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REVIEW

Mechanism of Amine Oxidation by Coenzyme PQQ

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Chemical behavior of coenzyme PQQ and its analogues toward several amines is reviewed. Product analyses and kinetic studies so far reported indicate that the oxidation of primary amines by pyrroloquinoline quinone derivatives proceeds via the ionic mechanism through the *carbinolamine* intermediate from which two competing reaction pathways, direct α-proton abstraction and transamination, occur to give the quinol and the aminophenol products, respectively. The similar ionic mechanism through the *carbinolamine-type* intermediate is proposed for the reactions between PQQ and α,ω-diaminoalkanes, hydrazines, and aminoguanidine, where the redox reaction (quinol formation) and adduct formation reactions are controlled by alkylene chain length, electronic nature of the substituents, and pH conditions of the solution, respectively. PQQ catalyzes *oxidative decarboxylation* and *oxidative dealdolation* (C_α–C_β bond fission) of α-amino acids, which can be also interpreted by the similar ionic mechanism.

INTRODUCTION

Since PQQ (4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, methoxatin, **1**) was first reported as the second organic cofactor of mammalian copper-containing amine oxidases (*1, 2*), several research groups have demonstrated that PQQ or a closely similar compound is possibly contained as the cofactor in various amine oxidases (*3–10*), bacterial amine dehydrogenases (*11, 12*), and methylamine oxidase (*13*). Enzymatic studies on the action of these enzymes have been also carried out to support such a possibility of being quinoproteins (*14–22*). Therefore, the long history of arguments on the coenzyme structure of the amine-oxidizing enzymes seemed to be brought to an end. But very recently, Vellieux *et al.* and Janes *et al.* independently reported new redox cofactors, pro-PQQ (6-hydroxy-4,5-indolequinone derivative **2**) and topaquinone (**3**), for methylamine dehydrogenase and bovine serum amine oxidase, respectively (*23–25*), giving rise to the similar argument again. Despite of such a discrepancy, the reactions between PQQ and amines are still attracting much attention, because they are involved in several biologically important processes (*26*). For example, nutritional importance of PQQ has been attributed to its functional effect on the crosslinking of collagen and elastin in connective tissue biogenesis and on the regulation of intracellular spermine and spermidine levels (*27*). From these points

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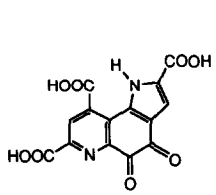

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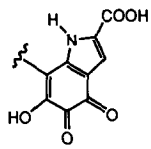

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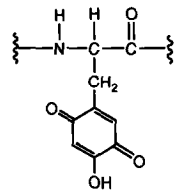

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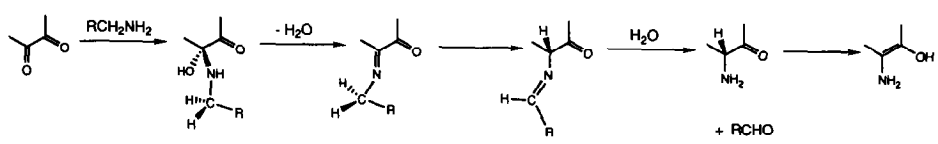
of view, it must be worth while to review the chemical behavior of coenzyme PQQ toward several amine substrates.

Because of the lack of structural information on the coenzyme, amine oxidation mechanism in enzymatic systems had not been clearly demonstrated, though some of the earliest investigations focused on the role of pyridoxal phosphate (*28*) and a ring-modified flavin (*29*). Abeles *et al.* demonstrated the mechanism of C–H bond activation for plasma amine oxidases by using mechanism-based inhibitors and provided probes of the active-site structure (*30*). During the last few years, Klinman *et al.* investigated the enzymatic mechanism of bovine plasma amino oxidase by taking account of the *o*-quinone structure of the coenzyme, and proposed a transamination mechanism involving a Schiff base formation between one of the quinone carbonyl and amines and the following 1,3-prototrophic shift to give the intermediate **4** (Scheme 1). In order to explain the result that the imine hydrolysis is not involved at all in the rate-determining step, they also proposed that enolization within the imine intermediate is a relatively slow process, occurring only after imine hydrolysis to produce aldehyde and the aminophenol product of the cofactor (*31*). Kagan *et al.* presented the similar transamination mechanism for lysyl oxidase, where formation of the carbanion intermediate **5** was detected by using nitromethane as a trapping reagent (Scheme 2) (*15, 16*).

In nonenzymatic systems, oxidation of amines by quinonoid compounds has been well demonstrated to proceed via electron-transfer mechanism within a charge-transfer complex with high oxidation potential quinones and also via transamination mechanism through imine intermediates with quinones of moderate oxidation potentials (*32, 33*). Thus, it is very curious to know which category can be applied to the amine-oxidation by PQQ, or whether any other type of mechanism exists or not. In this review, we would like to present several nonenzymatic reactions between coenzyme PQQ and amines and discuss their mechanism.



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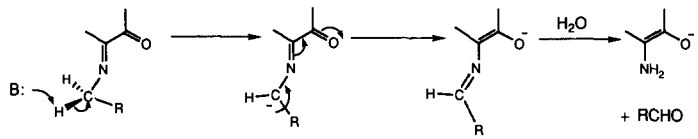
SCHEME 1



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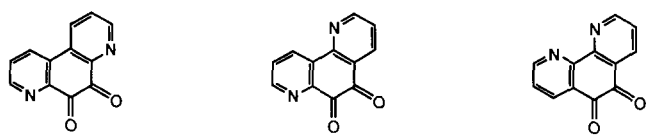
SCHEME 2

OXIDATION OF AMINES BY COENZYME PQQ

About a decade ago, soon after the finding of the new coenzyme, model studies on the amine-oxidation by PQQ was started by Bruice *et al.* using phenanthrolinequiones, **6–8** (*34, 35*). The oxidation of primary amines such as cyclohexylamine and glycine by those quinones proceeds smoothly to provide 6-amino-5-hydroxyphenanthrolines, so-called aminophenol, as a sole isolable reduced product, and the order of reactivity is **6** > **7** > **8** in spite of their almost identical redox potentials. On the other hand, the reactivities of secondary and tertiary amines are relatively low. These findings and the observations of general base catalysis by the amine substrate itself and of consecutive first-order kinetics suggest that the oxidation of primary amines proceeds via the transamination mechanism through the imine intermediate (*35*). The similar mechanism has been well established also in the case of 3,5-di-*tert*-butyl-1,2-benzoquinone as mentioned above (*33, 36–38*).

