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Enantioselective epoxidation of chalcone catalysed by the artificial enzyme poly-L-leucine: kinetic mechanism

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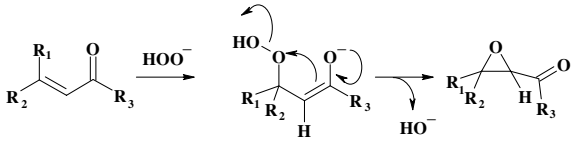
Abstract—An insight into the kinetics, mechanism and optimum reaction conditions of the Juliá-Colonna enantioselective epoxidation has been gained using a soluble polyleucine catalyst (PLL), chalcone as the substrate, and hydrogen peroxide (HOO^-) as the oxidant. PLL shows saturation kinetics for both chalcone and HOO^- and has a behaviour that fits a steady state random bireactant system with one of the pathways (HOO^- binding first) being kinetically preferred to the other (chalcone binding first) in the formation of the ternary complex PLL:HOO^-:Chalcone.

1. Introduction

Treatment of α,β-unsaturated ketones with hydrogen peroxide under basic conditions yields epoxy ketones via an oxidation known as the Weitz–Scheffer reaction.[1] It involves the addition of a hydroperoxide anion to the enone unit to form a hydroperoxide enolate.[1] Loss of the hydroxide ion with concomitant ring closure and formation of the epoxide occurs in the second step of the transformation (Scheme 1). The basic transformation was originally studied in detail by Rapoport some 20 years ago.[2]



Scheme 1.

There are a number of different ways of inducing asymmetry into the Weitz–Scheffer reaction.[3,4] The methodology introduced by Juliá and Colonna involving the use of a polyamino acid as a catalyst for the reaction[5] has been developed in recent years to become the methodology of choice for the preparation of chiral, optically active epoxides derived from α,β-unsaturated ketones.[6] In fact, subsequent to the Juliá and Colonna work,[5] Lantos et al. used the same procedure to prepare a precursor of a leucotriene antagonist up to a multi-hundred gram scale.[7a] Optically active epoxides obtained by the same procedure were also used as precursors in novel routes to flavonols.[7b] Advances in the field have led to an expansion in the range of enones, which can be epoxidized with good stereoselectivity.[6,7c,d] Interestingly, poly-D-amino acids and poly-L-amino acids have opposite enantioselectivity, normally yielding (*2S*,*3R*)-epoxides and (*2R*,*3S*)-epoxides, respectively.[6]

Much of the earlier work was undertaken with a heterogeneous catalyst, most often prepared by inducing the polymerisation of an amino acid *N*-carboxyanhydride with an amine such as 1,3-diaminopropane.[8] Recently two research groups have introduced an organic solvent-soluble version of the Juliá–Colonna catalyst.[9] These soluble catalysts, for example the PEG bound polyleucine **1** (PLL) are destined to be extremely important in deciphering the mechanism of the Juliá–Colonna asymmetric epoxidation reaction.[10] Already it has been shown that the catalyst performs best when presented in a helical conformation;[11] it is also apparent that the 'active site' of the catalyst is adjacent to the *N*-terminus of the polypeptide.[12]

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Herein, we report the use of the PEG bound polyleucine catalyst **1** to investigate some kinetic parameters of the Juliá–Colonna reaction, employing chalcone (R_1 = H; R_2 = R_3 = Ph; Scheme 1) as the substrate in THF, which dissolves the PLL catalyst up to, at least, 5 mg/mL.[13] It has been shown that under these reaction conditions the epoxychalcone product can be obtained in high enantiomeric excess (ee $\geqslant$96%).[9]

$$MeO - PEG_{5000} - NH - [COCH(CHMe_2)NH]_{15}H(\mathbf{1})$$

2. Results and discussion

Chalcone epoxidation involves four reagents: the PEG bound polyleucine **1**, the substrate chalcone, hydrogen peroxide and the base. The base is essential for inducing the dissociation of hydrogen peroxide to the hydrogen peroxide anion, which is the actual oxidant (Scheme 1). To obtain complete (or very high) dissociation of hydrogen peroxide, different bases were tested[14] with 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) eventually being chosen for its stability and high basicity. Whilst recognising the much higher basicity of BEMP compared to that of hydrogen peroxide,[14] a BEMP to H_2O_2 ratio of 3 to 1 was utilised to ensure complete dissociation of the oxidant. The catalytic properties of PLL were determined by spectrophotometrically monitoring the disappearance of chalcone. The influences on the reaction rate of such parameters as catalyst, substrate and oxidant concentration, temperature, and the presence of water in the reaction medium were systematically studied. A linear correlation between the rate of substrate oxidation and catalyst concentration was found in the range investigated (1–5 mg/mL of PLL in THF). Water increased the rate (30%) up to 0.8% concentration (v/v in THF) and then decreased it (at 2.3% water, the rate was 36% of that in THF only) (Fig. 1). The same trend was also observed when the experiments were carried out in acetonitrile (data not shown).

