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Kinetics of chalcone oxidation by peroxide anion catalysed by poly-L-leucine†

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An insight into the kinetics, mechanism and optimum reaction conditions of the Juliá–Colonna epoxidation has been gained using a soluble polyleucine catalyst.

The Weitz–Scheffer oxidation of α,β-unsaturated ketones involves the addition of hydroperoxide to the enone unit to form a hydroperoxide enolate.[1] Loss of 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 studied in detail by Rapoport some twenty years ago.[2]

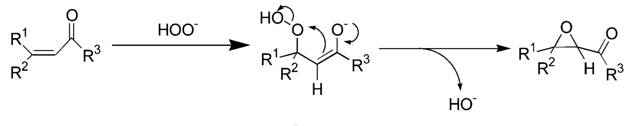
There are a number of different ways of inducing asymmetry into the Weitz–Scheffer reaction.[3,4] The methodology introduced by Juliá and Colonna involving the use of a polyamino acid as a catalyst for the reaction[5] has been developed in recent years[6] to become the methodology of choice for the preparation of chiral, optically active epoxides derived from α,β-unsaturated ketones.[7]

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 (PLL, **1**) 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]

In this communication 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^{-1}.[13] It has been shown that under these reaction conditions the epoxychalcone product is obtained with high optical purity (e.e. ≥ 96%).[9]

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

Chalcone epoxidation involves four reagents: the PEG bound polyleucine (**1**), the substrate chalcone, hydrogen peroxide,[14] and the base. The base is essential to induce 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[15] and 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) was eventually chosen for its stability and high basicity. Whilst recognising the much higher basicity of BEMP compared to that of hydrogen peroxide,[15] a BEMP to H_2O_2 ratio of 3 : 1 was utilised to ensure complete dissociation of the oxidant.



Scheme 1

The catalytic properties of PLL were determined by spectrophotometrically monitoring the disappearance of chalcone. The influence on the reaction rate of such parameters as catalyst, substrate and oxidant concentration, temperature, and the presence of water in the reaction medium was systematically studied. A linear correlation between the rate of substrate oxidation and catalyst concentration was found in the range investigated (1–5 mg ml^{-1} 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 39% of that in THF only). Increase of 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) and the Arrhenius plot gave an activation energy of 17.03 kJ mol^{-1}. Based on these preliminary experiments, the conditions used for the subsequent investigations were 3.5 mg ml^{-1} of PLL in THF with no water present and 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) and the results are shown in Fig. 1. 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. The velocity data reported in Fig. 1 were also plotted as a function of H_2O_2 concentration at different fixed concentrations of chalcone (Fig. 2). Now it can be seen that the initial part of all of the velocity curves is sigmoidal.[16]

Sigmoidal saturation curves, such as those shown by PLL (Fig. 2), have often been considered to involve cooperative interactions

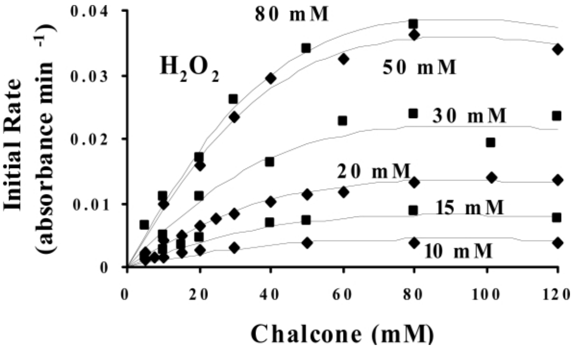


Fig. 1 The effect of chalcone concentration (5–120 mM) on the initial rates of chalcone oxidation, at different fixed concentrations of H_2O_2 (10–80 mM), catalysed by PLL (3.5 mg ml^{-1}) in THF. The disappearance of chalcone was spectrophotometrically monitored at 420 nm in a cuvette with a 0.5 cm 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. The curves were theoretical ones (see text) and the S.D. (%) 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. The e.e. values (determined by chiral HPLC) of the epoxychalcone produced in the kinetic experiments were 90–96%, depending on reactant concentrations.

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† Electronic supplementary information (ESI) available: double reciprocal plots of the data in Fig. 1. See http://www.rsc.org/suppdata/cc/b4/b401497a/

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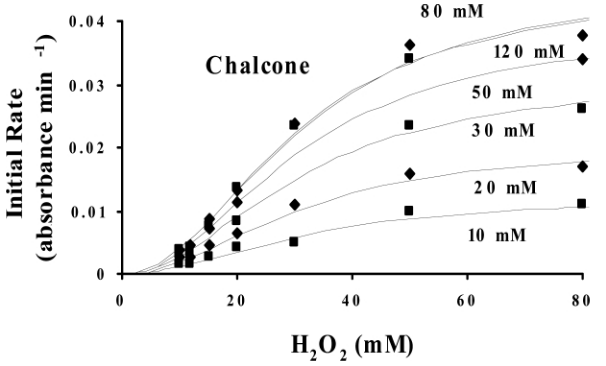


Fig. 2 The effect of H_2O_2 concentration (10–80 mM) on the initial rates of chalcone oxidation, at different fixed concentrations of chalcone (10–120 mM), catalysed by PLL. The S.D. (%) of the theoretical curves were 5.7, 2.9, 2.0, 0.4, 6.2, 3.1 for 10, 20, 30, 50, 80, 120 mM chalcone, respectively

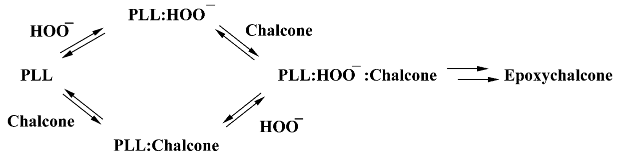
between enzyme subunits. However, both substrate activation at low concentrations (sigmoids of Fig. 2) and substrate inhibition at high concentrations (Fig. 1), could be explained on the basis of a mechanism proposed by Ferdinand for a two-substrate enzyme.[17] It is a steady state random bireactant 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 ref 17,18).

The kinetic data obtained with PLL were used to calculate the theoretical curves based on the equation developed by Ferdinand[17] for such a mechanism (see eqn. (1)). It can be seen that the curves of rates *versus* chalcone concentrations satisfactorily fit to hyperbolas (Fig. 1), whereas those of rates *versus* H_2O_2 concentrations fit to sigmoids (Fig. 2). The double reciprocal plots† of the theoretical curves of reaction rates *versus* chalcone concentrations (up to 40 mM) gave straight lines that intersected at a single point on the abscissa (data not shown), from which a K_m value for chalcone of 110 mM could be derived. The apparent maximum specific activities were between 0.37 and 1.81 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ PLL depending on the fixed concentration of H_2O_2. The sigmoidal character of the lines of Fig. 2 only permits a very rough estimate of the apparent K_m value for H_2O_2 (around 30 mM). These data, though approximate, should be useful for optimisation of the synthetic uses of PLL.

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

In 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,[17] *i.e.* 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).

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.



Scheme 2

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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 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.

15 The bases tested were 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diaza-phosphorine (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.

16 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[15] and the high BEMP/PLL ratio ($\geq$ 50, on a molar basis) should always assure complete deprotonation of the catalyst.

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