formulation

The catalysts were originally prepared by polymerization of *N*-carboxyanhydrides (NCA) and the degree of polymerization was determined from the molar ratio of the amino acid-NCA versus the amine used as the initiator. Initially, three polyamino acids, poly-L-alanine, poly-L-β-benzylglutamate and poly-L-β-butylglutamate, were used for the oxidation of (*E*)-chalcone 1, (Figure 1) and then the study was extended to a much broader series of amino acids [3]. Ala, Leu and Ile were the most efficient catalysts and higher polymer homologues compared favourably, in terms of reaction rates and enantiomeric excesses (e.e.), with the shorter ones (Table 1). As expected, D-amino acids provided epoxides with the opposite absolute stereochemistry.

The polyamino acids still functioned as catalysts when anchored on a polystyrene matrix, giving high yield and e.e. Substituted chalcones afforded satisfactory results under the standard conditions (Table 2). Optically active epoxides were prepared by Augustyn *et al.* as precursors of flavonols [3].
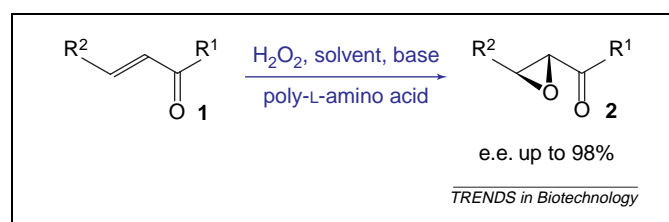

Figure 1. Scheme of poly-L-amino acid catalyzed oxidation of α,β-unsaturated ketones by hydrogen peroxide under basic conditions. $R_1 = R_2 = Ph$, chalcone 1.

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Table 1. Epoxidation of chalcone 1 with various polyamino acids

Catalyst (MW)	Time (h)	Yield (%)	e.e. (%)
L-Ala (~1000)	28	75	93
D-Ala (~1000)	37	53	90
D,L-Ala (~1000)	24	5	0
L-Ala (~3000)	28	77	96
L-Val (~1000)	168	6	10
L-Val (~3000)	144	4	33
L-Leu (~1500)	28	60	84
L-Leu (~4500)	28	44	88
L-Isoleu (~1500)	72	76	95
L-Phe (~2000)	72	32	1
L-β-Bn-Asp	456	8	3
L-β-Bn-Glu	144	12	12
[L-Leu-L-Ala]$_{10}$	24	67	95
[L-Val-L-Ala]$_{10}$	96	39	88
L-Ala-L-Ala	144	41	2

The original experimental procedure has been greatly improved and simplified. A fundamental enhancement was the use of urea-hydrogen peroxide complex (UHP), together with an organic base such as 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), in a non-aqueous two-phase system [3]. The rate of epoxidation of the model substrate chalcone 1 was increased by two orders of magnitude and a larger number of substituted enones were able to react.

Absorption of polyleucine onto silica gel reduced the amount of catalyst needed by 75% and made the recovery of the reaction product easier [4,5]. Recently, a soluble version of the Julià–Colonna catalyst has been developed using aminopoly(ethyleneglycol) (PEG-NH$_2$) to initiate the polymerization of *N*-carboxyanhydride precursors [6,7]. The resulting peptide conjugate is soluble in tetrahydrofuran (THF) and enables the stereoselective oxidation of chalcone in homogeneous solution. Using these improved methods the scope of Julià–Colonna epoxidation has been extended to ketones with aliphatic substituents, symmetrical α,β-unsaturated ketones, dienes and *bis*-dienes [3,8].

At present, the limitations of the Julià–Colonna asymmetric epoxidation are: (i) electron-deficient alkenes other than α,β-unsaturated ketones are generally unreactive, apart from a vinyl sulfone that gave the corresponding epoxide with a modest e.e. [9]; and (ii) trisubstituted alkenones are generally unreactive. Exceptionally, cyclic enones do form epoxides, often in high yield and excellent e.e. [9].

The epoxidation of electron-deficient alkenes by this methodology complements the well-known Sharpless epoxidation of allylic alcohols catalyzed by titanium tartrates and Shi epoxidation with chiral dioxiranes.

