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# THE COFACTOR PYRROLOQUINOLINE QUINONE

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## INTRODUCTION

Studies in microbial enzymology have shown that nonphosphorylative substrate oxidation by bacteria frequently proceeds via cytoplasmic membrane-bound dehydrogenases. Like their eukaryotic counterparts in the mitochondria, these NAD(P)-independent dehydrogenases were initially viewed as flavoproteins. However, in the 1960s work on the cofactor of bacterial methanol dehydrogenase (5) and glucose dehydrogenase (28) revealed that this view was not correct (the research groups working in separate laboratories were unaware of each other's work so that they did not realize that the cofactor of both enzymes was the same). Despite these initial indications of a novel cofactor, it was not until 1980 that the structural elucidation of the cofactor of methanol dehydrogenase was reported. Independent studies (14, 54) revealed that the cofactor was pyrroloquinoline quinone (2,7,9-tricarboxy-1*H*-pyrrolo[2,3-*f*]quinoline-4,5-dione) (PQQ). Virtually at the same time, several other bacterial quinoprotein (PQQ-containing) dehydrogenases were discovered. The finding that some bacteria excrete substantial amounts of PQQ into their growth medium (12) provided material for studies that sought to unravel the intricacies of the cofactor.

A more recent breakthrough was the discovery of covalently bound PQQ (41) in eukaryotic enzymes. Several well-known mammalian and plant enzymes have this form of the cofactor (in addition to metal ions or pyridoxal phosphate, which previously were thought to be the only cofactor). Where the cofactor in these organisms comes from is presently unknown, but findings from bacterial studies may be relevant: Tyrosine and glutamic acid are the precursors for biosynthesis of free PQQ in methylotrophic bacteria (31, 70); free PQQ is not required for synthesis of covalently bound PQQ in glutamate decarboxylase of *Escherichia coli* (62); this implies that synthesis of covalently bound PQQ may occur *in situ*.

Nutritional studies on PQQ were undertaken rather late; the first papers have only now begun to appear, ten years after its structural elucidation. In this review we attempt to evaluate these experiments. Based on what is known of the properties, distribution, and biosynthesis of PQQ, arguments (pro and con) for its role as a vitamin are presented. More specific information on the topic can be found in a number of reviews (13, 16, 17, 27) and in the *Proceedings of the First International Symposium on PQQ and Quinoproteins* (18).

## PROPERTIES

### *Characteristics*

PQQ has a flat, aromatic tricyclic ring structure (32, 75). If one ignores nomenclature rules, the compound can be viewed as a quinoline and a pyrrole

ring or an indole and a pyridine ring (Figure 1). The redox behavior of the compound is reflected in the *o*-quinone grouping, which can be oxidized to the quinone (PQQ), half-reduced to the radical or the semiquinone (PQQH·) or fully reduced to the quinol ($PQQH_2$) form. Three rather strongly acidic carboxylic acid groups are responsible for the high solubility of the compound at any redox level in neutral buffers. Therefore, PQQ is quite different from the well-known (*p*-) quinones, namely ubiquinone and menadione, which are lipid-soluble compounds operating in biomembranes.

As indicated by the bands in the absorption spectrum (Figure 2, Table 1), PQQ solutions will have a slightly yellow, a brick-red, or a dark red color, depending on the concentration. However, while the absorption spectrum of the cofactor and that of quinoproteins sometimes are quite different from each other, those of the enzymes more or less resemble the absorption spectra of pyridoxal phosphate-containing enzymes. When irradiated with the appropriate light, aqueous PQQ solutions emit greenish fluorescent light. Color, intensity of fluorescence and absorption spectrum vary when the temperature of the solution is changed. These changes are due to the presence of fluorescent covalently hydrated PQQ (Figure 1), which is in a temperature-dependent equilibrium with the nonfluorescent unhydrated form and which has a different absorption spectrum.

Compared with other redox cofactors, PQQ has a relatively high redox potential (+90 mV at pH 7), in accordance with its *o*-quinone structure (this difference should not be overemphasized when enzyme classes are compared, because the protein environment of a cofactor can induce a large shift).

