Reaction of Coenzyme PQQ with Amino Acids. Oxidative Decarboxylation, Oxidative Dealdolation (C_α–C_β Fission) and Oxazolopyrroloquinoline (OPQ) Formation[1]

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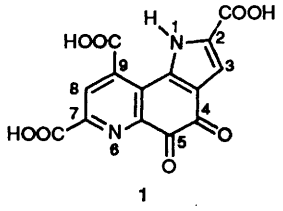
The reactions of coenzyme PQQ (pyrroloquinolinequinone) and several amino acids have been investigated *in vitro*. PQQ catalyses the *oxidative decarboxylation* of α-amino acids to afford the corresponding aldehydes under aerobic conditions. During the catalytic cycles, PQQ is gradually converted into oxazolopyrroloquinoline (OPQ) derivatives to be deactivated. Product analyses indicate that the reaction proceeds *via* an ionic mechanism that involves a carbinolamine-type adduct as a key intermediate. From this intermediate, direct decarboxylation (major path) and dehydration followed by decarboxylation and hydrolysis or by intramolecular cyclization (minor paths) competitively occur to give the quinol, the aminophenol and the OPQ derivative, respectively. In the reactions with β-hydroxy amino acids, tyrosine and tryptophan, oxidative dealdolation (C_α–C_β fission) proceeds effectively. The similar ionic mechanism that involves the carbinolamine-type intermediate is suggested by the product analyses under both aerobic and anaerobic conditions.

PQQ(4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, **1**) has been shown to be a novel prosthetic group of several non-flavin or nicotinamide-dependent dehydrogenases.[2] In addition to the enzymological importance, the growth stimulating activity,[3] the pharmaceutical activities[4–6] and the nutritional importance[7] of PQQ have recently received much attention in various research fields. The application of PQQ to several therapeutic[8] and cosmetic agents and to biosensors[10] has also been aimed at various industrial areas. Therefore, elucidation of the chemical behaviour of PQQ towards biologically-important substances has become increasingly important.

To address this issue, we have investigated the chemical reactions of PQQ with such substances to find many interesting aspects of PQQ-functions,[11–20] of these, the reaction with amino acids is one of the most attractive subjects. For example, a major factor which makes it difficult to identify free PQQ is its high reactivity toward nucleophiles such as amino acids which exist in biological fluids. This behaviour has, however, been applied to develop a detection method for PQQ, namely, the redox-cycling assay using glycine and NBT (Nitroblue Tetrazolium).[21] In order to characterise the product, a few research groups have investigated the reaction between PQQ and amino acids,[22–25] and some of them reported that PQQ is converted into an oxazole derivative (so-called oxazolopyrroloquinoline, OPQ).[22,25] However, little is known about the products and the mechanism. In this paper, we aim to investigate the reaction between PQQ and amino acids in more detail so as to obtain further information on the chemical properties of PQQ.[26]



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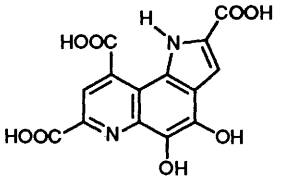
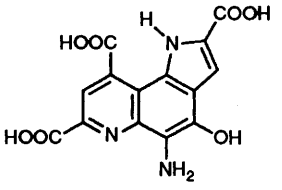
Results and Discussion

Oxidative Decarboxylation.—The reaction of PQQ with the simplest amino acid, glycine, was investigated under anaerobic conditions (Ar). Fig. 1 shows the spectral change along the course of the reaction. A typical absorption of the reduced species of PQQ at around 318 nm remarkably increased as the reaction proceeded [Fig. 1(*a*)]. Introduction of air into the final reaction mixture immediately regenerated PQQ [Fig. 1(*b*)], indicating that the reoxidation of the reduced PQQ by molecular oxygen is much faster than the reaction between PQQ and glycine under the reaction conditions. It should be noted that a small absorption at around 420 nm [indicated by the arrow in Fig. 1(*b*)] remained unchanged even after the aeration. Product analysis by HPLC under anaerobic conditions (Fig. 2) indicated that the major product from PQQ was the quinol $\mathbf{1H_2}$ while the aminophenol **2** was a minor one. In addition to those reduced species of PQQ, another product was detected at a retention time of *ca.* 24 min (indicated as compound **a** in Fig. 2, molar ratio; $\mathbf{2}$:$\mathbf{1H_2}$:**a** = 1:11:1, see Experimental section).



$\mathbf{1H_2}$



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The kinetics for the reaction of PQQ and glycine under anaerobic conditions were examined in a similar manner as described for the reaction of 7,9-didecarboxymethoxatin (7,9-didecarboxylated PQQ) and benzylamine by Bruice *et al.*[22] The reaction was carried out at 30 °C under pseudo-first-order conditions of [glycine] = 4.0×10^{-2} to 1.6×10^{-1} mol dm^{-3} ≫ [PQQ] = 4.0×10^{-5} mol dm^{-3}. At pH 9.1, 9.5, 9.9 and 10.3, the increase in A_{318} yielded first-order kinetics and a plot of k_{obs} *vs.* $[\text{glycine}]_{total}$ gave an upwardly-curving line [Fig. 3(*a*)]. A linear line with positive intercept was obtained in the plot of $k_{obs}/[\text{glycine}]$ *vs.* [glycine] [Fig. 3(*b*)], indicating that the reaction is both second and first order in the amino acid concentration [eqn. (1a)]. From the intercept, k_1 is calculated to be 6.3×10^{-2} dm^3 mol^{-1} s^{-1} as an average of the four values

