Characteristic Reaction Mode of Cofactor PQQ toward Nucleophiles

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Abstract

The characteristic reaction mode of coenzyme PQQ toward nucleophiles such as alcohols, amines, and hydrazines are surveyed. The selectivity of addition position of nucleophiles to the carbonyl group of PQQ was affected by reaction conditions and substituents of nucleophiles. Reaction mechanisms were discussed.

Key words: Cofactor, Coenzyme, PQQ, Quinoprotein, Redox Reaction

1 Introduction

In the early 1980s, it was established PQQ (pyrroloquinolinequinone) as the novel redox cofactor in addition to the well-known NAD(P)H and flavin cofactors [1]. Although the existence of this novel cofactor had been suggested in early 1960s, the structure was elucidated in 1979 [2,3]. The finding of this novel cofactor led to the discovery of several kinds of quinone-containing enzymes (quinoproteins) [4]. During 1980s, PQQ or closely related compounds had been believed to be the cofactor of all kinds of quinoproteins [4,5]. However, recent advances in biological analyses have demonstrated that PQQ is not the only one. Trihydroxyphenylalanine (TOPA) [6], tryptophan tryptophylquinone (TTQ) [7], and a covalently modified tyrosine crosslinked to a cysteinyl sulfur (Tyr-Cys) [8] have been found from bovine serum amine oxidase, methylamine dehydrogenase, and galactose oxidase, respectively.

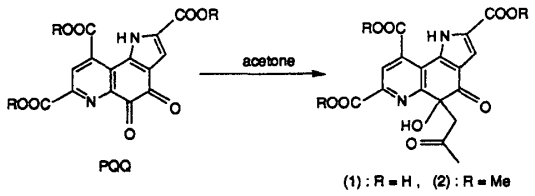
In addition to the enzymological significance, PQQ was demonstrated to be a growth-stimulating substance for microorganisms [9] and a nutritionally important compound for mammals [10]. Furthermore, the biomedical and pharmacological activities of PQQ have been demonstrated in relation to the electron-transfer ability [11,12]. Quinoproteins have also been applied as biocatalysts [13] and biosensors [14]. To date, several kinds of biologically important heterocyclic quinones are known [15], but PQQ and TTQ are the only two that act as redox catalysts in biological systems.

In this paper, our attention is focused on the characteristic reaction modes of the newly found heterocyclic ortho-quinone cofactor PQQ [16]. One of the most interesting aspects of the chemical properties of PQQ is its high reactivity toward nucleophiles [17].

2 Adduct Formation of PQQ with Acetone

Acetone adds easily to the quinone carbonyl carbon of PQQ and its trimethyl ester derivative under basic conditions to form the aldol-type adducts (1) and (2) [2,18]. PQQ was isolated at

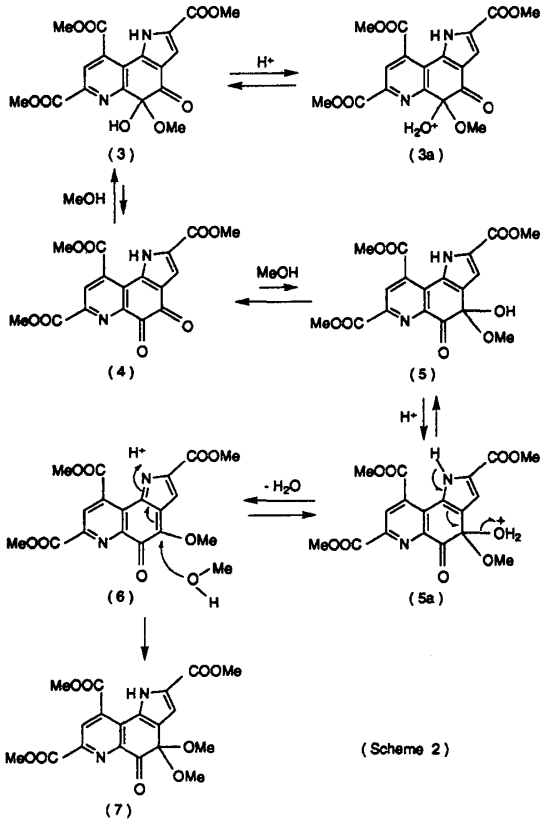
first from the methanol dehydrogenase of methylotropic bacteria as the acetone adduct [2]. The addition position (C-5) was determined by X-ray crystallographic analysis. The same addition reactions were also observed for the model compounds [19-21].



(Scheme 1)

3 Nucleophilic Addition of Alcohols to PQQ

Addition of alcohols to the quinone function of PQQ was studied spectrophotometrically [22], and the addition position was assumed to be C-5 without a direct proof. Recently, the C-5 hemiacetal (3) was isolated in the reaction of the trimethyl ester of PQQ (4) with methanol under neutral conditions and its crystal structure was determined. On the other hand, treatment of the quinone (4) with methanol under acidic conditions gave the dimethyl acetal (7) as the major product, for which the addition position of methanol was decided to be C-4 by X-ray crystallographic analysis [18].