Scale-up

Studies of the scale-up of polyleucine-catalyzed epoxidation reactions have been undertaken by Degussa (Hanau; http://www.degussa.com) and Bayer (Leverkusen; http://www.bayer.com). The first studies produced silica-supported polyleucine on a substantial scale. L-Leucine-*N*-carboxyanhydride (L-leucine NCA) was prepared from L-leucine and phosgene in THF on a 0.5-kg scale and the crude product was crystallized from hexane/toluene to provide material for the polymerization reaction. Silica supported polyleucine was prepared in two ways. In one procedure, L-leucine NCA was heated with initiator (1,3-diaminopropane, DAP) in dimethoxyethane at 90 °C for one day before cooling and addition of silica gel to the suspension. Alternatively, polymerization of L-leucine NCA was conducted in toluene at 0–20 °C using DAP as the initiator, before adsorption of the polypeptide onto silica over a period of three days. The product from the second procedure required sequential washing with water and acetone to provide material, which catalyzed the epoxidation of chalcone 1 at an acceptable rate [10].

More recently, Gerlach and Geller reported the preparation of L-leucine NCA on a multi-100-g scale, which was polymerized using DAP in toluene. The poly-L-leucine was used directly for the epoxidation of a chalcone on a 100-g scale. By optimizing conditions, only a small amount of catalyst (0.35 mol %) was used, together with a phase-transfer agent (such as tetrabutylammonium bromide) in hexane and aqueous sodium hydroxide (1.3 equiv.) containing hydrogen peroxide (5 equiv.) at 25 °C over 20 h. The reported yield of epoxychalcone 2 (95% e.e.) was 75%. The recovered catalyst could be re-used successfully [11].

The use of polymer-bound oligo-L-leucines in a continuously operated membrane reactor has been demonstrated [7] and the use of silica-bound polyleucines in continuous flow fixed-bed reactors is under investigation.

Kinetics

The kinetics of Julià–Colonna epoxidation have been investigated using PEG bound poly-L-leucine (PLL), which is soluble in several organic solvents [12,13]. The experimental system involved four reagents: the PLL catalyst, the substrate chalcone 1, hydrogen peroxide, and a strong base (2-*t*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine, BEMP) for inducing the dissociation of hydrogen peroxide to the hydrogen peroxide anion, which is the actual oxidant. The kinetics were determined in THF by spectrophotometrically monitoring the disappearance of chalcone.

Table 2. Poly-L-leucine and poly-D-leucine catalyzed epoxidations of chalcone derivatives

R$_1$	R$_2$	Time (h)	Solvent	Yield (%)	e.e. (%)
Ph	2-Thio	48	Toluene	96	80
Ph	3-Thio	48	Toluene	30	70
Ph	2-Naphthyl	40	CCl$_4$	83	99
2-Pyridyl	Ph	16	CH$_2$Cl$_2$	84	72
4-Pyridyl	2-Naphthyl	16	CH$_2$Cl$_2$	67	96
2-Furyl	2-Naphthyl	50	CH$_2$Cl$_2$	75	96
2-Quinolyl	Cyclo-Pr	18	CH$_2$Cl$_2$	94	79
Cyclo-Pr	2-Naphthyl	28	CH$_2$Cl$_2$	73	98
t-Bu	t-Bu	15	Toluene	100	95
Ph	t-BuO	15	Toluene	66	95

The results indicated that PLL behaves as an enzyme-like catalyst, at relatively low concentrations of substrates, and shows saturation kinetics for both chalcone ($K_M = 110$ mM) and HOO$^-$ ($K_M = 30$ mM) [12,13]. The apparent maximum specific activity was up to 1.81 μmol min^{-1} mg^{-1} of PLL.

Additionally, substrate inhibition at high concentrations of chalcone and substrate activation at low concentrations of hydrogen peroxide were observed [12,13]. As suggested by Ferdinand for a two substrate enzyme [14], both phenomena can be explained on the basis of a steady-state random bireactant mechanism, which implicates alternative pathways to the ternary complex and, importantly, postulates that one of the pathways (HOO$^-$ binding first) is kinetically preferred to the other (chalcone binding first) (Figure 2) [12,13].