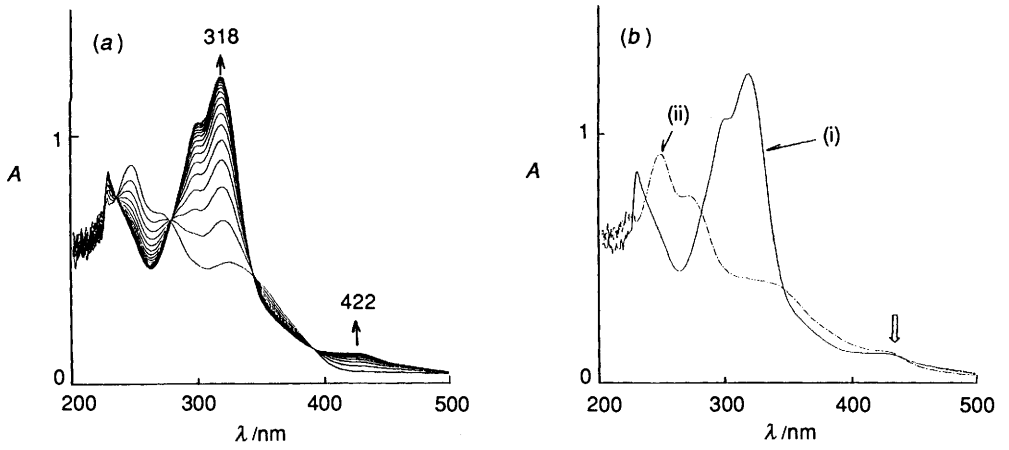


Fig. 1 (*a*) Spectroscopic change along the progress of the reaction of PQQ (4.0×10^{-5} mol dm^{-3}) and glycine (4.0×10^{-2} mol dm^{-3}) in 0.5 mol dm^{-3} carbonate buffer at pH 9.5, 30 °C under anaerobic conditions. (*b*) The final spectrum (i) and the spectrum after aeration (ii).

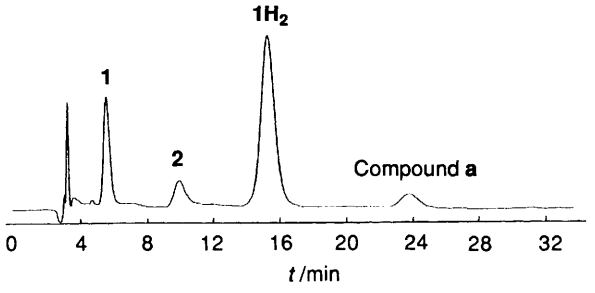


Fig. 2 HPLC analysis of the anaerobic reaction

obtained under each pH. The second-order term of the reaction corresponds to the existence of general-acid catalysis by $^{+}NH_3CH_2COO^{-}$, as is found for the reaction of 7,9-di-decarboxymethoxatin and benzylamine.[22] In fact, the plot of the slopes of the lines, obtained under each pH, *vs.* a_H (hydrogen activity) showed a straight line [Fig. 3(*c*)] with k_2/K_a as the slope from which k_2 is calculated to be 2.8 dm^6 mol^{-2} s^{-1}.

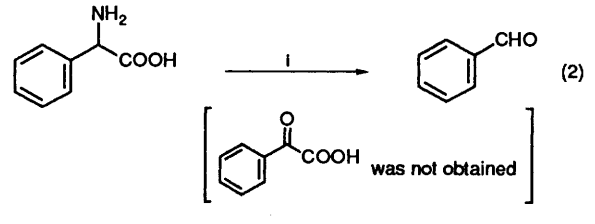
$$k_{obs} = k_1[NH_2CH_2COO^-] + k_2[NH_2CH_2COO^-][^+NH_3CH_2COO^-] \quad (1a)$$

$$k_{obs}/[NH_2CH_2COO^-] = k_1 + (k_2a_H/K_a)[NH_2CH_2COO^-] \quad (1b)$$

K_a: acid–base dissociation constant for

$$^{+}NH_3CH_2COO^- \rightleftharpoons NH_2CH_2COO^- + H^+$$

The products from amino acids were then examined using DL-2-phenylglycine as a substrate. When phenylglycine was treated with a catalytic amount of PQQ under aerobic conditions (at 30 °C, pH 8.8), remarkable formation of benzaldehyde was detected by HPLC, and its yield reached *ca.* 530% based on PQQ after *ca.* 6 h [eqn. (2), Fig. 4]. In this reaction, however,



(2)

Reagents and Conditions: i, PQQ (6.1 mol dm^{-6})/CTAB, pH 8.8, 30 °C, aerobic conditions

benzoylformic acid (oxidative deamination product) could not be detected.

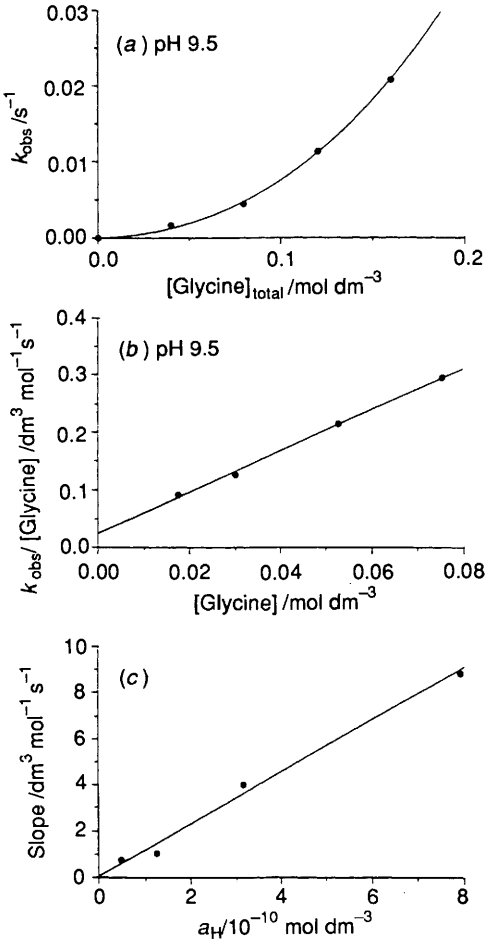


Fig. 3 (*a*) Plot of the pseudo-first-order constants (k_{obs}) *vs.* $[glycine]_{total}$ for the reaction of PQQ and glycine at pH 9.5, 30 °C under anaerobic conditions. (*b*) Plot of k_{obs}/[glycine] *vs.* [glycine]. (*c*) Plot of the slope *vs.* hydrogen activity (a_H).

