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The mechanism of polyleucine catalysed asymmetric epoxidation

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Catalysis in the Juliá–Colonna epoxidation of α,β-unsaturated ketones is due to binding of the hydroperoxide enolate intermediate by the three *N*-terminal amidic N–H groups of α-helical poly-leucine; the *N*-terminal pair forms an oxy-anion hole, whilst the third aids displacement of hydroxide.

The Juliá–Colonna reaction[1] has now been sufficiently developed, such that it is a reliable technique for the asymmetric epoxidation of a diverse range of electron-deficient alkenes[2] and is one of a growing family of asymmetric synthetic methods based on "unnatural" peptides.[3] Despite the importance of the enhanced Juliá–Colonna reaction, hitherto there has been only one attempt to decipher the mechanism.[4]

A broad body of evidence indicates that the mechanism of both achiral Weitz–Scheffer, and Juliá–Colonna epoxidation, proceeds *via* fast reversible addition of hydroperoxide, followed by slow, intramolecular nucleophilic displacement of hydroxide (Scheme 1). Formation of the transition state **4** requires overlap of the enolate π-system with the anti-bonding orbital of the O–O bond of the peroxide substituent and possibly enhancement of the nucleofugacity of the hydroxyl moiety by protonation or coordination.[5]

In the accompanying paper it was shown that the rate of isomerisation of (*Z*)-3-[$^2H_1$]-phenyl vinyl ketone (and hence rate of hydroperoxide addition) was increased by about an order of magnitude under typical two phase PLL-catalysed conditions and the rate of epoxidation about five fold, both relative to the uncatalysed reaction. Thus the predominant mode of catalysis by PLL is stabilisation of the enolate conformers **3**, **4**, which could be achieved by an oxy-anion hole formed from the amidic groups of poly-leucine.[5]

Leucine has close to the highest helix propensity of all natural amino acids and X-ray crystal structures of even short sequences show the α-helical conformer.[6] The catalytic significance of the α-helical structure of poly-leucine in solution is demonstrated by CD[7] and FT-IR[8] studies correlating reaction rate and the proportion of α-helix present. Catalysis follows enzyme-like Michaelis–Menten kinetics characteristic of a saturatable active site model.[9] Studies by Berkessel[4] and Roberts, indicate that the catalytic site is close to the *N*-terminus, but does not require a terminal amine/ammonium substituent,[10] because an *N*-terminal acetyl or Boc group has only a marginal effect on catalytic efficiency.[11] Five contiguous homochiral leucine residues appears to be the minimum catalytically competent moiety.[4,8,11]

The four *N*-terminal N–H groups of an α-helical polypeptide (**5**, Fig. 1) do not participate in intra-residue hydrogen bonding, hence they are available for creating an oxy-anion hole, which will also benefit from charge stabilisation by the helix macrodipole. The first three amidic N–H groups of residues form an isosceles triangle with an apical angle (NH-2,3,4) of *circa* 100°, inclined at the pitch of the helix (Table 1, entries 2, 3). Thus without steric constraints, there are three pairs of N–H groups capable of creating an oxy-anion hole for the enolate **4**. This can be bound with the benzyl hydroperoxide moiety, *endo* or *exo* relative to the centre of the helix to give a total of 6-possible binding modes, but only the *endo* binding modes enable the third N–H group to assist in the displacement of hydroxide from the hydroperoxide substituent. NH-2 and NH-4 are equidistant from NH-3 and hence each pair could provide

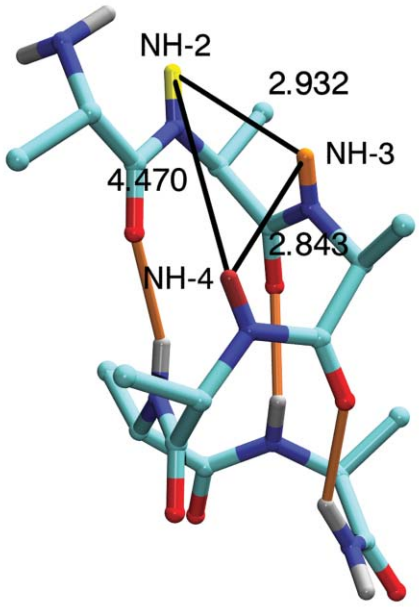
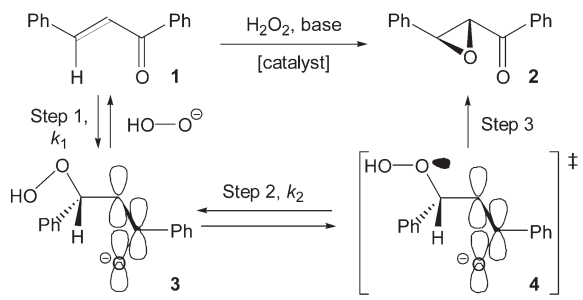