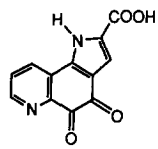


6 **7** **8**

A little latter, we reported the PQQ-catalyzed oxidation of amines under aerobic conditions, so-called aerobic autorecycling oxidation of amines, by applying a cationic micellar system (*39*), where amine substrates are efficiently converted into the corresponding carbonyl compounds (Eq. [1]). This is the first example of the PQQ-catalyzed oxidation reaction of amines, suggesting its possible contribution to amine-metabolism in living systems. Investigation on the role of the micelle was also carried out by using several surfactants and aliphatic amines to indicate that the reaction proceeds in the electrostatic layer of the micelle (*40, 41*). Namely, PQQ is bound onto the micellar surface via electrostatic interaction between carboxylate groups of PQQ and the charged head group of the micelle. On the other hand, an apolar alkyl group of the substrate is inserted into the hydrophobic core and the polar amino group is located into the interfacial layer of the system to approach to the quinone moiety of PQQ.

$$RR'CHNH_2 + O_2 + H_2O \xrightarrow[\text{pH 7, 30°C}]{\text{PQQ (1 mol\%), CTAB}} RR'C{=}O + NH_3 + H_2O_2 \qquad [1]$$

The model studies were further extended by using 7,9-didecarboxymethoxatin **9** to demonstrate that it is capable of the oxidation of primary amines such as ethylamine, glycine, benzylamine, and glycinamide, while secondary amines (e.g., morpholine) and tertiary amines (e.g., *N,N*-dimethylbenzylamine) are not oxidized (*42*). Interestingly, the oxidation of the primary amines by **9** under anaerobic conditions converts the latter to the corresponding quinol with some formation of the aminophenol product. It is assumed that these products arise via two competing covalent addition base-catalyzed elimination mechanism through the carbinolamine and the imine intermediates, respectively (Scheme 3). The similar results were obtained in the oxidation of amines by PQQ itself in the micellar system (*43*). Detailed kinetic analysis has been carried out by using 9-decarboxymethoxatin **10** to indicate that the product ratio of the quinol and the aminophenol is controlled by pH conditions of the solution (*44*).



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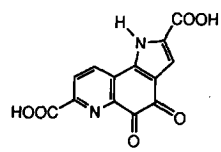

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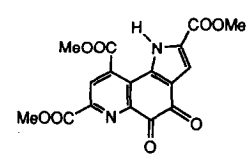

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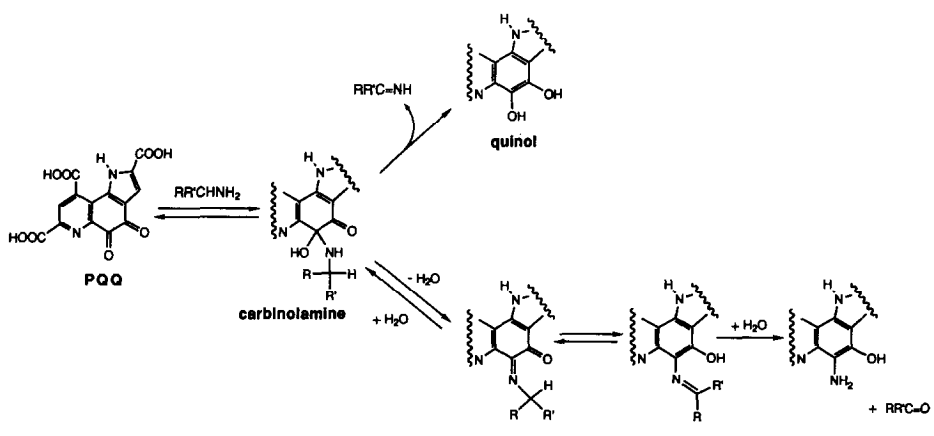
However, there are some limitations in the studies in aqueous solutions. First, the reduced species of the quinol and the aminophenol are easily reoxidized by molecular oxygen in aqueous media (*45*), and the aminophenol is converted into



SCHEME 3

the quinol in the presence of a small amount of the quinone even under anaerobic conditions (autocatalytic conversion of the aminophenol into the quinol) (*44*). These features of the reduced PQQ make the quantitative product analysis of PQQ very difficult. Second, the quinone moiety of PQQ is well demonstrated to be hydrated in relatively higher extent even in a neutral to weakly alkaline aqueous solution (*46*), which also makes the kinetic studies more complicated (*44*). Third, the imine intermediates are not so stable in aqueous media that we could not isolate and characterize those intermediates in such systems. These problems can be circumvented by using PQQTME (trimethyl ester of PQQ, **11**) in organic media as follows.

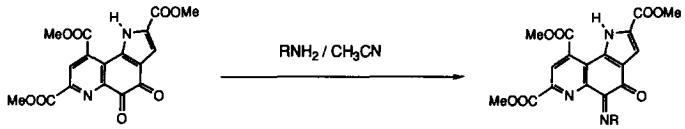
The important iminoquinone derivatives **12** and **13** can be easily isolated and characterized in the reaction of **11** with ammonia and *tert*-butylamine, respectively (Eq. [2]). The corresponding aminophenol $\mathbf{12H_2}$ can be also prepared by the reduction of **12** with methylhydrazine (Eq. [3]) (*47*). The reduced species $\mathbf{11H_2}$ and $\mathbf{12H_2}$ are stable enough in organic media to perform the product analysis in more detail. For example, product ratio of $\mathbf{11H_2}$ to $\mathbf{12H_2}$ has been determined to be about 7:3 in the reaction of **11** (2.0 mM) and benzylamine (20 mM) in CH_3CN under anaerobic conditions (Eq. [4]) (*48*). More interestingly, alkylaminophenol **14** is produced together with $\mathbf{11H_2}$ and $\mathbf{12H_2}$ in the reaction with *n*-propylamine under the same conditions, where the product ratio of these products is quite different depending upon the amine concentration (Eq. [5] and Table 1). In the case of *N*-methylpropylamine, only $\mathbf{11H_2}$ is isolated but its reactivity is relatively low as expected (Table 1). Cyclopropylamine, on the other hand, gives the iminoquinone **15** as a single product (Eq. [6]). However, no redox reaction occurs in the case of tertiary amines such as triethylamine. Under aerobic conditions, catalytic oxidation of benzylamine proceeds quantitatively to give *N*-benzylidenbenzylamine ($PhCH{=}N{-}CH_2Ph$; a condensation product of $PhCH{=}NH$ and $PhCH_2NH_2$). The catalytic activity of **11** is much higher than that of PQQ itself in the aqueous reaction. In such a catalytic reaction, **11** is gradually converted into the redox inactive oxazole **16** as in the case of the aqueous reaction (*42*).