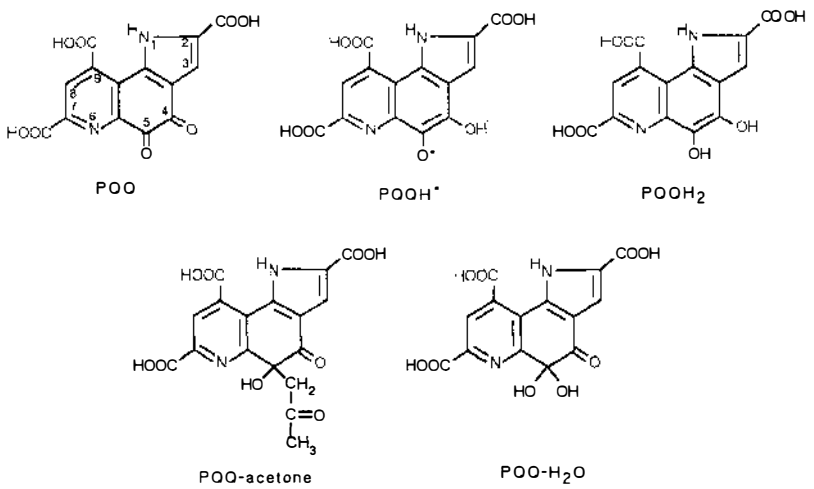


*Figure 1* Structure of the redox forms and adducts of PQQ.

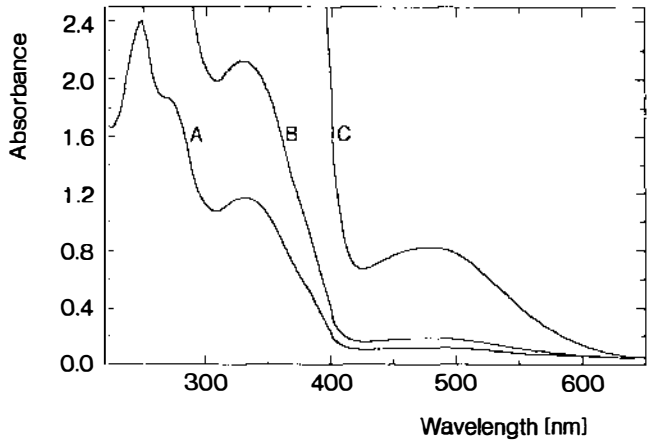


*Figure 2* Absorption spectra of solutions with different concentrations of PQQ in 0.1 M sodium phosphate, pH 7.2: 95 $\mu$M PQQ (*A*), 195 $\mu$M PQQ (*B*), and 875 $\mu$M PQQ (*C*).

Several sites in the molecule are suitable for liganding metal ions (Figure 3). Complexation is reflected in spectral shifts that occur when certain metal salts are added to a PQQ solution, as has been demonstrated with $Ca^{2+}$-(21) and $Cu^{2+}$-ions (36). Information about some of these complexes has been obtained by X-ray structure determination (32, 75) (it should be realized, however, that the mode of interaction between metal ion and cofactor may be quite different in the so-called metalloquinoproteins).

## *Stability*

PQQ is a heat-, acid-, and photo-stable compound. Because it sticks to laboratory glassware and chromatographic media, one must devise appropriate strategies to prove the absence or presence of biosynthetic capacity of an

**Table 1** Some properties of PQQ

| | |
|---|---|
| Relative molecular weight | 330 |
| Absorption maxima | 249, 325, 475 nm |
| Fluorescence maximum | 460 nm |
| Molar absorption coefficients (measured in water, pH 4.3) | 25,200 $M^{-1}\cdot cm^{-1}$ (at 249 nm)<br>17,810[a] $M^{-1}\cdot cm^{-1}$ (at 236 nm)<br>21,420[a] $M^{-1}\cdot cm^{-1}$ (at 257 nm)<br>10,770[a] $M^{-1}\cdot cm^{-1}$ (at 342 nm) |
| Redox potential (at pH 7) of the $PQQ/PQQH_2$ couple | + 90 mV |

[a] Values taken at the isosbestic points of the PQQ/PQQ-$H_2O$ spectra so that they are temperature independent.

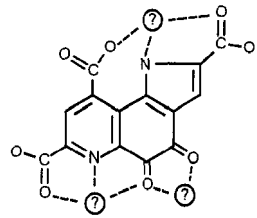

*Figure 3* Potential binding sites of PQQ for metal ions.



organism for the cofactor (69). Contamination with PQQ can be abolished by keeping the equipment at 500°C for several hours or by washing the equipment with alkaline fluids containing hydrogen peroxide (ring opening occurs under the latter condition). Partial destruction of the molecule is achieved upon heating it with nitric acid.

PQQ is easily converted into condensation products with nucleophiles. Studies with amino acids revealed that the type of amino acid, the concentration of $NH_3$, the pH, and the presence of certain metal ions have a large effect on the rate of and the tendency toward condensation (or toward a catalytic reaction, leading to decarboxylation of the amino acid with concomitant $PQQH_2$ formation) (73).

