Oxidative C_α–C_β Fission (Dealdolation) of β-Hydroxy Amino Acids by Coenzyme PQQ

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Coenzyme PQQ is demonstrated to catalyse the oxidative C_α–C_β fission (dealdolation) of β-hydroxy amino acids under very mild conditions.

The reaction of coenzyme PQQ with amino acids has attracted much recent attention, since dopa decarboxylase (EC 4.1.1.28), glutamic acid decarboxylase (EC 4.1.1.15) and tryptophan decarboxylase (EC 4.1.1.28), which are believed to be PLP-dependent enzymes, have recently been suspected to be quinoproteins (PQQ-containing enzymes).[1–3] However, the major factor which makes it difficult to identify the covalently bound PQQ (or its analogue) is the high reactivity of the cofactor towards nucleophiles such as amino acids and conversion into adducts.[4] From this viewpoint, a few research

Scheme 1 *Reagents and conditions*: PQQ (3.3 mol %)–CTAB (cetyltrimethylammonium bromide), 30 °C, aerobic conditions

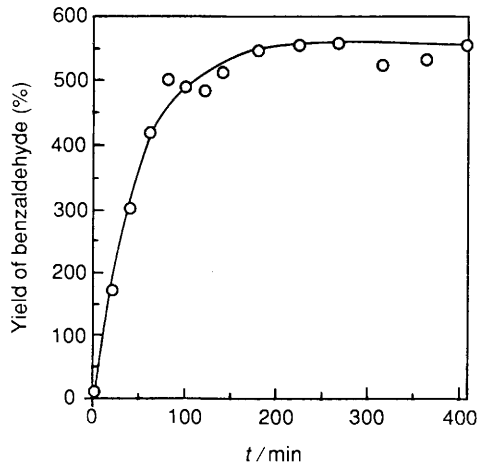


Fig. 1 Time course of benzaldehyde formation in the reaction of DL-3-phenylserine (4.6×10^{-2} mol dm^{-3}) with PQQ (1.5×10^{-3} mol dm^{-3}) in the presence of CTAB (1.3×10^{-2} mol dm^{-3}) at pH 10.3, 30 °C under aerobic conditions

groups have investigated the reaction between coenzyme PQQ and amino acids,[5] and some of them reported that PQQ is converted into an oxazole derivative.[5a,d]

As a model reaction of the quinoproteins, we have already demonstrated the efficient oxidative decarboxylation of 2-phenylglycine by PQQ in a cationic micellar system and suggested the possible contribution of PQQ to amino acid metabolism in biological systems.[6] In this paper, we demonstrate the first example of the oxidative C_α–C_β fission (dealdolation) of β-hydroxy amino acids by coenzyme PQQ.

When DL-3-phenylserine was treated with a catalytic amount of PQQ at 30 °C under aerobic conditions (pH 10.3), effective formation of benzaldehyde was observed (Scheme 1). In this reaction, mandelaldehyde, which is an expected product of the oxidative decarboxylation, and glycine, which is a common product of the dealdolation reaction catalysed by PLP and metal ion,[7] could not be detected. In Fig. 1 is shown the time course of the formation of benzaldehyde, in which the yield of benzaldehyde increases to reach about 550% based on PQQ and then gradually levels off. The decrease of the observed rate seems to be due to the deactivation of PQQ in the catalytic cycles because the colour of the reaction mixture gradually turned from red (the colour of PQQ) to yellow. Fig. 2 shows spectral changes for the reaction, where the disappearance of PQQ (broad shoulder at around 320 nm) is accompanied with an increase in the absorbance at around 420 nm. The time course of the appearance of the latter absorption (inset, Fig. 2) is very close to that of the formation of benzaldehyde (Fig. 1). Thus, it could be said that the compound having λ_{max} at 422 nm is the deactivated product of PQQ. Product analysis was then carried out on a preparative scale to show that the deactivated product was the oxazole derivative **1**.† It should be noted that the formation of **1** is also

† ^{1}H NMR (CD_3SOCD_3): δ 7.38 (1H, br s, 3-H), 8.42 (1H, s, 8-H), 9.35 (1H, s, oxazole ring H) and 13.29 (1H, br s, pyrrole H); IR (KBr): ν_{max}/cm^{-1} 1710 (ester C=O), 1644 (C=N), 1528 (C=C) and 1370 (C–O); UV-VIS (pH 9.7): λ_{max} = 422 nm.