TABLE 1

Products of the Reaction between **11** and Propylamine Derivatives

Amine (mM)	Time (h)	Total yield (%)	Ratio (%)		
			$\mathbf{11H_2}$	$\mathbf{12H_2}$	**14**
n-Propylamine					
(3.7)	2	94	88	12	0
(5.1)	2	98	69	31	0
(20)	2	100	53	33	14
(100)	2	97	0	7	93
(200)	2	88	0	0	100
N-Methylpropylamine					
(200)	24	100	100	0	0

Note. [**1**] = 2.0 mM, in CH_3CN at room temperature under Ar.



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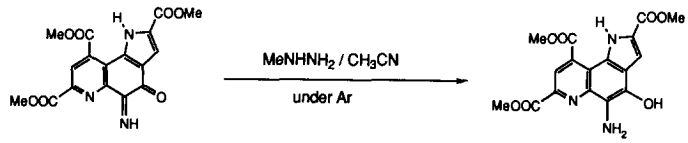
12 : R = H 94%
13 : R = t-Bu 84%

[2]



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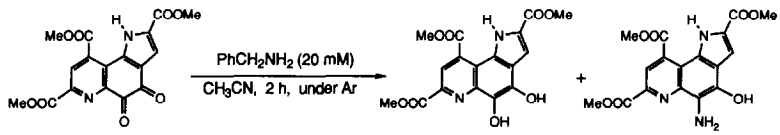
12H2 63 %

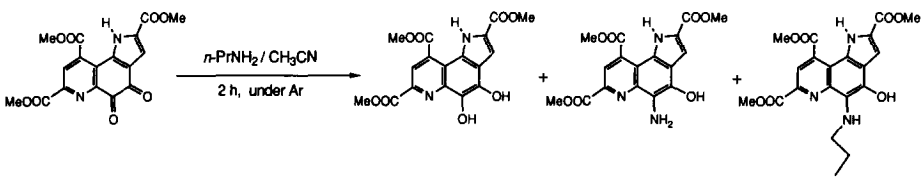
[3]



11 (2.0 mM)

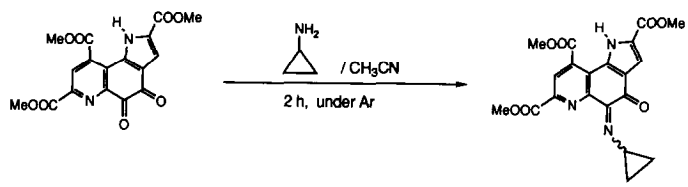
11H2 : 12H2 (7 : 3)

[4]



11 11H2 12H2 14

[5]



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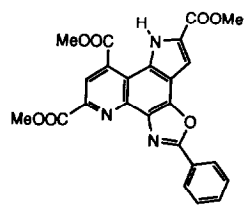
15 quant.

[6]



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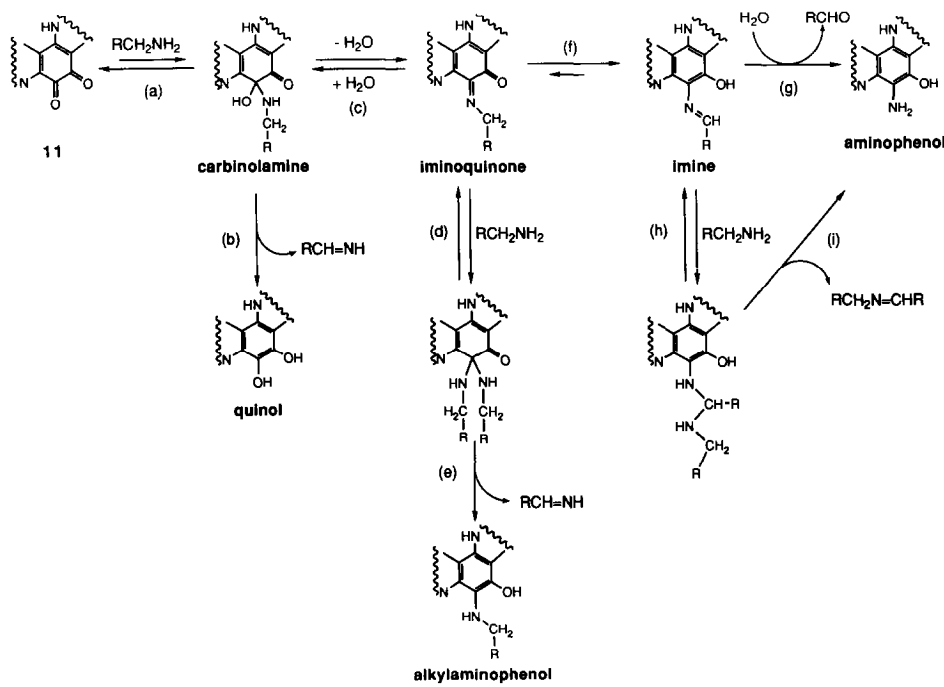
These results can be interpreted by the ionic mechanism shown in Scheme 4. In the reaction of **11** with *n*-propylamine, concentration of the amine substrate alters the reduced products. At the lower concentration of the amine, direct α-proton removal (addition–elimination) mainly occurs to give the quinol (path b). On the other hand, when a relatively large amount of the amine exists, equilibrium of the step c goes right to accumulate the iminoquinone intermediate from which the propylaminophenol product **14** is derived via the similar type of addition–elimination mechanism (paths d and e). As a minor process, rearrangement of the iminoquinone intermediate occurs to give the imine intermediate (path f) which is finally converted into the aminophenol $\mathbf{12H_2}$ by hydrolysis and/or imine exchange reaction (path g and/or paths h and i). In the case of *N*-methylpropylamine, only the addition–elimination path (paths a and b) is allowed to afford the quinol $\mathbf{11H_2}$, but the reactivity is considerably low because of its steric hindrance at the addition step.