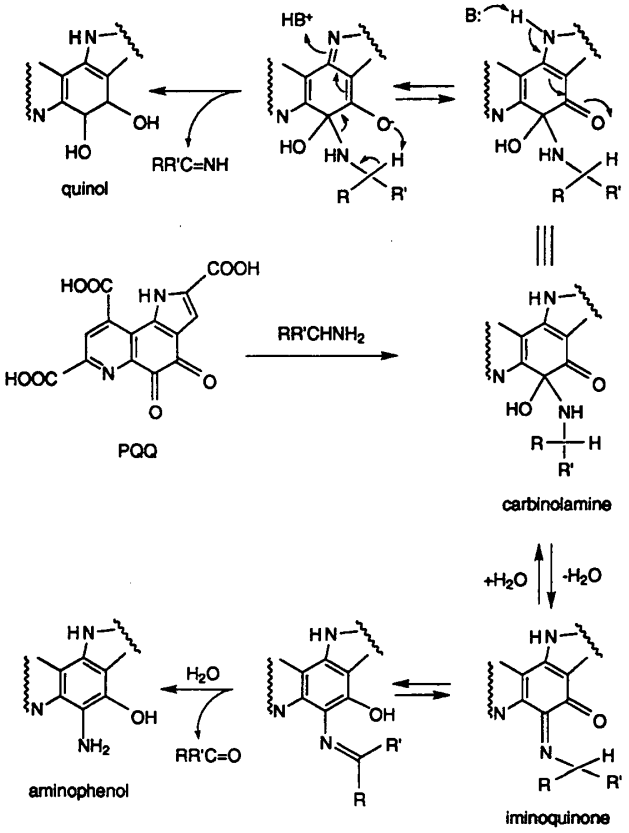


(Scheme 2)

The calculated values of the heat of formation clearly indicate that the C-5 hemiacetal is more stable than the C-4 adduct (5) (1-3 kcal/mol). Because the hemiacetal (3) is readily returned to the original quinone, the hemiacetal formation is considered to be completely reversible, and the reaction seems to be controlled thermodynamically. Therefore, it is reasonable that hemiacetal (3) is formed as the only isolable product under neutral conditions. In the presence of acid, elimination of water from the protonated intermediate (5a) proceeds much faster than the protonated hemiacetal (3a) because of the facile release of the pyrrole proton (H-1) to give the conjugated intermediate (6). Attack by a second molecule of methanol gives the C-4 acetal (7). There is no conjugative effect upon elimination of water from the C-5 hemiacetal (3a). Once the C-4 acetal (7) is formed, it cannot revert to the C-4 hemiacetal (5) in the presence of excess methanol. Therefore, the C-4 acetal (7) is gradually accumulated under the acidic conditions. This mechanism is supported by the results of the acetal formation reactions of other quinones.

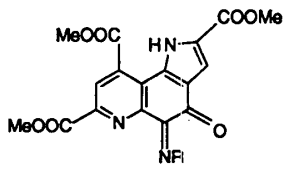
4 Nucleophilic Addition of Amines to PQQ

Because PQQ was believed to be the organic cofactor of copper-containing amine oxidases, bacterial methylamine dehydrogenases, and methylamine oxidase, many investigations have been focused on the reaction of PQQ with amines. Although the situation of PQQ as the cofactor of the amine oxidases has turned out to be incorrect, the chemical behavior of PQQ toward amines is still noteworthy because PQQ has been reported to play an important role in the crosslinking of collagen and elastin and in the regulation of intracellular spermine and spermidine levels [23]. Otherwise, PQQ has been demonstrated to be an excellent turnover catalyst for the oxidative deamination of amines in non-enzymatic systems [24] (this is the first exsample of quinone-catalysed amine oxidation).

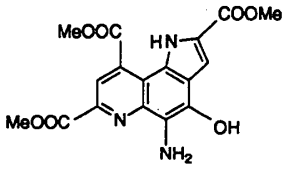


(Scheme 3)

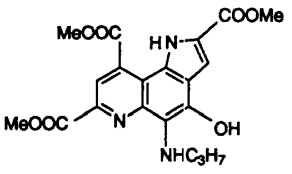
Kinetic studies and product analyses indicated that the amine oxidation reaction proceeds through the ionic transamination mechanism via the C-5 adduct (carbinolamine) as shown in Scheme 3 [25]. In this case, several products were obtained depending on the substrates and on the reaction conditions.