Fig. 1 Hexa(L-leucine) carboxamide, backbone **5** viewed from the side of Leu-4. Cα hydrogen, Cβ, Cγ1 & Cγ2 and attached hydrogens omitted. Standard CPK colours except, Leu-2, -3, -4 amidic hydrogens, yellow, orange and brown respectively. Hydrogen bonds are shown as narrow light orange rods and distances are in Angstroms.



Scheme 1 Conformational constraints for epoxidation, illustrated by the intermediate **3** and transition state **4** for PLL (poly-L-leucine) catalysed epoxidation of (*E*)-chalcone **1**.

Table 1 Geometry of amidic hydrogens

		Distances/Å			Angle/°
Entry		2,3	3,4	2,4	θ 2,3,4
1	Raw helix	2.778	2.778	4.152	96.7
2	Minimised **5**	2.932	2.843	4.470	101.4
3	Complex **6**	2.556	2.734	4.000	98.2
4	Complex **7**	2.346	3.051	3.986	94.3

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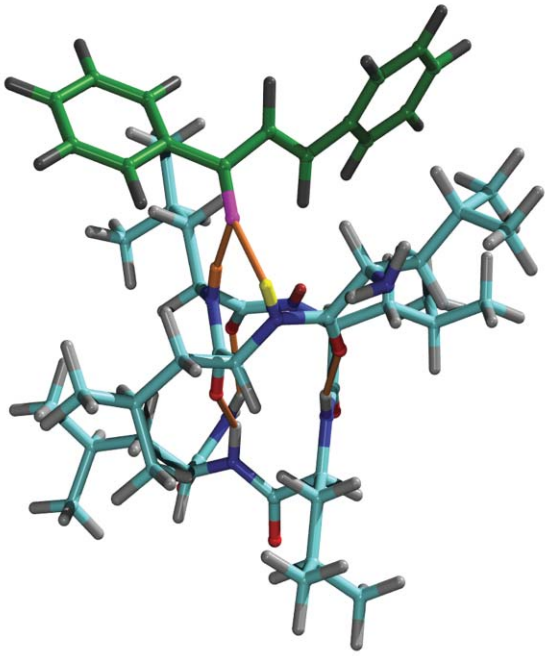

Fig. 2 Hexa(L-leucine) carboxamide, chalcone complex **6** viewed from the side of Leu-1 and 2. Standard CPK colours except as Fig. 1 and ligand: carbon, green; oxygen, magenta; hydrogen, dark grey.

equivalent hydrogen bonding, but for NH-3, NH-4 binding, NH-2 is on the wrong face for assisting hydroxide displacement. The geometry of the isosceles triangle precludes appreciable asymmetric induction from NH-2, NH-4 binding with assistance from NH-3.

Hexaleucine carboxamide with C=O 1 to N–H 5 as the only distance constraint (2 Å) was used as a model for the active site.[12] It gave a robust structure which retained all three inter-residue hydrogen bonds during most minimisations or conformational searches.[13] The structures of the chalcone complex **6** and the 3-hydroperoxy-chalcone enolate complex **7** (Figs. 2 and 3) were minimised without constraints and each is representative of an ensemble of structures of similar energy and gross geometry. Hydrogen bonding motif data are shown in Tables 1 and 2.

The α-helical conformer of hexaleucine carboxamide was created using average distances for all amino acids, it therefore expanded slightly upon minimisation to accommodate the bulky leucine side chains and contracted upon binding chalcone to give the complex **6** (Table 1, entries 1–3; Fig. 2).