The oxidation of benzylamine may proceed in the similar manner. However, in this case, the quinol $\mathbf{11H_2}$ is the major product even in the presence of a large excess of the amine, and the alkylaminophenol-type product is not obtained at all. These results might be attributed to the difference of acidity of the α-proton of the substrates. Namely, in the case of benzylamine, because of higher acidity of the α-proton, the direct α-proton removal (path b) and the rearrangement of the iminoquinone to the imine (path f) proceed efficiently to give the quinol and the aminophenol, respectively, thereby the attack of the second amine to the



SCHEME 4

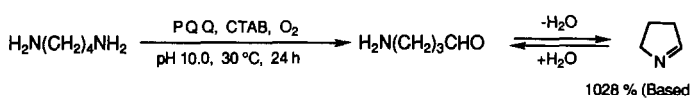
iminoquinone intermediate is prevented. An opposite effect, lower acidity of the α-proton, may be responsible to the different behavior of cyclopropylamine (*49*). In this case, dehydration from the carbinolamine intermediate (formation of **15**) predominates over the direct α-proton removal, but the following rearrangement (path f) is suppressed. Addition of the second amine to the iminoquinone intermediate is also prevented by the steric hindrance of cyclopropyl group existed around the C-5 position. Therefore the iminoquinone **15** is obtained as a single product. Under aerobic conditions, the quinol $\mathbf{11H_2}$ and the aminophenol $\mathbf{12H_2}$ are easily reoxidized by molecular oxygen to regenerate **11** and **12**, respectively, constructing an efficient catalytic system. The oxazole **16** is formed gradually by the intramolecular cyclization from the imine intermediate and the following aromatization during the course of the catalytic cycles.

Formation of the quinol could be interpreted by electron-transfer mechanism as is considered in the reaction between quinones of high oxidation potentials such as DDQ or chloranil and amines (*32*). But the lower reactivities of the secondary and tertiary amines may rule out such a possibility. As Bruice and his co-workers have already stated (*35*), order of reactivity of amines is tertiary > secondary > primary in the oxidation reaction via electron-transfer mechanism. Furthermore, the cyclopropyl ring remains intact in the reaction of **11** with cyclopropylamine (Eq. [6]). If the reaction of **11** and amines proceeded via electron-transfer mechanism, a ring opening product should be formed. It is well demonstrated that cyclopropylamine radical cation produced by one-electron oxidation readily gives a ring-opening product (*49*).

REACTION OF COENZYME PQQ WITH DIAMINES

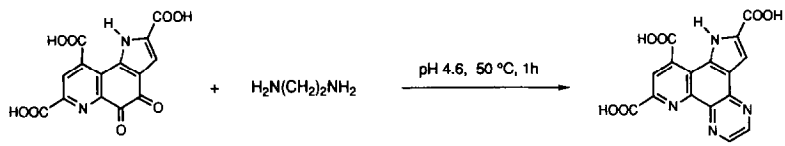
α,ω-Diaminoalkanes are known to be specific substrates and/or irreversible inhibitors of diamine oxidase and of lysyl oxidase depending upon the alkylene chain length (*50, 51*). These enzymes have been also suspected to be quinoproteins due to the resemblance to the other copper-containing amine oxidases (*4, 5, 7, 17*), but details of the inhibition mechanism has not been known for a long time because of the lack of structural information on the coenzyme.

Model studies on the reaction between PQQ and several α,ω-diaminoalkanes provided a possible mechanism of such an inhibition (*20, 52*). Spectrophotometric examination of the anaerobic reaction with the diamines having 4 to 10 methylene carbons indicates that only the redox reaction occurs under alkaline conditions (quinol, 320 nm; aminophenol, 302 nm). So it can be said that these diamines having longer alkylene chain behave as substrates. As in fact, treatment of 1,4-diaminobutane with a catalytic amount of PQQ (1 mol %) in the CTAB micellar system gives the oxidative deamination product, 4-aminobutylaldehyde, in 1028% yield based on PQQ (Eq [7]).



[7]

On the other hand, PQQ shows a different behavior toward ethylenediamine. At pH 4.7, a slow increase in the absorption at 286 nm is observed and the final spectrum is quite different from that of the reduced species of PQQ (Fig. 1). Product analysis of the reaction in a preparative scale indicates that this one having λ_{max} at 286 nm is a pyrazine derivative **17** (Eq. [8]). Also in this case, the structure of the product can be further confirmed by performing the reaction using PQQTME (**11**) in an organic medium (*52*). At higher pH conditions (pH 7–10), both the adduct formation and the redox reaction competitively proceed to give a mixture of the reduced PQQ and the pyrazine derivative.



17 : λmax = 286 nm

[8]

Consequently, the reaction pathway is altered by changing the alkylene chain length of the substrates. If the alkylene chain length is longer than four, the redox reaction proceeds predominantly to produce the reduced products of PQQ. But in the case of 1,2-diaminoalkanes, the second amino group is close enough in the C-5 adduct intermediate to attack the C-4 position, providing the cyclic adduct. These results are in good accordance with an inhibitory action of diamines in enzymatic systems (*20*) and must be a good evidence for the existence of the C-5 carbinolamine and/or the iminoquinone intermediates in the amine-oxidation by PQQ (Scheme 5). The similar adduct of PQQ with 2,3-diaminonaphthalene has been used as a chromophore in a PQQ-detection method (*53*).

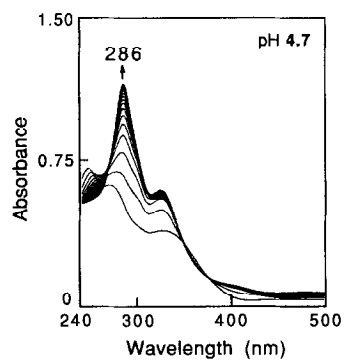
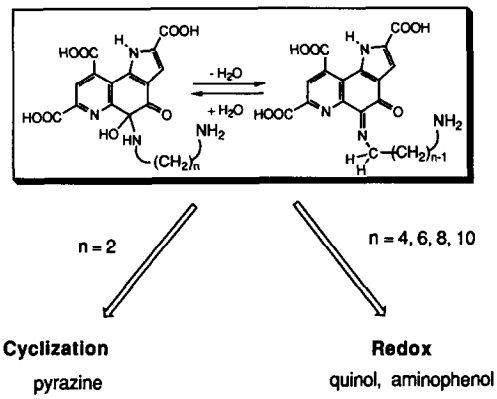


FIG. 1. Spectral change along the progress of the reaction of PQQ (4.0×10^{-5} M) with ethylenediamine (4.0×10^{-2} M) in the presence of CTAB (2.0×10^{-3} M) under anaerobic conditions at 35°C and pH 4.7.