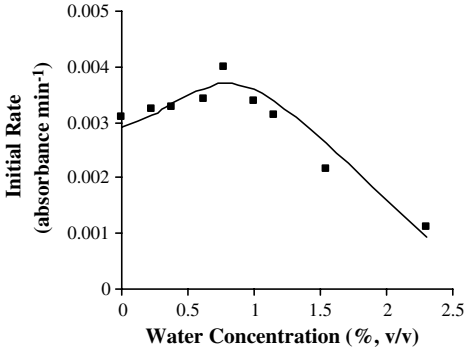


Figure 1. Effect of water concentration in THF (%, v/v) on the initial rates of chalcone oxidation. Conditions: PLL 3.5 mg/mL; 15 mM chalcone; 15 mM H_2O_2.

A rationale for this phenomenon might be that, as already shown for enzymes in organic solvents,[15] up to a certain concentration, water acts as a 'lubricant' increasing the flexibility and activity of PLL. However, higher concentrations of water would compete with HOO^- for binding to PLL, thus decreasing the activity. Increasing the temperature, tested in the 15–35 °C range, increased the oxidation rate (at 35 °C the rate was 64% higher than that at 15 °C) with the Arrhenius plot giving an activation energy of 17.03 kJ mol^{-1} (Fig. 2).

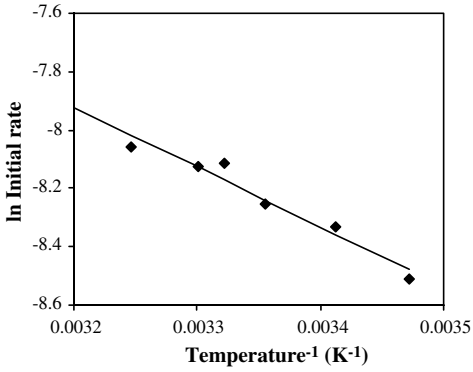


Figure 2. Natural logarithm of the initial rates of chalcone oxidation versus the reciprocal of absolute temperature (Arrhenius plot). Conditions: PLL 3.5 mg/mL; 20 mM chalcone; 20 mM H_2O_2.

Based on these preliminary experiments, the conditions used for the subsequent investigations were 3.5 mg/mL of PLL in THF with no water present at a temperature of 25 °C. The effect of chalcone concentration (5–120 mM) was studied at different fixed concentrations of H_2O_2 (10–80 mM) with the results shown in Figure 3. It can be seen that the phenomenon of substrate saturation, typical of enzyme-catalysed reactions, is present at all the fixed concentrations of H_2O_2. It can also be seen that high concentrations of chalcone inhibit the reaction.[16] It should be emphasized that the ee values (determined by chiral HPLC) of the (2*R*,3*S*)-epoxychalcone produced in the kinetic experiments were 90–96%, depending on the reactant concentrations.

The rate data reported in Figure 3 were also plotted as a function of H_2O_2 concentration at different fixed concentrations of chalcone (Fig. 4). It can be seen that the initial part of all of the rate curves is sigmoidal.[17] Sigmoidal saturation curves, such as those shown by PLL (Fig. 4), have often been considered to involve cooperative interactions between enzyme subunits. However, both substrate activation at low concentrations (sigmoids of Fig. 4) and substrate inhibition at high concentrations (Fig. 3), could be explained on the basis of a mechanism proposed by Ferdinand for a two-substrate enzyme.[18] This is a steady state random bireactant mechanism, which implicates alternative pathways to the ternary complex and, very importantly, postulates that one of the pathways is kinetically preferred to the other (for a detailed discussion of the mechanism see refs. 18–20).

The kinetic data obtained with PLL were used to calculate the theoretical curves based on the equation developed by Ferdinand[18] for such a mechanism (see Eq. 1).[21] It can be seen that the curves of rates versus chalcone concentrations satisfactorily fit to hyperbolas

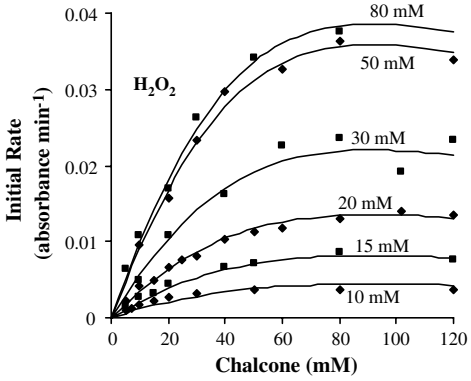


Figure 3. Effect of the chalcone concentration (5–120 mM) on the initial rates of chalcone oxidation, at different fixed concentrations of H_2O_2 (10–80 mM), catalysed by PLL (3.5 mg/mL) in THF. The curves were theoretical ones (see text) and the SD (%) were 5.7, 2.8, 2.4, 4.7, 1.9, 1.6 for 10, 15, 20, 30, 50, 80 mM H_2O_2, respectively.