This type of mechanism is sequential [14]; that is, all substrates must bind to the catalyst to form a central complex (PLL–HOO$^-$–chalcone) before the formation of the hydroperoxide enolate of chalcone (*cf.* model complex 5), which eventually forms epoxychalcone 2 (Figure 2).

Mechanistic aspects

The Weitz–Scheffer epoxidation [15] of electron-deficient alkenes by alkaline hydrogen peroxide is the achiral progenitor of Julià–Colonna epoxidation. Early studies of Weitz–Scheffer epoxidation showed that the rate had a first-order dependency on the concentration of both hydroperoxide ion and the electron-deficient alkene [16]. These data can be accommodated by two explanations: slow hydroperoxide addition followed by fast epoxide ring closure, or the converse, fast addition followed by slow ring closure. It was surmised that the second explanation was correct based on isomerization studies. However, unambiguous evidence was provided by a study in which (Z)-3-[^{2}H$_1$]-phenylprop-2-enone (Z)-3 was subjected to

both Weitz–Scheffer and Julià–Colonna epoxidation conditions with ^{1}H-NMR monitoring (Figure 3). The stereochemistry of the label in the alkene was scrambled by fast rotation of the enolates 4 before slow formation of the epimeric epoxides (E)- and (Z)-5. Moreover, the rate of isomerization was at least ten times faster under Julià–Colonna than Weitz–Scheffer conditions [17]. The slow rate of epoxide ring formation from the peroxyenolate 4 is a consequence of the poor nucleofugacity of hydroxyl, which requires both the peroxymoiety and the O–O bond to be perfectly orientated before ejection of hydroxyl. By contrast, peracid epoxidation of labelled ethyl acrylate occurs with retention of configuration [18].

Soluble PEG-polyleucine containing as few as five residues has catalytic activity (rate and enantioselectivity), which is comparable with insoluble polyleucine with 20+ residues [19,20]. The fact that the epoxidation activity is independent of the nature of the initiating amine, which resides at the *C*-terminus as a carboxamide, indicates that the catalytic site is located at the *N*-terminus. Leucine has close to the highest α-helix propensity of all natural amino acids and the predominance of this conformation in solution for the soluble catalysts has been shown using circular dichroism (CD) [19]. The four *N*-terminal N-H groups of a α-helical polypeptide chain are not engaged in intrachain hydrogen bonds and, consequently, they provide an ideal set of acceptors for hydrogen bonding (an oxyanion hole) to the enones 1, 3 and, more importantly, the peroxyenolates 4. Two models have been
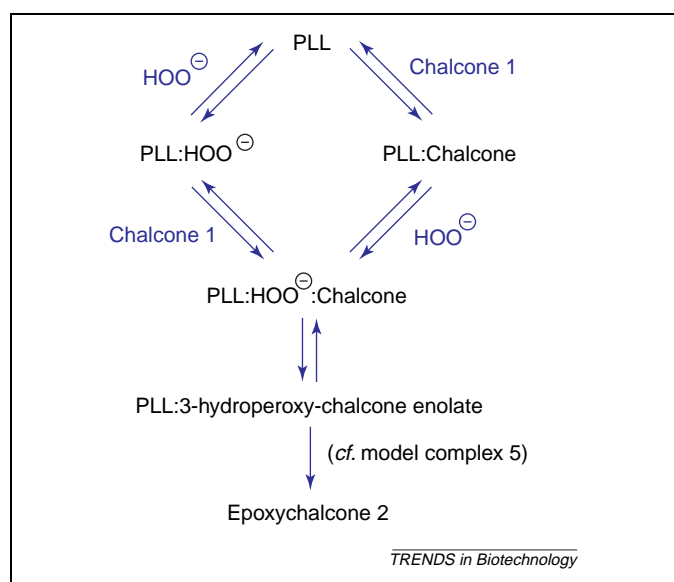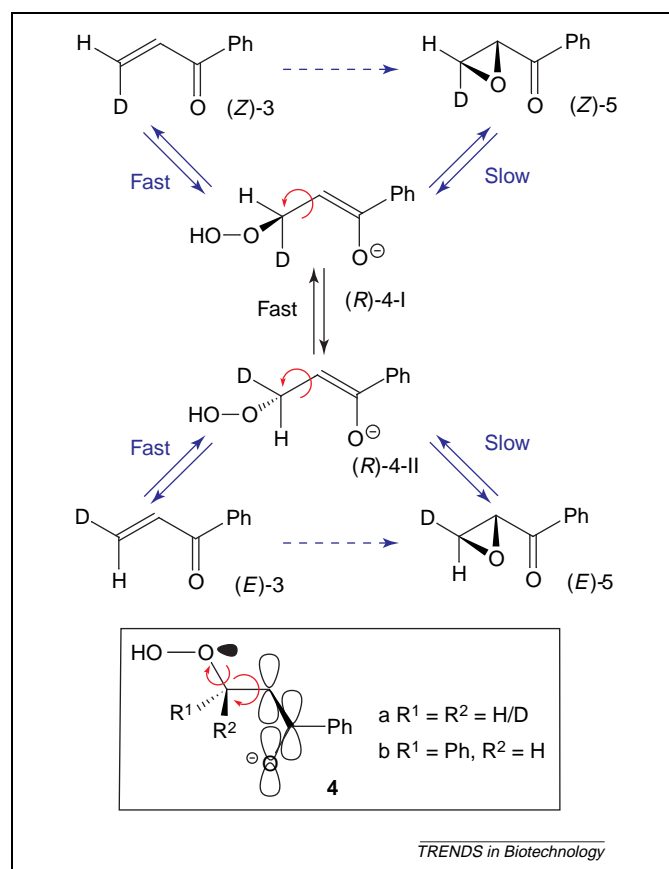