Oxazolopyrroloquinoline (OPQ) Formation.—When the reaction of PQQ and glycine was carried out under aerobic conditions, the absorption at around 422 nm, which has already been mentioned in Fig. 1(*b*), increased remarkably as the reaction proceeded (Fig. 5). This spectral change corresponds to the formation of the oxazole derivative of PQQ (oxazolopyrroloquinoline, OPQ, **3a**).[22,25] In the ^{1}H NMR spectrum of the isolated product in $[^2H_6]Me_2SO$, the aromatic protons appear at δ 7.32, 8.34 and 9.31. Esterification of the product further confirmed the OPQ structure. Application of Duine's

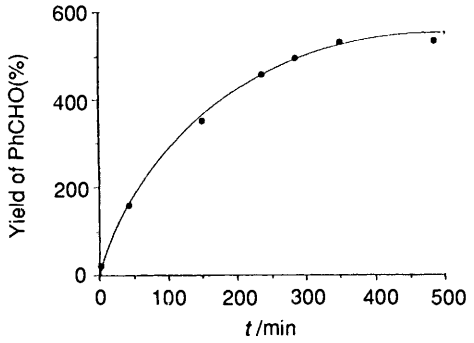


Fig. 4 Time course of benzaldehyde formation in the reaction of PQQ (1.52×10^{-3} mol dm^{-3}) and DL-2-phenylglycine (4.55×10^{-2} mol dm^{-3}) in the presence of CTAB (1.25×10^{-2} mol dm^{-3}) in 0.5 mol dm^{-3} phosphate buffer at pH 8.8, 30 °C under aerobic conditions

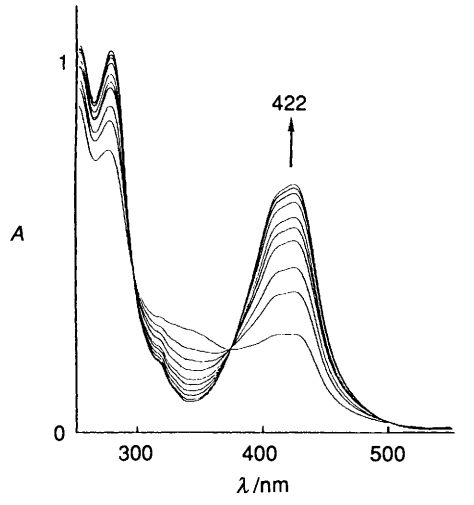
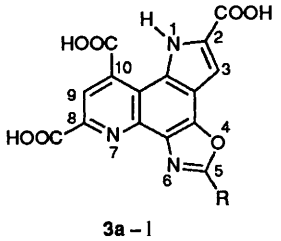
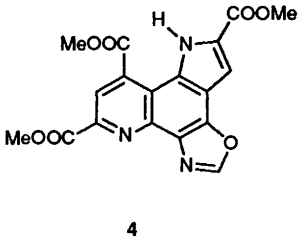


Fig. 5 Spectroscopic change along the progress of the reaction of PQQ (1.52×10^{-3} mol dm^{-3}) and glycine (4.55×10^{-2} mol dm^{-3}) in 0.5 mol dm^{-3} phosphate buffer at pH 6.6, 30 °C under aerobic conditions. The spectra were taken on diluted aliquots (40-fold) from the reaction mixture.

method[27] with Me_2SO_4 and K_2CO_3 in DMF to **3a** gave the trimethyl ester **4**, which is well characterized by ^1H NMR, IR, mass and UV–VIS spectroscopy and elemental analysis (see Experimental section). Unfortunately, it is not possible to determine the exact positions of the nitrogen and the oxygen atoms in the oxazole ring, but it might be reasonable to assign the nitrogen to the 6-position, since the C-5 carbonyl carbon of PQQ has been shown to be very susceptible towards nucleophilic addition (see Scheme 1).[28] The retention time of the isolated product **3a** in the HPLC analysis was identical to that of compound **a** in Fig. 2.



3a–l



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The OPQ-formation was also investigated in the reaction of PQQ and other amino acids (Table 1). In all cases, α-carboxyl groups of the substrates do not exist on the oxazole ring of the products, but the β- and γ-carboxyl groups of aspartic acid and

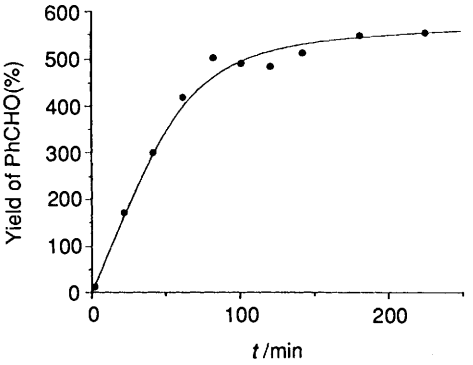
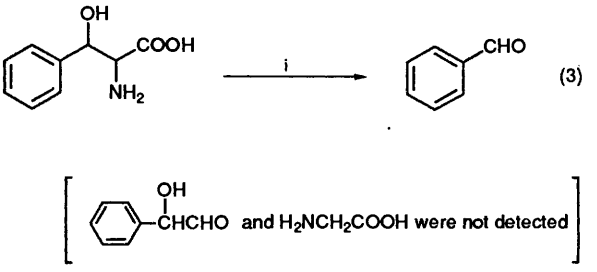