SCHEME 5

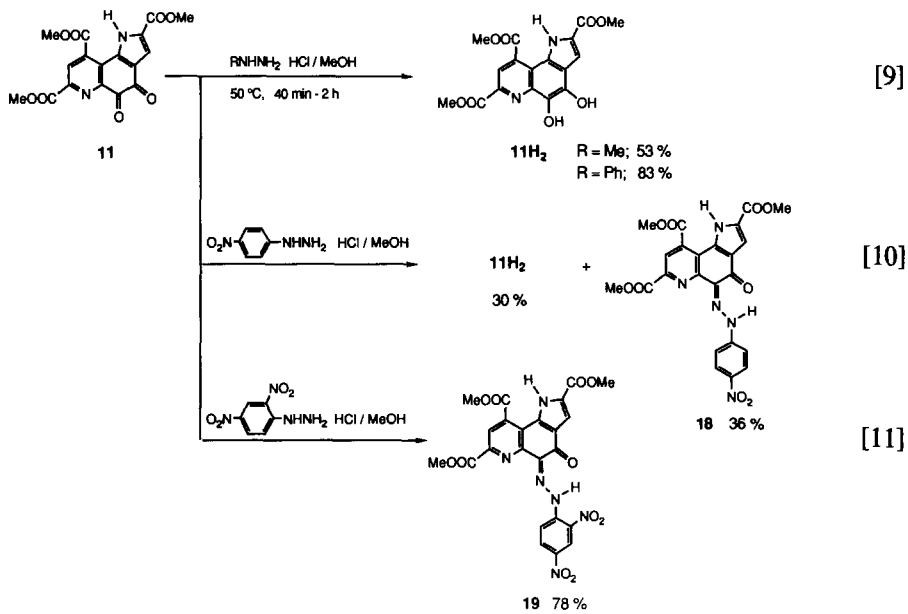
REACTIONS OF COENZYME PQQ WITH HYDRAZINES AND AMINOGUANIDINE

One of the most pronounced character of the second organic cofactor of copper-containing amine oxidases is high reactivity toward carbonyl reagents such as phenylhydrazine derivatives and conversion into a chromophore having a strong absorption at around 450 nm (*54*). This tendency has been applied to development of several detection methods of the covalently bound coenzyme (*1, 3, 4, 5, 6, 9, 11, 13, 25*). Although there is a discrepancy in the results of these methods, the reaction between PQQ and hydrazine derivatives is very intriguing not only from the viewpoint of the quinoprotein inhibition but also in connection with the amine-oxidation mechanism.

The first example of a nonenzymatic reaction between PQQ and phenylhydrazine was reported by Duine *et al.*, where PQQ is readily reduced to the quinol under anaerobic conditions (*55*). On the other hand, PQQ is converted into the C-5 hydrazone derivative in the reaction with 2,4-dinitrophenylhydrazine under acidic conditions (*1, 56*). It is noteworthy that both hydrazine derivatives convert the organic cofactor into the corresponding hydrazone derivatives in enzymatic systems (*57*). The addition–elimination mechanism has been suspected in the reaction of phenanthrolinequinones **6–8** and 7,9-didecarboxymethoxatin **9** with hydrazine itself, but there has been little evidence reported (*35, 42*). The following results shed light on the mechanism in more detail (*58*).

Kinetic studies on the redox reaction (quinol formation) of PQQ and hydrazines reveal that the order of reactivity is phenylhydrazine ~ methylhydrazine > *N,N'*-dimethylhydrazine ≫ *N,N*-dimethylhydrazine, which does not correlate with two-electron redox potentials of the hydrazines. Examination of the reactivity of some PQQ model compounds (*59, 60*) indicates that it is somewhat related to sensitivity of the quinone functional group toward nucleophilic addition such as hydration but does not reflect two-electron redox potentials of the model compounds. More-

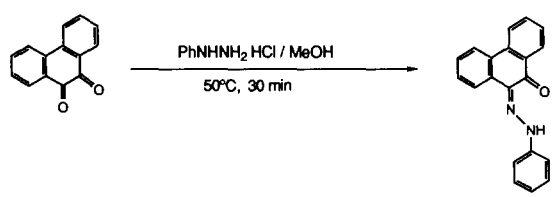
over, in the reaction of PQQTME (**11**) with phenylhydrazine and methylhydrazine in methanol, the quinol $\mathbf{11H_2}$ is obtained as a major product (Eq. [9]), while introduction of a strong electron-withdrawing group such as nitro group into the phenyl ring of the substrates alters the reaction product. Namely, almost 1 : 1 mixture of the quinol and the C-5 hydrazone derivatives **18** is obtained in the reaction with 4-nitrophenylhydrazine (Eq. [10]), and the C-5 hydrazone **19** becomes the main product in the case of 2,4-dinitrophenylhydrazine (Eq. [11]). In the case of a simple *o*-quinone such as phenanthrenequinone, on the other hand, only the hydrazone formation occurs even in the reaction with phenylhydrazine under the same conditions (Eq. [12]).



[9]

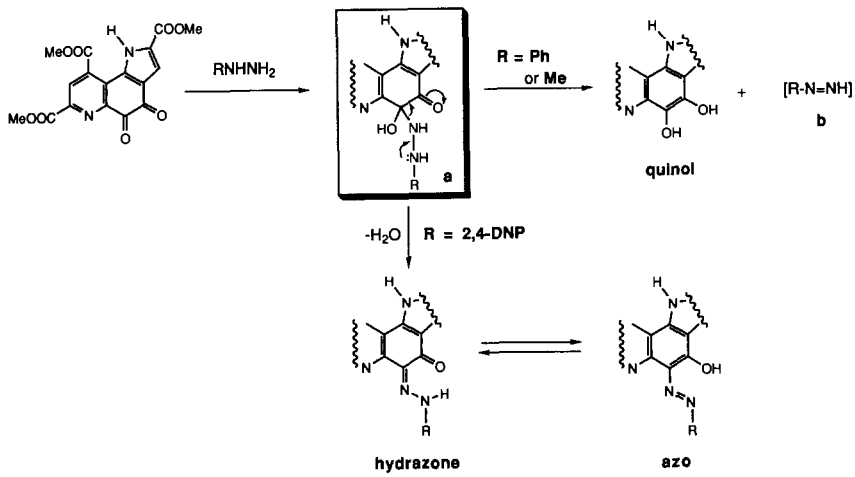
[10]