Figure 2. Kinetic scheme for chalcone oxidation by hydrogen peroxide anion catalyzed by poly-L-leucine (PLL). Oxidation takes place through a steady-state random bireactant mechanism, which implicates alternative pathways to the ternary complex and postulates that one of the pathways (HOO$^-$ binding first) is kinetically preferred to the other (chalcone binding first). For details on PLL:3-hydroperoxy-chalcone enolate see the model complex 5 in Figure 4.

Figure 3. Mechanistic scheme for Weitz–Scheffer and Julià–Colonna epoxidation: fast hydroperoxide addition to chalcone, followed by fast rotation of enolates 4 and slow ring closure to the epimeric epoxides (E)- and (Z)-5.

proposed for binding of the chalcone derived peroxyenolate 4b [20,21]. In the Berkessel model [20], the peroxyenolate 4b is bound by NH-1 (the *N*-terminal amino group) and NH-3, with the hydroperoxide moiety bound by NH-2 and to a lesser extent NH-1. However both *N,N*-dimethyl- and *N*-acetyl-terminated polyleucine are catalytically active, which indicates that the mode of binding to NH-1 must be different in these cases. The second model (Figure 4) postulates binding of the peroxyenolate 4b to NH-2 and NH-3 with the hydroperoxide bound to NH-4 5 [21]. The advantage of this model is that only amidic N-H groups are acting as hydrogen donors and these are much more efficient donors than an amino group like NH-1. Indeed, based on pK_a arguments alone a single amidic NH should fully donate a proton to an enolate. The hydrogen bonding motif for the enolate moiety is similar to that which templates the formation of Leigh's peptidic rotaxanes [22].

There has been much interest in the past few years in chiral amplification for practical synthetic reasons and for its possible role in the origin of life. Julià–Colonna epoxidation was the first example of a chiral amplification system that does not involve a metal. When scalemic mixtures of L- and D-leucine NCAs were polymerized, the polyleucine formed showed high chiral amplification. For example, the catalysts prepared from a 1.2:1 mixture of L-leucine-NCA and D-leucine-NCA (9.1% e.e.) gave chalcone epoxide 2 (41% e.e.), and a 2.5:1 mixture (42.9% e.e.), gave chalcone epoxide 2 with almost the same degree of conversion and enantiomeric excess as the polymer prepared from enantiomerically pure L-leucine–NCA alone [23].