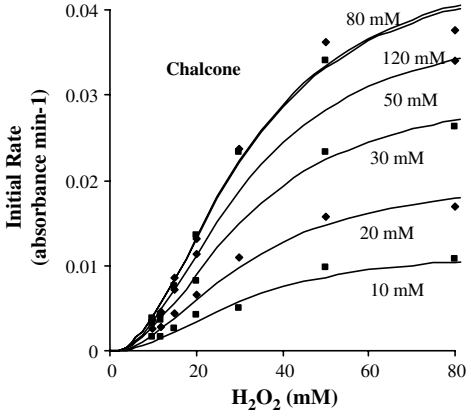


Figure 4. Effect of H_2O_2 concentration (10–80 mM) on the initial rates of chalcone oxidation, at different fixed concentrations of chalcone (10–120 mM), catalysed by PLL. The SD (%) of the theoretical curves were 5.7, 2.9, 2.0, 0.4, 6.2, 3.1 for 10, 20, 30, 50, 80, 120 mM chalcone, respectively.

(Fig. 3), whereas those of rates versus H_2O_2 concentrations fit to sigmoids (Fig. 4).

$$v = (ia^2 + ja)/(k + la^2 + ma) \quad (1)$$

The double reciprocal plots of the theoretical curves of the reaction rates versus chalcone concentrations gave straight lines, which intersected at a single point on the abscissa, from which a K_m value for chalcone of 110 ± 6 mM could be derived (Fig. 5). The apparent maximum specific activities were between 0.37 ± 0.02 and $1.81 \pm 0.11\ \mu mol\ min^{-1}\ mg^{-1}$ PLL depending on the fixed concentration of H_2O_2. The sigmoidal character of the lines of Figure 4 only permits a rough estimate of the apparent K_m value for H_2O_2 (30 ± 7 mM). Moreover, this also precludes the accurate determination of the actual V_{max} of the reaction. In fact, the secondary plot of the reciprocal of the apparent V_{max} values versus the reciprocal of H_2O_2 concentration gave a curve and not a straight line (Fig. 6). Nevertheless, these data, though approximate, should be useful for the synthetic application of PLL.

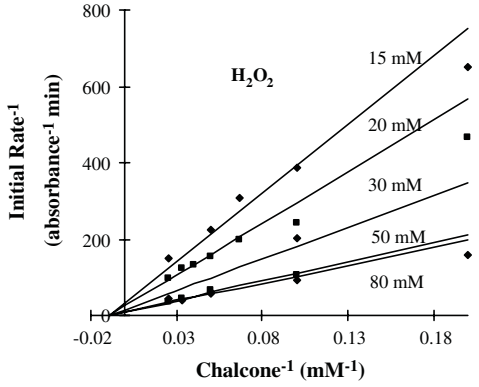


Figure 5. Double reciprocal plots of the data (points and lines) in Figure 3. Only the data up to 40 mM chalcone were considered.

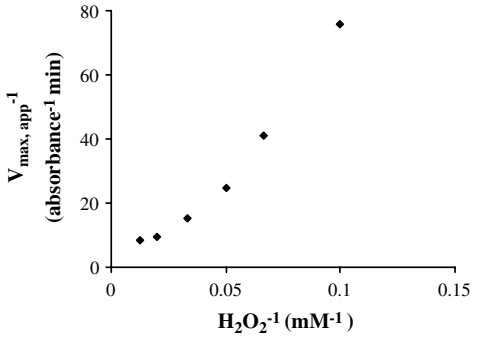
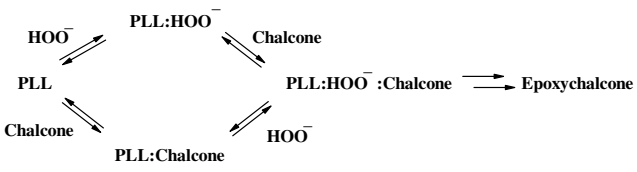


Figure 6. Secondary plot of the reciprocal of the apparent V_{max} values versus the reciprocal of H_2O_2 concentration (see Fig. 5).