Fig. 6 Time course of benzaldehyde formation in the reaction of PQQ (1.52×10^{-3} mol dm^{-3}) and DL-3-phenylserine (4.55×10^{-2} mol dm^{-3}) in the presence of CTAB (1.25×10^{-2} mol dm^{-3}) in 0.5 mol dm^{-3} carbonate buffer at pH 10.3, 30 °C under aerobic conditions

glutamic acid remained unchanged. Interestingly, serine, tyrosine and tryptophan gave the same product **3a** as a major product, indicating that α–β bond fission proceeds efficiently during the course of the reaction. This will be discussed later in more detail. In the case of cysteine, only the PQQ-catalysed oxidative coupling to cystine[15] was detected. The reaction with lysine, however, gave a complicated mixture of products. These results were almost comparable to those reported by Duine *et al.* using a combination of HPLC and UV–VIS analyses.[25]

Oxidative Dealdolation (*Oxidative* C_α–C_β *Bond Fission*). The PLP-catalysed dealdolation reaction of β-hydroxy amino acids is one of the important metabolic processes in living systems.[29] In the aerobic reaction with serine, tyrosine or tryptophan, PQQ was converted into the same oxazole derivative **3a** (Table 1), suggesting the similar possibility for the reaction of PQQ with these species. The reaction of PQQ and a series of β-hydroxy amino acids was therefore examined in more detail.

HPLC analysis on the anaerobic reaction between PQQ and serine indicated that the quinol $\mathbf{1H_2}$ was formed as a major product together with a small amount of the oxazole **3a** and a trace amount of the aminophenol **2** (molar ratio: $\mathbf{2}:\mathbf{1H_2}:\mathbf{3a}$ = trace:10:1). Product analysis was then carried out using DL-3-phenylserine under aerobic conditions. Interestingly, effective formation of benzaldehyde was also observed, but mandelaldehyde, which is an expected product of the oxidative decarboxylation, and glycine, which is a common product of the normal dealdolation reaction catalysed by PLP and metal ion, could not be detected at all [eqn. (3)]. Fig. 6 shows the time



Reagents and conditions: i, PQQ (3.3 mol %)/CTAB, pH 10.3, 30 °C, aerobic conditions

course of the formation of benzaldehyde, where the yield of benzaldehyde increases to reach *ca.* 550% based on PQQ, and gradually levels off afterwards. The rate retardation can be attributed to the OPQ-formation (deactivation of PQQ, see Table 3).

The pH-dependence of the reaction showed that a higher

Table 1 Formation of OPQ-derivatives in the reaction of PQQ and α-amino acids[a]

Amino acid	pH	Product	R	Yield (%)[b]
Glycine	6.6	**3a**	H	100
Alanine	6.5	**3b**	CH_3	20[e]
Phenylglycine	10.0[c]	**3c**	Ph	52
Aspartic acid	6.3	**3d**	CH_2COOH	55
Glutamic acid	6.5	**3e**	CH_2CH_2COOH	62
Histidine	6.7	**3f**	5-imidazolylmethyl	80
Serine	7.7	**3a**	H	53
		3g	CH_2OH	44
Tyrosine	10.0[c]	**3a**	H	77
Tryptophan	6.7	**3a**	H	71
Cysteine	6.5	—[d]		

[a] [PQQ] = 1.52 mmol dm^{-3}, [amino acid] = 45.5 mmol dm^{-3}, 0.5 mol dm^{-3} phosphate buffer, 30 °C, 24 h, aerobic conditions. [b] Isolated yield based on PQQ. [c] The reaction was carried out at alkaline pH conditions (0.5 mol dm^{-3} carbonate buffer) because of poorer solubility of amino acid under the neutral conditions. [d] Only the PQQ-catalysed oxidative coupling of cysteine to cystine was observed. [e] A small amount of **3a** was detected by HPLC.

Table 2 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}, [CATB] = 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 the quinone.

yield of benzaldehyde was obtained at higher pH conditions (pH 10.7), whereas the optimal pH condition was below 9 in the oxidative decarboxylation of DL-2-phenylglycine (Table 2). Dissociation of the hydroxy group of 3-phenylserine may therefore be significant for the reaction. In fact, in the reaction of 2-amino-1-phenylethanol, a similar reaction took place to give the same product, benzaldehyde, and the catalytic efficiency of PQQ and the optimum pH conditions were found to be very similar to those of the reaction of 3-phenylserine. Furthermore, we might suggest that the present reaction is specific to PQQ, since catalytic activity of a simple *o*-quinone such as phenanthrenequinone was very low.

The OPQ derivative **3a** was also obtained in the reactions with threonine, phenylserine and 2-amino-1-phenylethanol at pH > 10 as in the cases of serine, tyrosine and tryptophan. The importance of dissociation of the hydroxy group of the substrate is also indicated by the fact that (i) the proportion of **3a** to **3g** increased drastically with increasing pH, and only **3a** was obtained at pH 9.7 whereas **3g** became the major product at lower pH conditions and (ii) protection of the hydroxy group of serine by methyl and phosphonyl groups prevented the formation of **3a** but gave **3h** and **3i**, respectively.

Table 3 Formation of OPQ-derivatives in the reaction of PQQ and serine and its derivatives[a]

Amino acid	pH	Product	R	Yield (%)[b]
Serine	9.7	**3a**	H	100
		3g	CH_2OH	0
	7.7	**3a**	H	53
		3g	CH_2OH	44
	4.9[c]	**3a**	H	11
		3g	CH_2OH	41
	3.2[c,d]	**3a**	H	0
		3g	CH_2OH	53
Threonine	10.2	**3a**	H	70
Phenylserine[e]	10.5	**3a**	H	60
2-Amino-1-phenylethanol[e]	10.4	**3a**	H	78
O-Methylserine	9.8	**3h**	CH_2OCH_3	72
O-Phosphoserine	9.6	**3i**	$CH_2OPO_3H_2$	86