The enantiomeric excess of chalcone epoxide can be predicted using a Bernoullian model. If L and D are the proportions of each enantiomer and n is the number of residues involved in a catalytic competent sequence (in effect the active site), then:

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

and the amount of catalyst is

$$Catalyst_n = L^n + D^n$$

For $n=5$, predicted and experimental e.e. were correlated with $R^2 = 0.9975$. Moreover, five residues was found to be the minimum catalytically competent peptide for the amino–PEG polymers [19,20]. Interestingly, this effect only finds its maximum value with homochiral sites. If we imagine a catalytic site composed of the following residues *LDLD*, and its enantiomer *DLDL*, there must be identical amounts of each of these present, no matter what the initial ratio of the enantiomers.

Asymmetric prebiotic catalysis: did polyamino acids play a role?

It is not known how life first appeared. There is no geological evidence of the environmental conditions on the Earth at the time of the origin of life, nor any fossil register of the evolutionary processes that preceded the appearance of the first cells. Direct information is lacking, not only on the composition of the terrestrial atmosphere
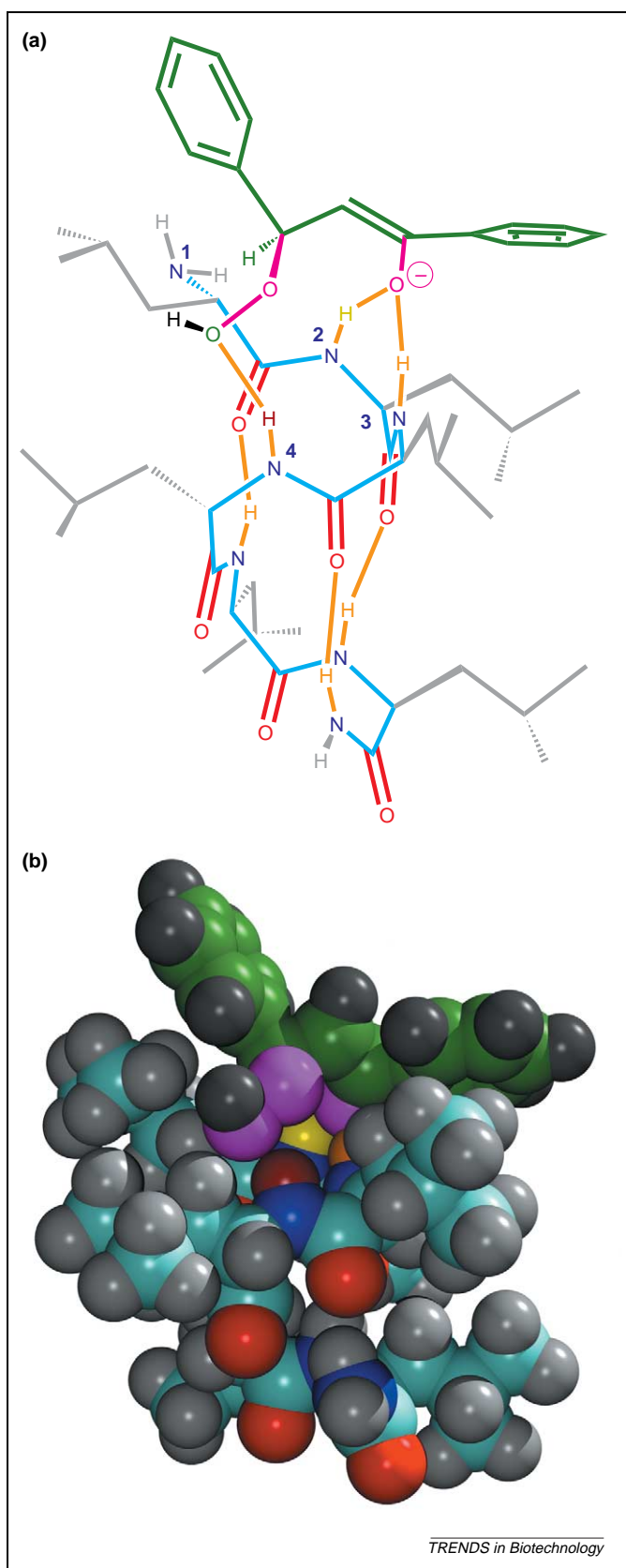
Figure 4. Model for the binding of the chalcone peroxyenolate to NH-2 and NH-3 and of the hydroperoxide moiety to NH-4 **(a)**. Hexa(L-leucine) carboxamide, 3-hydroperoxy-chalcone enolate complex 5, used in the modelling studies. Standard space-filling model Corey–Pauling–Koltun (CPK) colours except, chalcone enolate hydroperoxide, carbon, forest green; hydrogen, dark grey; enolate and hydroperoxide oxygens, purple. NH-2, NH-3 and NH-4 are coloured yellow, orange and brown, respectively, and are located in the centre of the picture. The colours match the ChemDraw depiction above **(b)**.