3. Conclusion

The present work indicates that in a THF solution, PLL behaves as an enzyme-like catalyst at relatively low concentrations of substrates. Thus it shows saturation kinetics for both chalcone and HOO^- and has a behaviour that apparently fits a steady state random bireactant system with one of the pathways (HOO^- binding first) being kinetically preferred to the other (chalcone binding first) (Scheme 2). This type of system is sequential,[18,19] 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, which eventually evolves to epoxychalcone (Schemes 1 and 2). It is clear that the (2*R*,3*S*)-epoxychalcone enantiomer will form when HOO^- and chalcone are correctly oriented in the PLL 'active site', otherwise racemic epoxides would be obtained. In spite of the



Scheme 2.

complexity of the steady-state velocity equation (Eq. 1) for this system (Scheme 2), the substrate response curves can be visualized in a qualitative way.[18,19] Considering the data of Figure 4, at zero HOO^- concentration the catalyst will be present only as PLL and PLL:chalcone. By increasing HOO^- starting from very low concentrations, PLL:HOO^-:chalcone will form faster via the PLL:chalcone intermediate than via PLL:HOO^-. As the HOO^- concentration is increased, the faster sequence gradually takes over and the slope of the initial rates increases sharply (sigmoid). By increasing further the HOO^- concentration, the PLL → PLL:HOO^- → PLL:HOO^-:chalcone route becomes predominant and the sloping off of the curve is observed as the rate approaches V_{max}. Considering the data of Figure 3, at the beginning the rate curves will rise in the usual way as the ternary complex is formed via the kinetically favoured intermediate PLL:HOO^-. However, when the concentration of chalcone becomes high enough to overcome the kinetic factors, a greater proportion of the reaction flux will proceed via the PLL:chalcone intermediate. As a consequence, the initial rate will pass through a maximum and then will decrease. We believe that the information obtained on the optimal conditions to be used and on the kinetics and mechanism of PLL-catalysed asymmetric epoxidation reaction will be helpful in broadening the applications of this interesting catalyst in organic synthesis.

4. Experimental

4.1. Materials

The PEG bound polyleucine (PLL) was bought from Lancaster (Eastgate, England). DBU, BEMP and the phosphazene base P_2-*t*-Bu were obtained from Fluka. All other reagents and compounds were of analytical grade.

4.2. Preparation of the urea hydrogen peroxide adduct

To avoid water addition to the reaction medium, the urea hydrogen peroxide adduct was utilised. The adduct (1 g) was added to THF (10 mL) and stirred overnight. The suspension was centrifuged and the precipitate (urea and remaining adduct) discarded. The supernatant was titrated and stored in the freezer. Hydrogen peroxide concentration was around 1 M and remained constant over time.

4.3. Kinetics

The PLL catalysed oxidation of chalcone by hydrogen peroxide was spectrophotometrically monitored by measuring the disappearance of chalcone at 420 nm in a cuvette with a 0.5-cm path length at 25 °C. For calculations, a molar extinction coefficient of 36.7 $L\,mol^{-1}\,cm^{-1}$ was utilised. The reported rates were corrected for the spontaneous chalcone oxidation by H_2O_2 and therefore represent only the PLL catalysed reaction.

4.4. Chiral HPLC

The ee values of the (2*R*,3*S*)-epoxychalcone formed by PLL catalysis were determined by chiral HPLC using a ChiralPack (Daicel) column, eluted with a 9/1 hexane/ethanol mixture, at a flow rate of 1 mL/min and with reading at 254 nm. The retention times for chalcone, (2*S*,3*R*)-epoxychalcone, and (2*S*,3*R*)-epoxychalcone were 12, 14, and 21 min, respectively.

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13. The catalyst solution in THF was slightly turbid and was clarified by centrifugation. The pellet, which represented 1.7% of the total catalyst, was unable to catalyse the epoxidation of chalcone.
14. The bases tested were 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) and the phosphazene base P_2-*t*-Bu. The latter two are 2×10^3 and 10^9 more basic than DBU, whose basicity is comparable to that of H_2O_2 (pK_a in water 11.75 for H_2O_2 and 11.9 for DBU). The use of the very strong phosphazene base P_2-*t*-

Bu was prevented by the fact that, contrary to the other two bases, it was unstable under the conditions used for the kinetic experiments.
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16. It was not possible to use chalcone concentrations substantially higher than 120 mM because of the too high substrate absorption and increase of spontaneous oxidation. However, extrapolation of the theoretical curves of Figure 3 at higher chalcone concentration, clearly indicated marked substrate inhibition.
17. This behaviour cannot be ascribed to different degrees of deprotonation of PLL amino terminus as a consequence of different BEMP concentrations in the medium, since the high basicity of BEMP[14] and the high BEMP/PLL ratio ($\geqslant$50, on a molar basis) should always assure complete deprotonation of the catalyst.
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21. For example, in the case of Figure 3, the symbols of Eq. 1 have the following meaning: v is the initial rate in the presence of a fixed concentration of hydrogen peroxide, a is the concentration of chalcone, and i, j, k, l and m are functions of hydrogen peroxide concentration and of the rate constants for the various steps of Scheme 2. For additional details see: King, E. L. *J. Phys. Chem.* **1956**, *60*, 1378–1381.