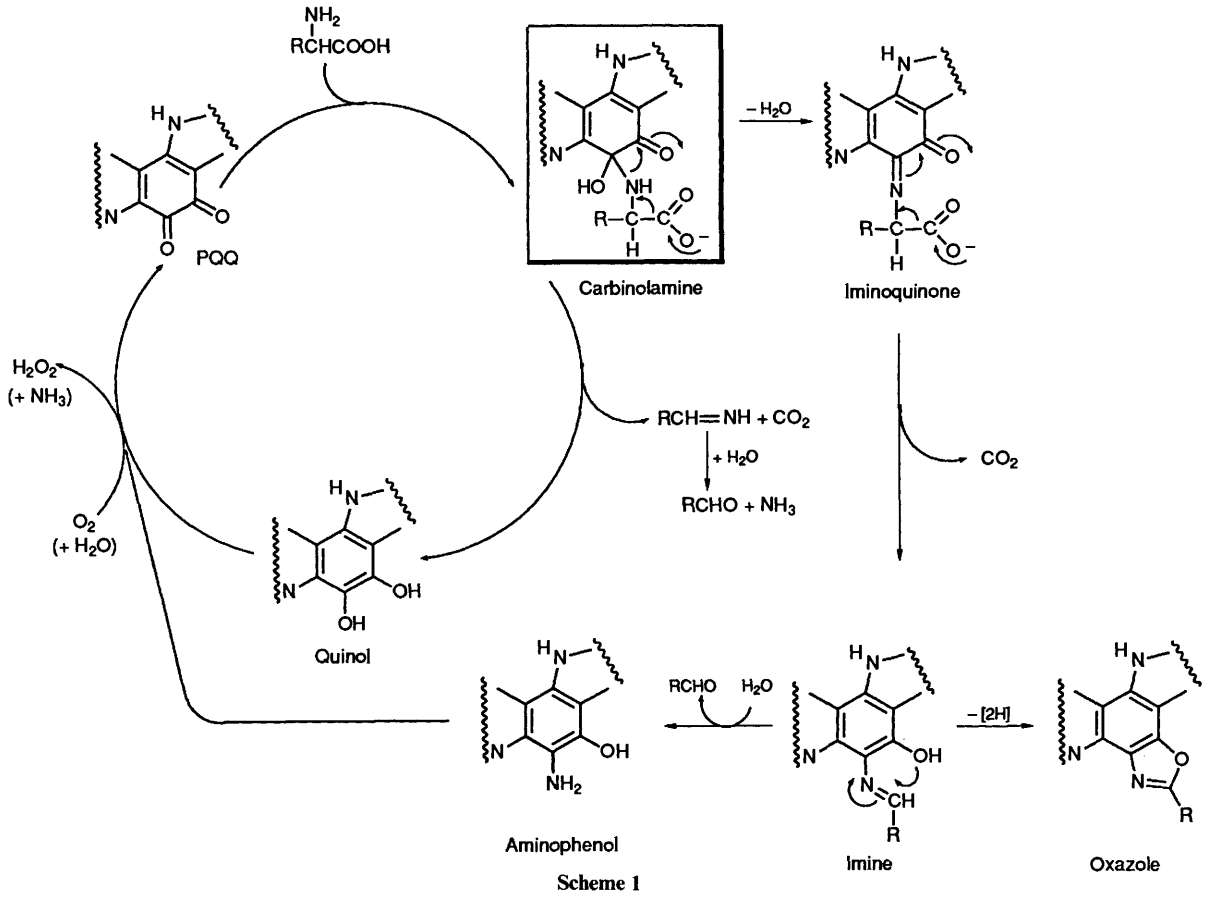
[a] [PQQ] = 1.52 mmol dm^{-3}, [amino acid] = 45.5 mmol dm^{-3}, 0.5 mol dm^{-3} phosphate buffer, 30 °C, 24 h, aerobic conditions. [b] Isolated yield based on PQQ. [c] 48 h. [d] 21% of PQQ was recovered. [e] The reaction was carried out in the presence of CTAB (12.5 mmol dm^{-3}) because of poorer solubility of the substrate under the conditions.

Reaction Mechanism.—Amine-oxidation by coenzyme PQQ or its analogues has been demonstrated to proceed *via* an ionic mechanism involving a carbinolamine-type intermediate, from which direct α-deprotonation and dehydration followed by rearrangement, and hydrolysis, proceed competitively to give the quinol and the aminophenol products, respectively.[30,31] The present results suggest that the reaction of PQQ and amino acids proceeds in a similar manner as shown in Scheme 1. The carbinolamine-type adduct is also proposed to be a key intermediate from which direct decarboxylation occurs effectively to afford the quinol $\mathbf{1H_2}$ by the assistance of the second substrate, $^+NH_3CH_2COO^-$ (general-acid catalysis). As a minor process, dehydration and successive decarboxylation from the adduct intermediate may proceed to give an imine that is finally converted into the aminophenol by hydrolysis and also into the OPQ derivative by intramolecular cyclization and aromatization. Reoxidation of the reduced species of PQQ thus formed proceeds smoothly to construct the efficient catalytic system.[32]