during the period of the origin of life but also on the temperature, ocean pH values and other general and local environmental conditions, which might or might not have been important for the emergence of living systems. However, the remarkable correlation between the compounds that are produced in Miller-type electric discharge experiments and those found in 4.6×10^9 year old carbonaceous meteorites appears too striking to be fortuitous and suggests that amino acids, nucleobases, sugars and membrane-forming compounds were part of the chemical environment from which life first evolved [24]. Analysis of the four stereoisomers of α-methyl amino acids (DL-α-methylisoleucine and DL-α-methylalloisoleucine) obtained from a sample of the Murchison meteorite have demonstrated that the L-enantiomers occur in excess (e.e. 7.0% and 9.1%, respectively), which suggests that the exogenous delivery of organic compounds during the early history of the Earth might have contributed to an enantiomeric enrichment of the prebiotic environment by the accumulation of significant amounts of non-racemic amino acids [25]. The abiotic accumulation of such asymmetric mixtures could have promoted enantioselective catalysis, as suggested by the role of L-proline in promoting aldol reactions in an asymmetric fashion [26], and of the influence of non-racemic alanine and isovaline in the water-based asymmetric prebiotic carbohydrate synthesis from glycolaldehyde and formaldehyde [27].

As shown by numerous experiments, clays, metal cations, imidazole derivatives and highly reactive derivatives of HCN, such as cyanamide, dicyanamide and cyanogen, might have catalyzed polymerization reactions. The possibility that selective adsorption of molecules onto mineral surfaces could have promoted their polymerization is supported by laboratory simulations using a variety of simple compounds and activated monomers [28]. The role of [D-His–D-Phe] in catalyzing the addition of HCN to aldehydes of the type RCHO (R=aryl or bulky alkyl) to give optically active cyanohydrins (e.e. 40–90%) [29] demonstrates the potential of simple peptides as catalysts in an asymmetric system with considerable prebiotic relevance. Direct concentration of dilute solutions of monomers to yield larger, more complex polyamino acids could also be accomplished by evaporation and by eutectic freezing of dilute aqueous solutions. Salt-induced peptide formation could provide an abiotic route for the formation of peptides directly from amino acids in concentrated NaCl solutions containing copper [30]. Clay minerals, such as montmorillonite, apparently promote the reaction, which could have taken place in evaporating tidal pools where the required concentrated salty brines would have been easily available. It has also been shown that the freezing of dilute solutions of activated amino acids at −20 °C produces peptides at higher yields than in experiments with highly concentrated solutions at 0 °C and 25 °C [31].

It does not strain the imagination to propose that as polymerized molecules became larger and more complex, some of them began to fold into configurations that could bind and interact with other molecules, expanding the list of primitive catalysts that could promote nonenzymatic reactions. Asymmetric local environmental conditions

could have existed due to transient instabilities or in chiral microenvironments, such as the surface of a crystal, which could have promoted the synthesis of small polyaminoacids containing small homochiral domains, such as the polyleucine catalyst formed by five L-residues, five D-residues and ten L-residues studied by Kelly *et al.* [23].