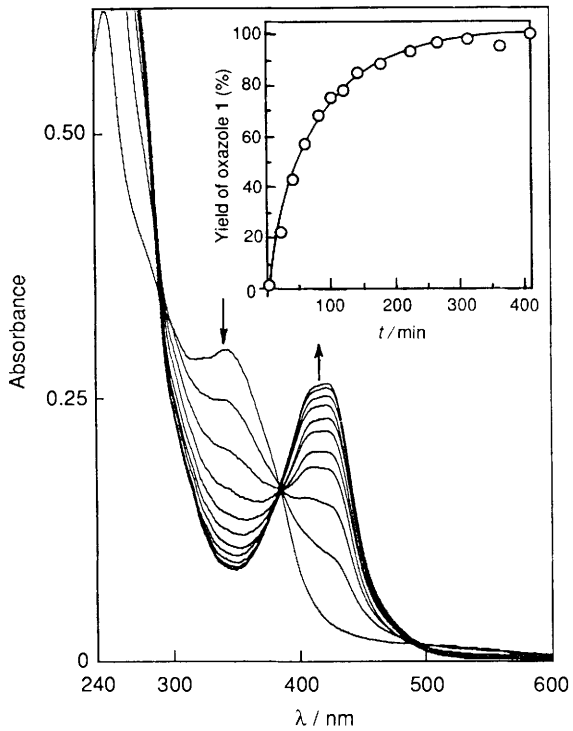
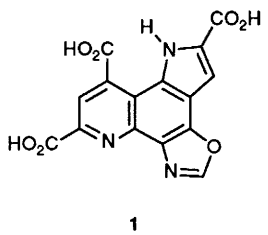


Fig. 2 Spectroscopic change along the progress of the reaction of PQQ (1.5×10^{-3} mol dm^{-3}) with DL-3-phenylserine (4.6×10^{-2} mol dm^{-3}) in the presence of CTAB (1.3×10^{-2} mol dm^{-3}) at pH 10.3, 30 °C under aerobic conditions. Each spectrum was taken by using the diluted aliquots (50-fold with the same buffer). Time course of the formation of oxazole **1** (determined by the appearance of the absorption at 422 nm) is shown in the inset.



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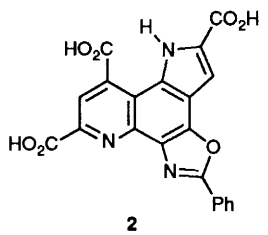

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accompanied with C_α–C_β fission of the amino acid residue. The same oxazole product **1** was also obtained in the reaction of PQQ with serine, threonine and tyrosine (the isolated yields of **1** were 55, 70 and 77%, respectively), and in the case of tyrosine, formation of *p*-hydroxybenzyl alcohol as an oxidation product was confirmed by HPLC.

The pH-dependence of the reaction showed that higher yields of benzaldehyde were obtained at a higher pH (pH 10.7), whereas the optimal pH condition was below 9 in the oxidative decarboxylation of DL-2-phenylglycine‡ (Table 1). So dissociation of the hydroxy group of phenylserine may be significant for the reaction. In fact a similar reaction also took place in the case of 2-amino-1-phenylethanol to give the same product (benzaldehyde and **1**) and the catalytic efficiency of PQQ and the optimum pH were found to be very close to those in the case of phenylserine (Table 1). It should also be mentioned that the present reaction is characteristic for PQQ because the catalytic activity of a simple *o*-quinone such as phenanthrenequinone was very low.

‡ The reaction of DL-2-phenylglycine could not be examined below pH 8, because of its poor solubility under these conditions.