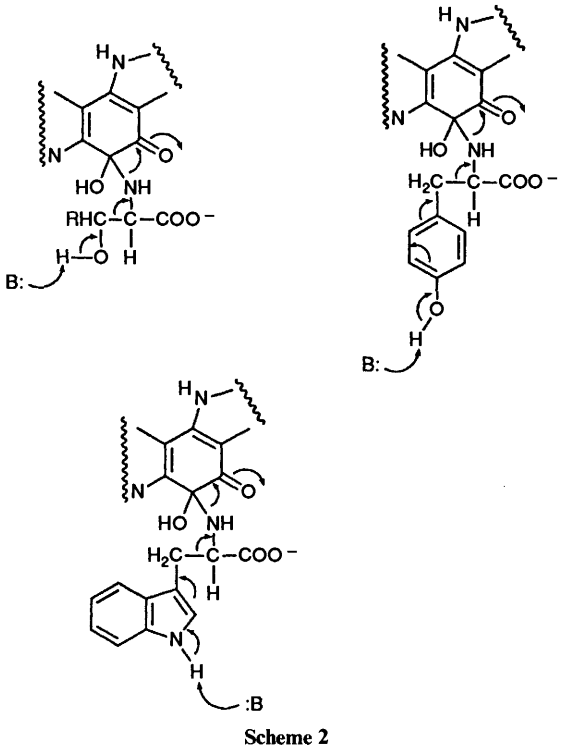
The oxidative dealdolation may also proceed through the carbinolamine adduct from which base-catalysed deprotonation from the hydroxy group at the β-position of the serine derivatives, from the phenolic hydroxy group of tyrosine or from the indole ring of tryptophan occurs to facilitate C_α–C_β bond cleavage as illustrated in Scheme 2, and these processes are suggested to be major pathways by the predominant formation of the quinol product (*vide ante*). As a minor process, dehydration proceeds from the carbinolamine intermediate to give the iminoquinone-type intermediate from which a similar type of C_α–C_β bond fission occurs to give the imine intermediate that is finally converted into the oxazole **3a** by intramolecular cyclization and aromatization.

During the course of our research, Duine and his co-workers reported the formation of oxazole **3a** 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.[25] They assumed that the main path of the reaction is the formation of the oxazole **3a** and that quinol is formed in the final aromatization step. However, the present results clearly indicate that the oxidative dealdolation of the substrate (formation of the quinol) is the major path and the deactivation of PQQ is the minor one.

PQQ used to be regarded as a coenzyme of dopa decarboxylase (EC 4.1.1.28), glutamic acid decarboxylase (EC 4.1.1.15) and tryptophan decarboxylase (EC 4.1.1.28).[33–35] Recent studies on these enzymes have, however, denied such possibilities.[36,37]



Scheme 1



Scheme 2

The present results do strongly suggest that PQQ plays an important role in amino acid metabolism somewhere in living systems.

Experimental

PQQ was prepared according to the reported method.[38] All amino acids were obtained commercially and used without further purification. Ultraviolet and visible absorption spectra were recorded on a Shimadzu UV-265 spectrophotometer equipped with a temperature-controlled cell holder, Shimadzu TCC-260. The pH values were determined on a Horiba pH meter F-8. Infrared spectra were recorded on a Hitachi 270–30 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL FT-NMR JNM-FX90Q (90 MHz) or a JEOL FT-NMR GSX-270 (270 MHz) spectrometer. *J* Values are given in Hz. Mass spectra were obtained on a JEOL JNX DX-303 HF mass spectrometer.

Kinetics and Product Analysis of the Anaerobic Reaction (General Procedure).—The kinetics for the reaction of PQQ and glycine were performed in 0.5 mol dm^{-3} carbonate buffer solution at 30 °C under anaerobic conditions. Typically, an aqueous buffer solution (1.5 cm^3) containing glycine (8.0 × 10^{-2} to 3.2 × 10^{-1} mol dm^{-3}) was mixed with an aqueous solution (1.5 cm^3) containing PQQ (8.0 × 10^{-5} mol dm^{-3}) in a Thunberg cuvette under anaerobic conditions. Both solutions were degassed by bubbling argon through them for 30 min prior to reaction. The progress of the reaction was followed by monitoring the appearance of the peak due to the reduced PQQ at around 320 nm.

The reaction mixture was acidified by adding degassed H_2SO_4 (6 mol dm^{-3}) under anaerobic conditions (in an AtmosBag) and was then subjected to HPLC analysis on a Waters Model 510 (pump), a Lambda-Max Model 481 (UV monitor) and a radial compression separation system (C_{18}). A degassed eluent (H_2O–MeOH–H_3PO_4, 54.5/45/0.5, v/v/v) was kept under an argon atmosphere during the HPLC analysis. Authentic samples of the quinol and the aminophenol derivatives of PQQ were prepared according to the reported methods,[31,32] and the factors of their peaks in HPLC analysis (at 300 nm) were determined in order to calculate the molar

ratio of the quinol to the aminophenol in the reaction mixture.

Product Analysis of the Aerobic Reaction (General Procedure).—An aqueous solution of PQQ (1.52×10^{-3} mol dm^{-3}), an amino acid or an amine (4.55×10^{-2} mol dm^{-3}) and CTAB (cetyltrimethylammonium bromide, 1.25×10^{-2} mol dm^{-3}), if necessary, in 0.5 mol dm^{-3} buffer (5.0 cm^3) was stirred at 30 °C under aerobic conditions, and the formation of PhCHO was followed by HPLC (Fig. 4, Fig. 6 and Table 2). Formation of the OPQ derivative was monitored by UV–VIS spectroscopy of diluted aliquots (40-fold) from the reaction mixture (Fig. 5). After 24 h, the reaction mixture was acidified by adding conc. HCl to pH 1–3 and was left standing for several days. The precipitate was collected by centrifugation, washed with diluted HCl and dried *in vacuo*. The yields are shown in Tables 1 and 3.