[11]



[12]

These results indicate that the reduction of PQQ proceeds via formation of the carbinolamine-type intermediate **a** which is followed by electron flow from the nitrogen of hydrazines into the quinone moiety of PQQ as shown in Scheme 6. If such electron flow is not fast enough, dehydration from the intermediate **a** predominantly proceeds to give the C-5 hydrazone or azo adduct. Electron-withdrawing substituents would retard such electron flow as in the case of 4-nitrophenylhydrazine and 2,4-dinitrophenylhydrazine to give the corresponding adducts,



SCHEME 6

while electron-donating substituents such as methyl and phenyl facilitate the reduction of PQQ. As in fact, both the redox reaction and the adduct formation are observed in the case of NH_2NH_2 (*42, 58*). It can be said that the pyrroloquinoline quinone structure of PQQ is favorable for the redox reaction because only hydrazone formation occurs in the case of phenanthrenequinone. The electron-withdrawing effect of the pyridine nucleus may help such electron flow in the stage of the intermediate **a**. A relatively small reactivity of MeNHNHMe compared to $MeNHNH_2$ may be due to the steric hindrance for the initial formation of the carbinolamine-type intermediate. The slow rate of the reduction by Me_2NNH_2 might be explained by the fact that the formation of unstable oxidation product **b** ($Me_2N^+=NH$) is less favored. The formation of reduced PQQ could not occur from the hydrazone since the hydrazone is stable enough and is not reduced in the presence of excess hydrazine.

So far, there have been a lot of spectroscopic studies using ir, uv–vis, and NMR to show that these kind of adducts between quinones and hydrazines are in azo–hydrazone tautomerism. The equilibrium between azo and hydrazone forms depends on several factors such as solvents, substituents, ring size, etc. (*61*). Thus, tautomerism of azo–hydrazone in the case of PQQ and phenylhydrazine derivatives seems to need further investigation.

Aminoguanidine is also known as an irreversible inhibitor of amine oxidases (*62*), but details of its inhibitory action in enzymatic system have not been clarifed either. Examination of the reaction between PQQ and aminoguanidine indicates that the redox reaction predominantly proceeds at pH 10.0 to give $PQQH_2$ (quinol), whereas deactivation of PQQ occurs at pH 6.7 to give a product having λ_{max} at 286 nm (Figs. 2a and 2b). Also in this case, product analysis using PQQTME (**11**) is very informative and indicates that this product is an aminotriazine derivative **20** (Eq. [13]) (*63*).

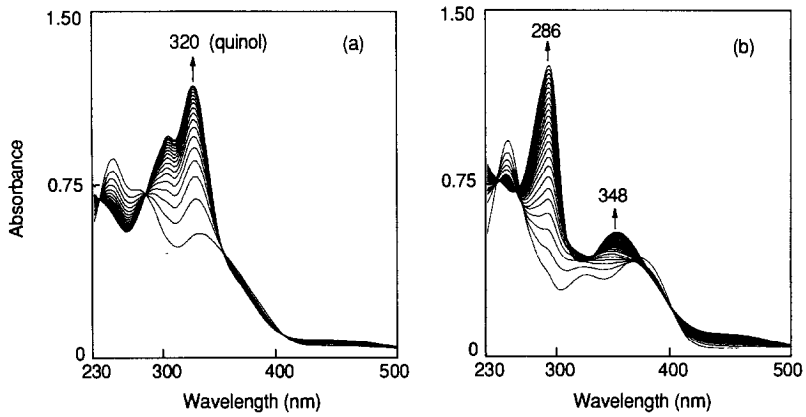
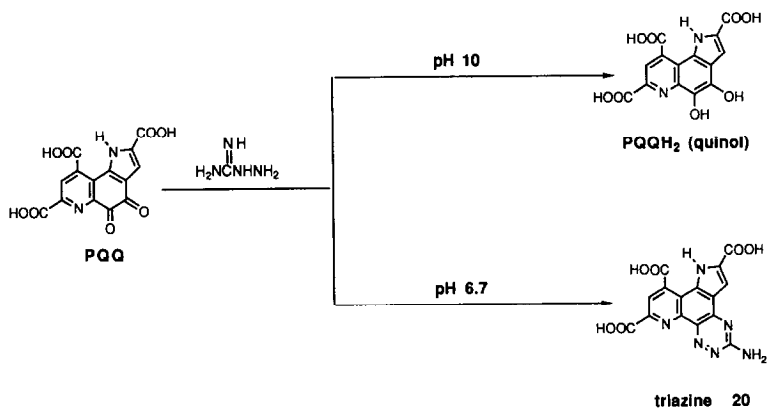


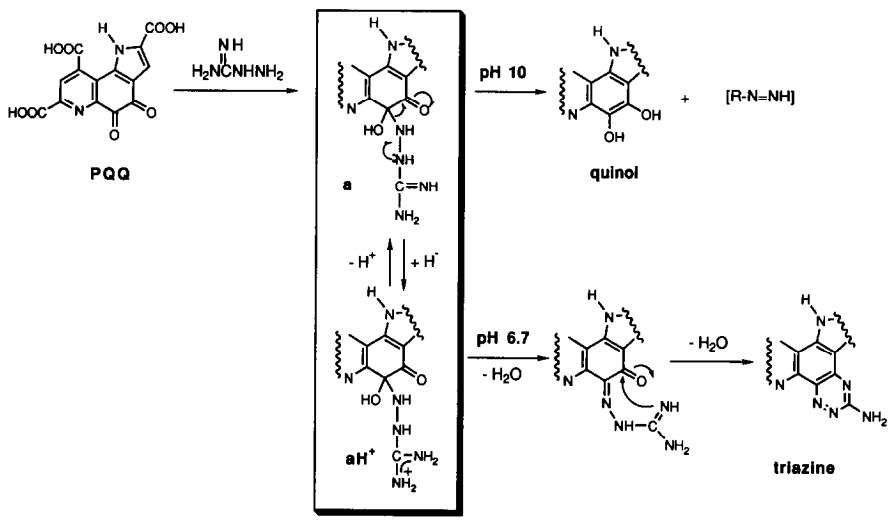
FIG. 2. Spectral change along the progress of the reaction between PQQ (4.0×10^{-5} M) and aminoguanidine (8.0×10^{-2} M) at 30°C under anaerobic conditions: (a) at pH 10.0 in 0.1 M carbonate buffer (μ = 0.2 with KCl); (b) at pH 6.7 in 0.1 M phosphate buffer (μ = 0.2 with KCl).