Although not all the reactions described so far might be truly prebiotic, they demonstrate how simple compounds like polyleucine could have enriched the primitive soup with components not readily synthesized by other abiotic mechanisms. Chalcone epoxide was probably not available in the primitive environment but enolase, which catalyzes the Michael addition of water to phosphoenolpyruvate 6 (Figure 5) in glycolysis, has been shown to be a highly conserved ancient enzyme that could have evolved during the RNA/protein world before the evolutionary development of DNA genomes [32]. It is unlikely that there is a direct genealogical link between extant enzyme-based systems and catalytic polyamino acids of abiotic origin and that the main reaction under consideration (Julia–Colonna epoxidation of electron deficient alkenes) took place in the prebiotic Earth. However, some of its underlying features could have a direct relevance to our understanding of the events that preceded the origin of life. First, a low molecular weight polypeptide constructed from a single amino acid displaying only a small excess of one enatimer, can be seen to transfer and amplify its chirality by acting as a catalyst in an asymmetric transformation. The corresponding prebiotic transformation, catalyzed by simple polyamino acids, could have been an asymmetric reduction reaction but evidence for it is not available. Second, the origin of enantiomeric homogeneity is far from being understood, but the characteristics of the epoxidation reaction described here chime with prescribed arguments [33], suggesting that in the primitive environment initial enantiomeric excesses were established for a compound or a group of compounds. It is possible that enantiomeric excesses were then amplified subsequently until enantiomeric homogeneity was achieved, thus providing the material from which primordial biological systems emerged.

Prospects for polyamino acids and related species for use as model enzymes in organic synthesis

Although polyamino acids (such as L-polyleucine) have attracted the most attention in terms of their potential as catalysts in asymmetric systems, other enzyme-like catalysts (also known as 'synzymes' or 'enzyme mimetics'), each comprising a few amino acid residues, have featured in the literature recently. For instance, tetrapeptides,
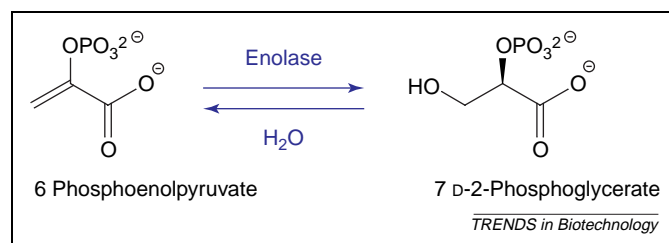
6 Phosphoenolpyruvate   7 D-2-Phosphoglycerate

TRENDS in Biotechnology

Figure 5. Enolase catalyzed Michael addition of water to phosphoenolpyruvate; this is a possible example of ancient catalysis.

designed by Miller and co-workers [34], effect enantio-selective acylation of some chiral cyclohexanol derivatives, mimicking the well-known transformations of lipases and other hydrolases in solvents of low water activity. The often exquisite selectivity of hydroxynitrile lyases (e.g. from *Prunus* and *Hevea* species) is replicated to a large extent by some selected cyclic dipeptides, as discussed for [D-His–D-Phe] [29].

L-Proline has been used to couple nitrosobenzene to aldehydes [35] and ketones [36] to produce the corresponding α-aminoxy compounds in high yield and with excellent optical purity. Importantly, it has been shown that optically active 5-pyrrolidino-2-yltetrazole can be used advantageously instead of proline in this fascinating transformation and related reactions [37]. The use of proline in asymmetric hydrogenation reactions has also been illustrated [38] but the reaction remains to be optimized.

Each of these systems represents an interesting system for study and, as a whole, demonstrates that there is an overwhelming case for targeting the understanding of the mechanisms-of-action of such enzyme-like catalysts, which could lead to the use of these relatively simple low molecular-weight readily-constructed systems instead of the corresponding macromolecular enzymes. Without an insight into the mechanism-of-action of these catalysts, the development of their catalytic potential is quite hit-and-miss. This is illustrated by the impasse facing scientists working with polyleucine and other polyamino acids.

Thus, it is clear from the previous discussion that polyamino acids have been developed to be extremely useful catalysts for asymmetric epoxidations. However, attempts to use the same species for other oxidations (e.g. sulfoxidations, Baeyer–Villiger oxidations) and other Michael reactions (e.g. leading to aziridines) have been unsuccessful. Only in the case of the addition of thiophenol to chalcone was poly-L-leucine seen to induce a small enantiomeric excess (45%) in the product, but the reaction was capricious and the rate of the background reaction was competitive (Lasterra-Sanchez, M.E. and Roberts S.M., unpublished). Not until a firm understanding of the interaction of the nucleophile (e.g. hydrogen peroxide) anion/the acceptor (e.g. chalcone/polyleucine) is in place, will other possibilities for catalysis become evident. This understanding will only emerge from the detailed inter-molecular interactions and techniques such as NMR spectroscopy will be needed to signpost the way forward.

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