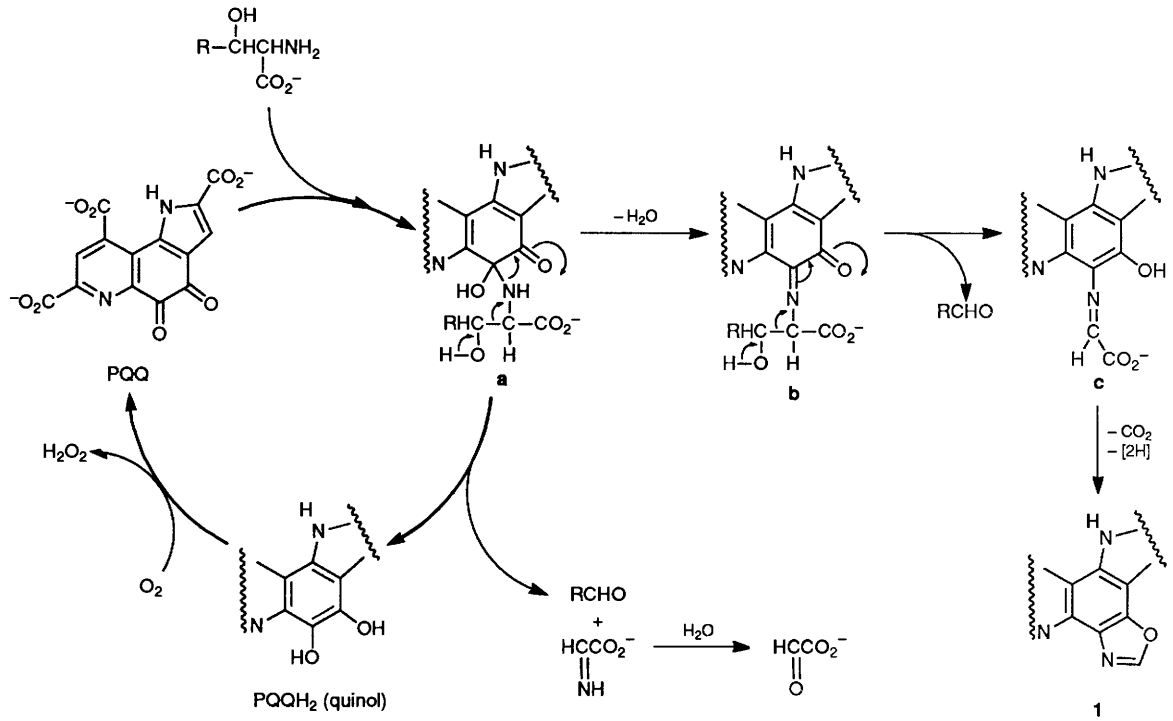

Table 1 The reaction of coenzyme PQQ with DL-3-phenylserine, DL-2-phenylglycine and 2-amino-1-phenylethanol[a]

Quinone	Substrate	pH	Yield of PhCHO (%)[b]
PQQ	DL-3-Phenylserine	6.6	53
PQQ	DL-3-Phenylserine	7.7	247
PQQ	DL-3-Phenylserine	9.5	343
PQQ	DL-3-Phenylserine	10.3	557
PQQ	DL-3-Phenylserine	10.7	678
Phenanthrenequinone	DL-3-Phenylserine	10.3	154
none	DL-3-Phenylserine	10.7	21
PQQ	DL-2-Phenylglycine	8.8	534
PQQ	DL-2-Phenylglycine	9.5	470
PQQ	DL-2-Phenylglycine	9.9	251
PQQ	DL-2-Phenylglycine	11.1	166
PQQ	2-Amino-1-phenylethanol	6.9	57
PQQ	2-Amino-1-phenylethanol	10.0	294
PQQ	2-Amino-1-phenylethanol	10.6	649

[a] [Quinone] = 1.52×10^{-3} mol dm^{-3}, [Substrate] = 4.55×10^{-2} mol dm^{-3}, [CTAB] = 1.25×10^{-2} mol dm^{-3}, 0.5 mol dm^{-3} buffer, 30 °C, 5 h, under aerobic conditions. [b] Yields were determined by HPLC based on quinone.

In the present reaction, the carbinolamine type adduct **a** is considered to be a key intermediate as in the case of the oxidation of amines,[8,9] hydrazines,[10] aminoguanidine[11] and α,ω-diaminoalkanes[12] (Scheme 2). From this intermediate oxidative C_α–C_β bond cleavage may occur to give benzaldehyde, glyoxylic acid§ and $PQQH_2$ (reduced PQQ in the quinol form) directly. Predominant formation of $PQQH_2$ was in fact confirmed by both UV-VIS spectroscopy and HPLC analysis under anaerobic conditions. However, formation of the aminophenol product was hardly detected by HPLC analysis.[8] Furthermore PQQ was found to be regenerated easily by aeration of the reaction mixture to construct efficient catalytic cycles. The C_α–C_β bond cleavage might also occur from the iminoquinone-type intermediate **b** to give the imine intermediate **c** from which decarboxylation, ring closure and aromatization take place to afford the oxazole **1**. Although the detailed mechanism of oxazole formation has to await further investigations, the existence of the hydroxy group at the β-position is necessary for the formation of the oxazole **1**, since the oxazole **2** was obtained as a deactivated product in the oxidative decarboxylation of phenylglycine.¶

During the course of our research, Duine and his co-workers reported the formation of oxazole **1** in the reaction of PQQ with a series of β-hydroxy amino acids, but they did not state the oxidation products from the amino acids.[5d] They assumed that the main path of the reaction is the formation of the oxazole **1** and $PQQH_2$ is formed in the final aromatization step. However, the present results clearly indicate that the oxidative dealdolation of the substrate (formation of $PQQH_2$) is the main path and the deactivation of PQQ is the minor one.

In conclusion, a new type of reaction, PQQ-catalysed oxidative C_α–C_β fission of β-hydroxy amino acids, was demonstrated for the first time. Although the organic cofactor of eukaryotic quinoproteins has been a controversial matter,[13,14] the present results are interesting not only from the viewpoint of biological studies of PQQ but also from the organic chemical one.

§ Formally the imino derivative of glyoxylic acid is a primary product which may be converted into glyoxylic acid itself by spontaneous hydrolysis. However, glyoxylic acid could not be detected because of its instability under the reaction conditions.

¶ ^{1}H NMR (CD_3SOCD_3): δ 7.52 (1H, br s, 3-H), 7.4–8.1 (5H, m, Ph), 8.18 (1H, s, 8-H), 14.25 (1H, br s, pyrrole H); IR (KBr): ν_{max}/cm^{-1} 1704 (ester C=O), 1640 (C=N), 1528 (C=C) and 1242 (C–O); UV-VIS (pH 9.7): λ_{max} 424 nm.

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