[13]

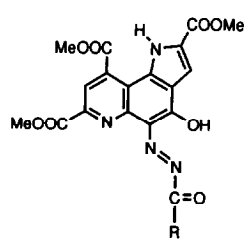
From these results, we have proposed the mechanism shown in Scheme 7. The amino group of aminoguanidine attacks the C-5 position of PQQ to form the carbinolamine-type intermediate **a** as in the case of amines and hydrazines. As shown in Fig. 2b, a characteristic uv–vis spectrum of the C-5 adduct of PQQ (*46*) was observed at the initial stage of the reaction. Electron flow (shown by arrows in Scheme 7) at the stage of the intermediate **a** will lead to the generation of $PQQH_2$. If such electron flow is not fast enough, dehydration from the intermediate predominantly proceeds to give the hydrazone adduct, which is converted into the 3-amino-1,2,4-triazine derivative **20** by subsequent intramolecular cyclization and aromatization.

It is interesting that the pH of the solution controls the reaction pathway. At lower pH where almost all the guanidino groups are protonated (indicated as $\mathbf{aH}^+$



SCHEME 7

in Scheme 7), the electron flow must be suppressed because of the strong electron-withdrawing effect of the protonated guanidino group, and the acid-catalyzed dehydration could be accelerated. Consequently, the adduct formation mainly proceeds at pH 6.7. On the contrary, the protonation of the guanidino group and the acid-catalyzed dehydration would be depressed at higher pH (pH 10) to allow the electron flow to give the quinol as a major product. Importance of the electron-withdrawing effect of the substituent attached to hydrazino group is also found in the reaction between PQQ and semicarbazide and acetohydrazide. In those cases, the azo adducts, **21** and **22**, are major products even at higher pH conditions (pH 10). In conclusion, the more electron-withdrawing nature the substituent attached to the hydrazino group has, the more preferable the adduct formation is.

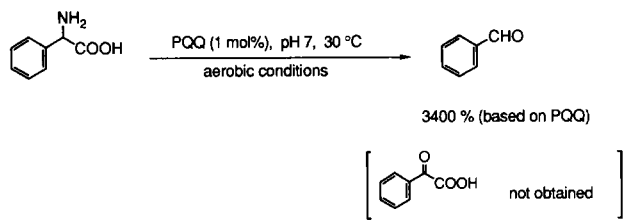


R = NH_2 : **21**
R = CH_3 : **22**

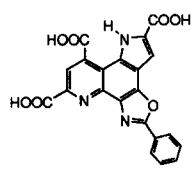
REACTION OF COENZYME PQQ WITH AMINO ACIDS

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 have been believed to be PLP-dependent enzymes, have been recently suspected to be quinoproteins (*64–66*). (However, there is a possibility to be criticized on the results as in the case of amine oxidases.) On the other hand, a major factor which makes it difficult to identify the covalently bound coenzyme is the high reactivity of the cofactor toward nucleophiles such as amino acids and conversion into adducts (*67*). From this viewpoint, a few research groups investigated the reaction between coenzyme PQQ and amino acids (*42, 68–70*), and some of them reported that PQQ is converted into an oxazole derivative (*42, 70*).

As an extension of the model studies, we first reported the PQQ catalyzed *oxidative decarboxylation* of α-amino acids in the cationic micellar system (*71*). For example, α-phenylglycine is efficiently converted into benzaldehyde in the presence of a catalytic amount of PQQ (1 mol %) and CTAB surfactant in a neutral aqueous solution under aerobic conditions (Eq. [14]). In this reaction, formation of the corresponding α-keto acid, benzoylformic acid, is not detected at all. Since the α-keto acid is not converted into benzaldehyde under the same conditions, it can be concluded that benzoylformic acid is not an intermediate of the reaction. On the other hand, PQQ is gradually converted into the redox inactive oxazole derivative **23**.

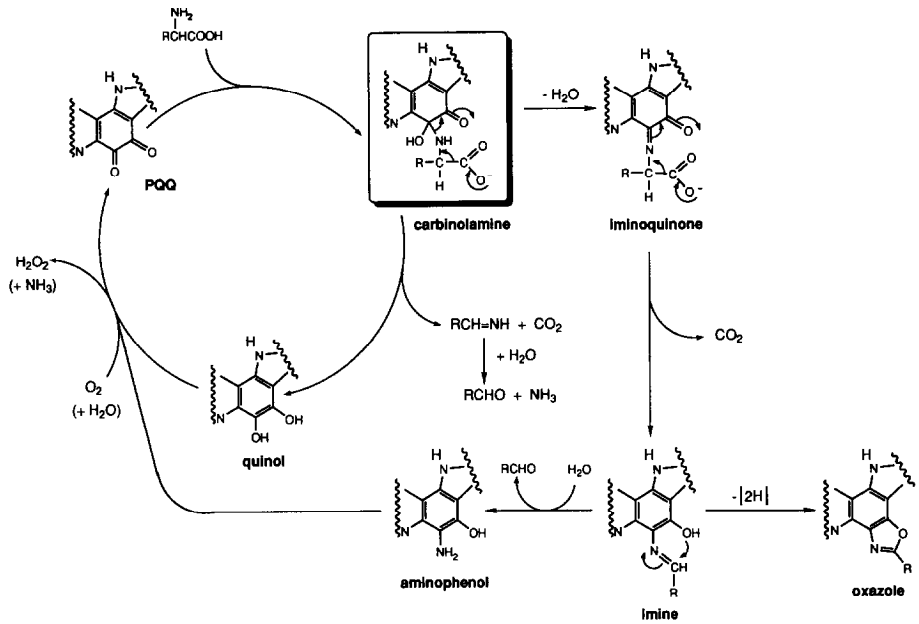


[14]



23

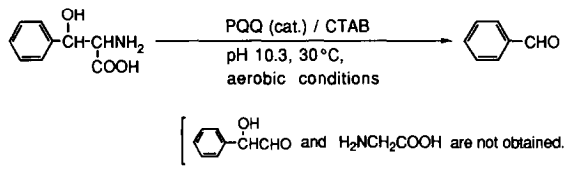
Product analyses of the *anaerobic* reaction using HPLC and uv–vis indicate that $PQQH_2$ (quinol) is formed as a major product together with a small amount of the aminophenol and the oxazole derivative. Although there are some problems on the product analysis of PQQ in aqueous reactions (*44*), the similar ionic mechanism can be applied (Scheme 8). The carbinolamine-type adduct is also considered to



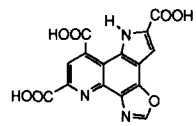
SCHEME 8

be an important intermediate, from which two competing reaction pathways are possible. The major one is direct decarboxylation from the intermediate to produce the quinol, and the minor one is dehydration to give the iminoquinone intermediate. Oxidative decarboxylation may also occur from the iminoquinone intermediate to give the imine intermediate which is finally converted into the aminophenol by hydrolysis and also into the oxazole by intramolecular cyclization and following aromatization.