Chalcone binding causes contraction of the interatomic distances predominantly by canting of the amidic N–H bonds inwards and splaying of the amidic carbonyl groups outwards to maintain planarity. The amide group of Leu-4 is not involved in binding, but is also twisted in the same way, because the *iso*-butyl side chain of Leu-3 has strong steric interactions with those of Leu-2 and Leu-6. This displaces the *iso*-butyl group of Leu-3, towards the axis of the helix. The hydrogen bond formed by N–H 3 is more linear and slightly shorter than that of N–H 2 (Table 2, entries 1, 2), but small movements of the enolate oxygen lengthen this hydrogen bond, while the hydrogen bond to N–H 2, becomes slightly shorter and more linear. This represents two energy minima separated by an insubstantial energy barrier.

The site for hydroperoxide attack is the alkene β-carbon atom which is buttressed by the C-α-proton of Leu-1 and to a lesser extent the *iso*-butyl side chain of Leu-3. Any rotation of the Cα–Cβ bond (χ_1) of Leu-1 increases steric interactions, because the *iso*-butyl group is already in close proximity to the 3-phenyl group of chalcone. In the preferred conformation, the *iso*-butyl side chain of Leu-1 only plays a minor steering role towards the β-phenyl group of 3-hydroperoxy-chalcone enolate. It permits rather than dictates the conformation, which is predominantly determined by interactions with the β-vinyl proton. This distinction is important for

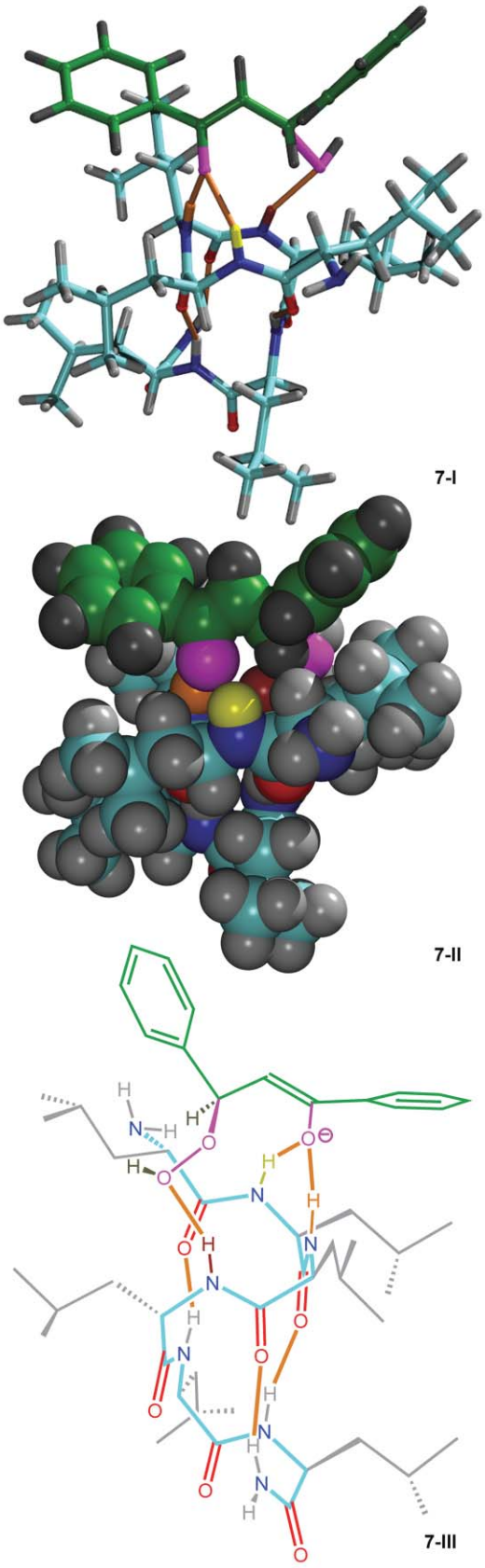

Fig. 3 Hexa(L-leucine) carboxamide, 3-hydroperoxy-chalcone enolate complex **7-I** viewed from the side of Leu-1 and 2. **7-II** as **7-I** but CPK with atom radii set at 0.8 of van der Waals radii. **7-III** view from the side of Leu-3 and 4. Colours as Figs. 1, 2, except that for **7-III** the side chains are shown in grey.