^1H NMR ([$^2H_6$]Me_2SO) **3a**: 7.32 (1 H, s, 3-H), 8.34 (1 H, s, 9-H), 9.31 (1 H, s, 5-H), 13.00 (1 H, br s, 1-NH); **3b**: 2.77 (3 H, s, $-CH_3$), 7.30 (1 H, s, 3-H), 8.04 (1 H, s, 9-H), 13.03 (1 H, br, 1-NH); **3c**: 7.38 (1 H, s, 3-H), 7.5–7.8 (5 H, m, Ph), 8.08 (1 H, s, 9-H), 14.15 (1 H, br, 1-NH); **3d**: 4.48 (2 H, s, $-CH_2-$), 7.32 (1 H, s, 3-H), 8.18 (1 H, s, 9-H), 13.4 (1 H, br, 1-NH); **3e**: 2.88 (2 H, t, $-CH_2-$, *J* 8.8), 3.32 (2 H, t, $-CH_2-$, *J* 8.8), 7.29 (1 H, s, 3-H), 8.04 (1 H, s, 9-H), 8.5 (1 H, br, –COOH), 13.9 (1 H, br, 1-NH); **3f**: 4.71 (2 H, s, $-CH_2-$), 7.29 (1 H, s, 3-H), 7.50 (1 H, s, imidazolyl-4-H), 8.12 (1 H, s, 9-H), 9.10 (1 H, s, imidazolyl-2-H), 13.2 (1 H, br, NH), 14.3 (1 H, br, NH); **3g**: 5.01 (2 H, s, $-CH_2-$), 7.31 (1 H, s, 3-H), 8.03 (1 H, s, 9-H), 13.3 (1 H, br, 1-NH); **3h**: 3.22 (3 H, s, $-OCH_3$), 5.03 (2 H, s, $-CH_2-$), 7.21 (1 H, s, 3-H), 7.88 (1 H, s, 9-H), 13.3 (1 H, br, 1-NH); **3i**: 5.47 (2 H, s, $-CH_2-$), 7.27 (1 H, s, 3-H), 8.17 (1 H, s, 9-H).

UV–VIS (λ_{max}/nm, in 0.5 mol dm^{-3} phosphate buffer, pH 7) **3a**: 251, 276, 422; **3b**: 253, 271, 414; **3c**: 209, 273, 422; **3d**: 254, 272, 417; **3e**: 254, 270(sh), 417; **3f**: 254, 270(sh), 419; **3g**: 253, 270(sh), 416; **3h**: 253, 270(sh), 414; **3i**: 254, 270(sh), 418.

Esterification of **3a**.—The oxazole derivative **3a** (5.0 mg) was treated with dimethyl sulfate (0.15 cm^3) and K_2CO_3 (50 mg) in DMF (2 cm^3) at room temperature for 8 h under N_2. Addition of water and acidification with conc. HCl to pH 2 afforded an orange solid which was collected by centrifugation, washed with water and dried *in vacuo*. From the supernatant, the product was also obtained by extraction with ethyl acetate and subsequent flash column chromatographic treatment (SiO_2, $CHCl_3$). In total, compound **4** was obtained in 85% yield: m.p. 250 °C (decomp.); ^1H NMR δ($CDCl_3$) 3.99 (3 H, s, $-COOCH_3$), 4.14 (3 H, s, $-COOCH_3$), 4.17 (3 H, s, $-COOCH_3$), 7.64 (1 H, d, *J* 2.2, 3-H), 8.49 (1 H, s, 9-H), 9.37 (1 H, s, 5-H), 12.81 (1 H, br s, 1-NH); ν(KBr)/cm^{-1} 3450 (NH), 1724 (ester C=O), 1662 (C=N), 1514 (C=C), 1262 and 1236 (C–O); λ_{max}(CH_3CN)/nm 257 (ε 27 800 dm^3 mol^{-1} cm^{-1}), 276 (21 400), 427 (15 800); *m*/*z* (EI) 383 (M^+) (Found: C, 56.0; H, 3.3; N, 10.8. Calc. for $C_{18}H_{13}N_3O_7$: C, 56.40; H, 3.42; N, 10.96%).