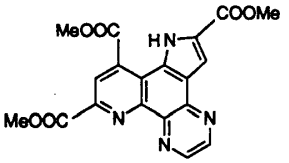
(8) : R = H
(9) : R = Bu^t
(10) : R = cyclopropyl



(8a)



(11)



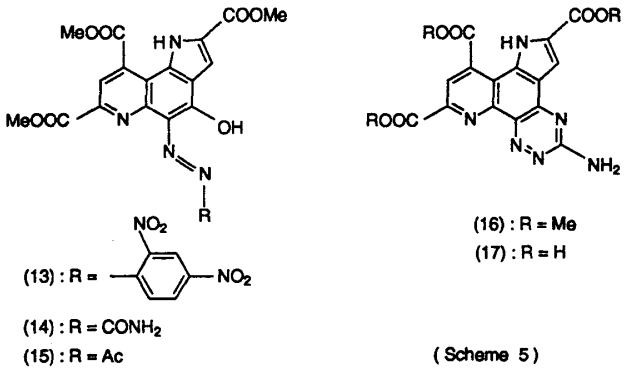
(12)

(Scheme 4)

The iminoquinone derivatives (8-10) are easily isolated from the reaction of the trimethyl ester of PQQ (4) with ammonia, t-butylamine, and cyclopropylamine, respectively. The aminophenol (8a) was generated by the reduction of iminoquinone with methylhydrazine. In the reaction of trimethyl ester of PQQ (4) with benzylamine in acetonitrile under anaerobic conditions, the aminophenol (8a) is directly formed together with the corresponding quinol. Interestingly, the alkylaminophenol (11) is produced together with the quinol and the aminophenol in the reaction with n-propylamine under the same conditions, where the product ratio is quite different depending upon the amine concentration [26]. Only the quinol is isolated in the case of N-methylpropylamine, but the reactivity is relatively low; and no redox reaction occurs for triethylamine. On the other hand, the pyrazine derivative (12) was obtained in the reaction of PQQ with ethylenediamine [27-29]. These results strongly support the ionic mechanism [30].

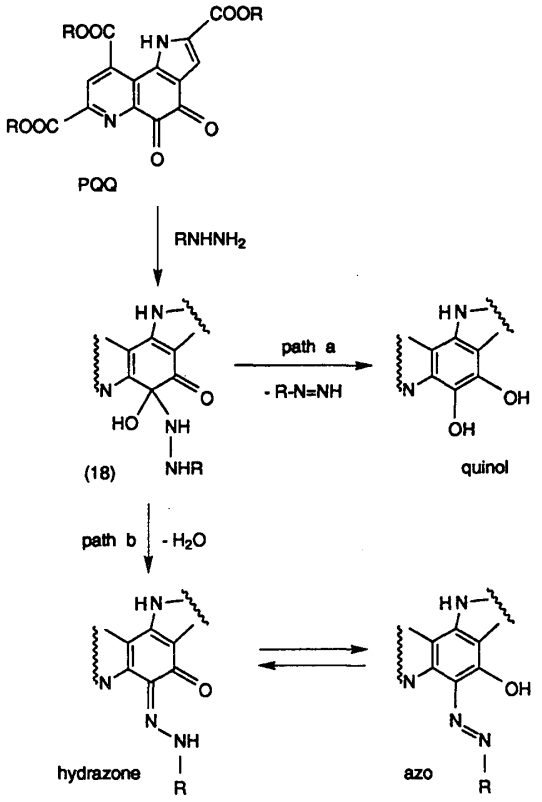
5 Nucleophilic Addition of Hydrazines to PQQ

Hydrazines are often used as carbonyl reagents in order to convert the carbonyl cofactors to more stable hydrazone derivatives [31]. The trimethyl ester of PQQ (4) is easily converted to the C-5 hydrazones (13), (14), and (15) by treatment with 2,4-dinitrophenylhydrazine, semicarbazide, and acetohydrazide, respectively [32,33]. The addition position of C-5 was confirmed by X-ray crystallography of the hydrazone (13) [34]. When aminoguanidine was used, intramolecular cyclization and aromatization of the corresponding hydrazone took place to give the triazine derivative (16) [33].



(Scheme 5)

It is interesting that the redox reaction (reduction of the quinone and oxidation of the hydrazine) occurs depending upon the acidity of the reaction media and the natures of the substrates and quinones. Aminoguanidine gave the triazine (17) in the reaction with PQQ under acidic conditions. On the contrally, the quinol was formed in an alkaline solution. The electronic nature of the substituent attached to the hydrazino group also affected to the reaction. The electron-withdrawing substituent was favorable to the adduct formation. On the other hand, the electron-withdrawing nature of the pyridine nucleus enhanced the redox reaction. The trimethyl ester of PQQ (4) was mainly converted to the quinol derivative in the reaction with phenyl hydrazine hydrochloride in methanol. All these results could be explained by an ionic mechanism via the C-5 carbinolamine-type intermediate (18) from which both the redox reaction (path a) and the adduct formation reaction (path b) proceed depending on the factors mentioned above.



(Scheme 6)

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