Table 2 H-bond geometry for complexes **6**, **7**

		Distances/Å			Angle/°
Entry	Complex, NH	N–H	O–H	N–O	θ NHO
1	**6**, 2	0.962	1.969	2.528	114.8
2	**6**, 3	0.963	1.719	2.671	169.4
3	**7**, 2	0.969	2.005	2.784	135.9
4	**7**, 3	1.060	1.594	2.647	171.3
5	**7**, 4	0.973	2.017	2.886	147.5

phenyl vinyl ketone which is epoxidised with the same relative stereochemistry as chalcone, but which lacks the β-phenyl group.[5] Cα–Cβ bond rotation of Leu-3 (from *t* to g^-) relieves hindrance of the alkene β-carbon atom by the *iso*-butyl side chain, but brings it into contact with the α-phenyl group of chalcone. Nevertheless there is ample space for delivery of hydroperoxide from above the plane of the π-system to the *re*-face of the β-carbon. Moreover NH 4 is capable of hydrogen bonding to hydroperoxide and delivering it to this face.

Compaction of the α-helix and splaying of the amide groups are seen more strongly in the 3-hydroperoxy-chalcone enolate complex **7**, because hydrogen bonding to an alkoxide is much stronger than towards a carbonyl (Table 1, entry 4). As with the chalcone complex **6**, one hydrogen bond is much shorter and more linear than the other (Table 2, entries 3, 4), but small movements of the enolate suffice to "exchange" their properties.

The idealized transition state for epoxide ring closure **4** requires that the C–O bond of the peroxide is parallel to the π-orbitals of the enolate (or 90° to the vinylic hydrogen or alkene bond) and that the O–O–C=C dihedral angle is 180° in a geometry which is reminiscent of the Baeyer–Villiger reaction.[14] In the complex **7**, the O–C=C–H, O–C=C–C, O–O–C=C dihedral angles are 96.8°, 83.2° and 163.2° respectively. The H-vinyl C–C–O dihedral angle is always close to 90°, due to interactions of the benzylic moiety with the vinylic C–H, whereas the orientation of the peroxide is more variable, due to the comparative weakness of the Leu-4 NH hydrogen bond to the distal oxygen of the peroxide.

Given these structures we view the chronicle of the reaction as follows. Polyleucine adopts an α-helical conformation, in which the terminal amidic NH groups are hydrogen bonded by hydrogen peroxide and/or water. Those bound at NH-2 and NH-3 are displaced by chalcone to give the complex **6** or an analogue with hydroperoxide bound at NH-4. This represents the correct orientation for cyclisation to occur with geometry similar to that of complex **7**. The electron withdrawing effect of the epoxide reduces the hydrogen bonding capability of chalcone epoxide **2** relative to chalcone **3** and the product is released. The origin of enantioselective catalysis is a combination of the hydrogen bonding motif and the helical conformation, which wraps round the substrate so as to shield the *si*-face of the alkene β-carbon.

The hydrogen donors and the acceptor atoms of the putative poly-leucine oxy-anion hole have geometry which is similar to that of oxy-anion holes found in modern proteins. Serine carboxy-peptidases utilise oxy-anion holes constructed from consecutive unfunctionalised amino acids, but these are invariably Gly-Gly or Ala-Gly (*e.g.* Prosite 00122[15]) and never adjacent to the *N*-terminus. Presumably evolution towards more highly organised active sites buried in a protein framework has made a consecutive *N*-terminal Leu-Leu oxy-anion hole less favourable. However enoyl-CoA hydratase, Δ^3, Δ^2-enoyl CoA isomerase and most likely all other members of the enolase family, stabilise enolates with non-sequential main chain oxy-anion holes[16] which may represent vestigial survival of this characteristic.

The geometry of the hydrogen bonding motif, in which the N–H bonds lie above and below the plane of the carbonyl group, rather than in the direction of "rabbit ear" lone pairs is fairly common for ketones in general and predominates for aryl-alkyl and diaryl ketones in the Isostar database.[17] This motif is evident in the X-ray crystal structures of some rotaxanes and is likely responsible for their assembly from acyclic precursors.[18]

The model presented here demonstrates that unfunctionalised polypeptides have the capability to form simple active sites, with substantial catalytic activity. This may be an illustration of a prebiotic form of catalysis.

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