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References

1 Presented in part at the 8th International Symposium on Vitamin B_6 and Carbonyl Catalysis, Osaka, Japan, 15–19 October, 1990.
2 J. A. Duine, R. A. van der Meer and B. W. Groen, *Annu. Rev. Nutr.*, 1990, **10**, 297.
3 M. Ameyama, E. Shinagawa, K. Matsushita and O. Adachi, *Agric. Biol. Chem.*, 1984, **48**, 3099.
4 A. Watanabe, N. Hobara and T. Tsuji, *Curr. Ther. Res.*, 1988, **44**, 896.
5 H. Nishigori, M. Yasunaga, M. Mizumura, J. W. Lee and M. Iwatsuru, *Life Sci.*, 1989, **45**, 593.
6 Y. Hamagishi, S. Murata, H. Kamei, T. Oki, O. Adachi and M. Ameyama, *J. Pharm. Exp. Ther.*, 1990, **255**, 980.
7 J. Killgore, C. Smidt, L. Duich, N. Romero-Chapman, D. Tinker, K. Reiser, M. Melko, D. Hyde and R. B. Rucker, *Science*, 1989, **245**, 850.
8 M. Katsumata, Y. Osawa, T. Nakagiri and S. Uchikuga, Jap. P 63-156724/1988 (*Chem. Abstr.*, 109, **20**, 176333u); M. Katsumata, Y. Osawa, T. Nakagiri and S. Uchikuga, Jap. P 63-48215/1988 (*Chem. Abstr.*, 110, **10**, 82492p); Y. Narutomi, M. Katsumata, Y. Osawa and S. Uchikuga, EP 262345/1988 (*Chem. Abstr.*, 109, **25**, 222458p); Y. Maruyama, A. Iwayama and S. Ohida, Jap. P 63-174931/1988 (*Chem. Abstr.*, 109, **25**, 222472p); M. Watanabe, N. Yoshihara and A. Iwayama, Jap. P 63-192717/1988 (*Chem. Abstr.*, 110, **9**, 69400n); S. Iwata, Jap. P 01-180827/1989 (*Chem. Abstr.*, 112, **19**, 172324f); A. Nakamura, Y. Inoue and S. Uragami, Jap. P 01-283221/1989 (*Chem. Abstr.*, 113, **7**, 52500j); Y. Matsutani, T. Sugawara, H. Kurobe, J. Takahashi, Y. Moriguchi and T. Endo, Jap. P 02-196720/1990 (*Chem. Abstr.*, 114 **3**, 17585q); M. Nakano, T. Kurashige, S. Uragami and C. Ito, Jap. P 02-268118/1990 (*Chem. Abstr.*, 114, **19**, 178386r); H. M. Kagan and S. N. Gacheru, USP 4997854/1991 (*Chem. Abstr.*, 114, **19**, 178404v).
9 K. Sugiyama, K. Takada and I. Yamamoto, EP 256472/1988 (*Chem. Abstr.*, 109, **18**, 155967p); K. Imada, K. Abe and T. Yamamoto, Jap. P 01-90115/1989 (*Chem. Abstr.*, 111, **18**, 159987j).
10 A. Murai and M. Yamamoto, EP 254319/1988 (*Chem. Abstr.*, 108, **23**, 203554e); M. Nakano, K. Sugioka, Y. Shoji and S. Uragami, Jap. P 01-126999/1989 (*Chem. Abstr.*, 113, **1**, 2559q).
11 Y. Ohshiro, S. Itoh, K. Kurokawa, J. Kato, T. Hirao and T. Agawa, *Tetrahedron Lett.*, 1983, **24**, 3465; S. Itoh, Y. Kitamura, Y. Ohshiro and T. Agawa, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 1907; M. Mure, S. Itoh and Y. Oshiro, *Chem. Lett.*, 1989, 1491; Y. Ohshiro and S. Itoh, *Bioorg. Chem.*, 1991, **19**, 169.
12 S. Itoh, N. Kato, Y. Ohshiro and T. Agawa, *Tetrahedron Lett.*, 1984, **25**, 4753; M. Mure, A. Suzuki, S. Itoh and Y. Ohshiro, *J. Chem. Soc., Chem. Commun.*, 1990, 1608.
13 M. Mure, K. Nii, T. Inoue, S. Itoh and Y. Ohshiro, *J. Chem. Soc., Perkin Trans. 2*, 1990, 315.
14 M. Mure, K. Nii, S. Itoh and Y. Ohshiro, *Bull. Chem. Soc. Jpn.*, 1990, **63**, 417.
15 S. Itoh, N. Kato, Y. Ohshiro and T. Agawa, *Chem. Lett.*, 1985, 135; S. Itoh, N. Kato, M. Mure and Y. Ohshiro, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 420.
16 S. Itoh, M. Mure, Y. Ohshiro and T. Agawa, *Tetrahedron Lett.*, 1985, **26**, 4225.
17 S. Itoh, K. Nii, M. Mure and Y. Ohshiro, *Tetrahedron Lett.*, 1987, **28**, 3975.
18 S. Itoh, M. Mure and Y. Ohshiro, *J. Chem. Soc., Chem. Commun.*, 1987, 1580.
19 S. Itoh, M. Kinugawa, N. Mita and Y. Ohshiro, *J. Chem. Soc., Chem. Commun.*, 1989, 694; S. Itoh, N. Mita and Y. Ohshiro, *Chem. Lett.*, 1990, 1949.
20 T. Hirao, T. Murakami, M. Ohno and Y. Ohshiro, *Chem. Lett.*, 1989, 785; T. Hirao, M. Ohno and Y. Ohshiro, *Tetrahedron Lett.*, 1990, **31**, 6039; T. Hirao, T. Murakami, M. Ohno and Y. Ohshiro, *Chem. Lett.*, 1991, 299.
21 M. A. Paz, R. Flückiger, A. Boak, H. M. Kagan, and P. M. Gallop, *J. Biol. Chem.*, 1991, **266**, 689.
22 P. R. Sleath, J. B. Noar, G. A. Eberlein and T. C. Bruice, *J. Am. Chem. Soc.*, 1985, **107**, 3328.
23 O. Adachi, K. Okamoto, E. Shinagawa, K. Matsushita and M. Ameyama, *BioFactors*, 1988, **1**, 251.
24 T. Ishida, M. Doi, K. Tomita, H. Hayashi, M. Inoue and T. Urakami, *J. Am. Chem. Soc.*, 1989, **111**, 6822.
25 M. A. G. van Kleef, J. A. Jongejan and J. A. Duine, *Eur. J. Biochem.*, 1989, **183**, 41.
26 For preliminary reports, see reference 12.
27 J. A. Duine, J. Frank, Jzn and P. E. J. Verwiel, *Eur. J. Biochem.*, 1980, **108**, 187.
28 J. A. Duine, J. Frank, Jzn and J. A. Jongejan, *Adv. Enzymol.*, 1987, **59**, 169.
29 A. E. Martell, *Acc. Chem. Res.*, 1989, **22**, 115.
30 E. J. Rodriguez and T. C. Bruice, *J. Am. Chem. Soc.*, 1989, **111**, 7947.
31 M. Mure, S. Itoh and Y. Ohshiro, *Tetrahedron Lett.*, 1989, **30**, 6875; S. Itoh, M. Mure, M. Ogino and Y. Ohshiro, *J. Org. Chem.*, 1991, **56**, 6857.
32 S. Itoh, Y. Ohshiro and T. Agawa, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 1911.

33 B. W. Groen, R. A. van der Meer and J. A. Duine, *FEBS Lett.*, 1988, **237**, 98.
34 R. A. van der Meer, B. W. Groen and J. A. Duine, *FEBS Lett.*, 1989, **246**, 109.
35 J. M. Penning, B. W. Goren, J. A. Duine and R. Verpoorte, *FEBS Lett.*, 1989, **255**, 97.
36 T. Takagishi, T. Tanaka, I. Imamura, H. Mizuguchi, M. Kuroda, H. Fukui and H. Wada, Proceeding of the 8th International Symposium on Vitamin B_6 and Carbonyl Catalysis, ed. T. Fukui, Pergamon, Oxford, UK, p. 487–489, 1991.
37 R. A. John, D. De Biase and B. Maras, Proceding of the 8th International Symposium on Vitamin B_6 and Carbonyl Catalysis, ed. T. Fukui, Pergamon, Oxford, UK, p. 483–485, 1991.
38 E. J. Corey and A. Tramontano, *J. Am. Chem. Soc.*, 1981, **103**, 5599.

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