Interestingly, efficient *oxidative* C_α–C_β *cleavage* (*dealdolation*) occurs in the case of β-hydroxy amino acids (*72*). For example, β-phenylserine is converted into benzaldehyde in the presence of a catalytic amount of PQQ and CTAB surfactant under alkaline aerobic conditions (Eq. [15]). In this reaction, mandelaldehyde, which is an expected product of the oxidative decarboxylation, and glycine, which is a common product of the dealdolation reaction catalyzed by PLP and metal ion (*73*), are not detected. It should be noted that in this case the nonsubstituted oxazole derivative **24** is obtained as a deactivated product of PQQ. The oxazole **24** is also produced in the reaction with a series of β-hydroxy amino acids such as serine, threonine, and tyrosine, and also in the reaction with β-hydroxy amine such as 2-amino-1-phenylethanol. Product analysis of the anaerobic reaction indicates that $PQQH_2$ (quinol) is a major product as in the case of the oxidative decarboxylation reaction. The similar ionic mechanism can be written for the oxidative C_α–C_β cleavage where the carbinolamine-type adduct is an important intermediate as indicated in Scheme 9. Duine *et al.* also reported the formation of

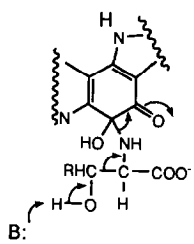


[15]



24

oxazole **24** 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 (*70*). They assumed that the main path of the reaction is the formation of the oxazole **24** and $PQQH_2$ is formed in the stage of the final aromatization step. However the results mentioned above 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.



SCHEME 9

CONCLUSION

In this review, we have introduced the reactions of coenzyme PQQ and its model compounds with several amines such as monoamines, α,ω-diaminoalkanes, hydrazines, aminoguanidine, and α-amino acids from the viewpoint of organic chemistry, and suggested that the carbinolamine-type adduct is the common key intermediate. From this intermediate, direct formation of the quinol product and dehydration giving the iminoquinone-type products proceed competitively, and these two reactions are controlled by several factors; acidity of the α-proton of amines, alkylene chain length of alkylenediamines, electric nature of the substituent of hydrazines, and pH conditions in the reaction with aminoguanidine. The iminoquinone-type adduct thus formed are finally converted into several products

such as the aminophenol, the pyrazine, the hydrazone, the aminotriazine, and the oxazole derivatives depending upon the substrates, respectively.

The quinol formation has been found to be the major pathway in the reactions with amines, phenyl- or methylhydrazine, aminoguanidine (at pH 10), and α-amino acids, constructing efficient catalytic redox systems under aerobic conditions (autorecycling catalysis). These reactions are characteristic for pyrroloquinoline quinone (PQQ). In other words, a simple *o*-quinone such as phenanthrenequinone does not work as a catalyst at all. The most important factor for such a big difference might be the existence of the electron-withdrawing effect by the pyridine nucleus. Namely, the pyridine nucleus facilitates the nucleophilic addition of the substrate to the quinone carbonyl and stabilizes the carbinolamine intermediate thus formed. Furthermore, if the substrate has an electron lone pair or a substituent in electron donating nature at the α-position, electron flow from there might be also accelerated by the electron-withdrawing effect of the pyridine nucleus to afford the quinol as a major product. Negative charge accumulated on the quinone oxygen at the C-4 position by conjugation with the pyrrole nucleus may also help as a general base catalyst for the quinol formation particularly in the case of the amine-oxidation (direct α-proton abstraction). As in fact, alkylation of the N-1 position of PQQTME leads its drastic inactivation in the amine-oxidation (*48*). So it can be said that PQQ (pyrroloquinoline quinone) is a well constructed molecule for the efficient amine-oxidation reaction. Importance of the pyrroloquinoline quinone structure will be further demonstrated by using several PQQ model compounds in the near future.

The transamination mechanism has been considered to be most plausible for the enzymatic reaction of mammalian copper-containing amine oxidases (*31*). But the present results clearly indicate that the direct quinol formation (addition-elimination mechanism) is the most energetically favorable process for the amine-oxidation by PQQ (pyrroloquinoline quinone). There may be two possibilities for this. The one is that the environment of the active site of the enzymes makes the transamination process more energetically favorable, and the other possibility is that the coenzyme is not PQQ. Since several compounds have been proposed as the coenzyme of amine-oxidizing enzymes recently, it seems to need some more time to get a conclusion.

Interaction of the coenzyme and copper ion is another interesting subject. It has been suggested that copper ion plays an important role in the electron-transfer step from the reduced coenzyme to molecular oxygen (reoxidation of the reduced coenzyme) (*74*). We have been also trying to approach to this subject from model systems (*75–77*).

ACKNOWLEDGMENTS

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BIOORGANIC CHEMISTRY 19, 226–228 (1991)

IN MEMORIAM

Professor Toshio Goto
April 24, 1929–August 29, 1990

It is with our deepest regret that we inform our readers of the unexpected death of Professor Toshio Goto, Laboratory of Organic Chemistry, School of Agriculture, Nagoya University, Japan. He died on August 29, 1990, at the age of 61.

Many readers are probably quite familiar with Professor Goto's pioneering work in natural product chemistry and bioorganic chemistry. His remarkable activities in this field include studies of the molecular association of flower pigments, bioluminescence, gangliosides, nucleosides, biotoxins, and steroids. He was graduated from the Department of Chemistry, Nagoya University, in 1954 under the guidance of Professor Yoshimasa Hirata and obtained a position as Research Assistant in this department. In 1957, Professor Goto joined the group of Professor Louis Fieser at Harvard University and spent 2 years working on the chemistry of steroids. He returned to Nagoya University, received his Ph.D. in 1960, and became an Associate Professor in the Hirata group in the next year. While there, he made major contributions to the structure elucidation of tetrodotoxin of fugu fish

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