## *Different Forms*

Both the cofactor and its variants occur in different redox forms. Thus one can argue for the existence of the iminoquinone form of PQQ and the aminophenol form of $PQQH_2$ (Figure 4) in the reaction cycle of the amine oxidoreductases and in the model system of PQQ and amino acids. Moreover, as in the case of pyridoxal phosphate, similar products of PQQ and an amino acid residue may occur in the quinoproteins. Several nucleophiles add to PQQ at the $C_5$-position, and these adducts may become reduced under certain conditions. Adducts are also converted into condensation products, as illustrated by the glycine-PQQ adduct, which forms a PQQ-oxazole (Figure 5). Under acid conditions, PQQ and tryptophan condense to a product that may have the structure shown in Figure 5. Thus a variety of PQQ products can be expected after a quinoprotein is hydrolyzed, thus making identification of the cofactor practically impossible (74).

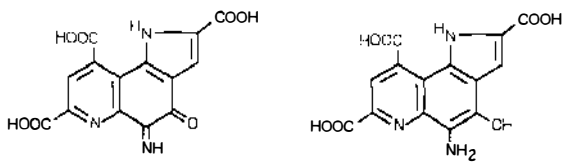

*Figure 4* The iminoquinone form of PQQ *(left)* and the aminophenol form of $PQQH_2$ *(right)*.

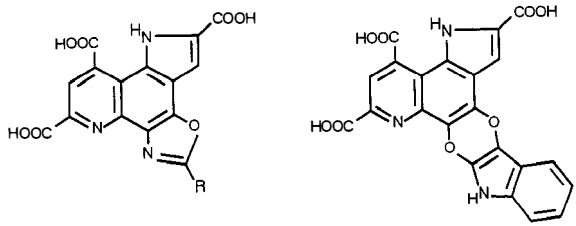


*Figure 5* PQQ-oxazole *(left)* and PQQ-trp condensation products *(right)*.



For all eukaryotic quinoproteins investigated so far, and under normal conditions of protein denaturation, PQQ is not detached from the protein. One of these enzymes, diamine oxidase from pig kidney, has been studied to determine how the cofactor is linked to the protein chain. A tryptic peptide was isolated in which the cofactor was attached to a lysyl residue with a pronase-scissile bond, most probably an amide bond between the ε-amino group of the lysine and one of the carboxylic acid groups of PQQ (68).

It has been claimed that methylamine dehydrogenase of Bacterium W3A1 has a modified PQQ, attached to the protein chain via ether and thioether bonds (44). However, in the case of the enzyme from *Thiobacillus versutus,* PQQ-phenylhydrazone was extracted by the hydrazine method (64). Studies on the three-dimensional structure of the latter enzyme show that the situation is not so simple (79). Surprisingly, the electron density map of the active site does not have a moiety that can be fitted with PQQ as such, although the product extracted with the hydrazine method shows that the constituents of PQQ are present. To accommodate these facts, a structure has been proposed for the cofactor, the so-called pro-PQQ, for which it is assumed that cyclization and oxidation occur during application of the analysis procedures for covalently bound PQQ. An important implication of this proposal is that

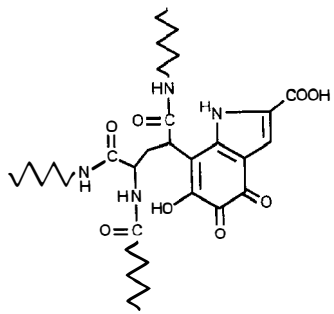


*Figure 6* The pro-PQQ structure, as proposed for methylamine dehydrogenase, with three points of attachment to the protein chain (78, 79).

enzymes said to contain covalently bound PQQ could in fact have a pro-PQQ-related structure instead of PQQ[1] (Figure 6) (79).

To summarize, in addition to the reduced forms of PQQ and its covalently hydrated form, adducts and condensation products can be expected to occur in complex environments. In eukaryotic organisms, all quinoproteins contain covalently bound PQQ. Because the cofactor can be detached with pronase, amide or ester bonds seem to be involved in binding.

## IDENTIFICATION AND DETERMINATION

Because the methods developed for the detection of free and covalently bound PQQ are fundamentally different, they are treated under separate subheadings. Details can be found elsewhere (13, 15, 16, 63, 66).



### *Methods for the Free Form*

Several different approaches for analysis have been developed, each of them with its merits and problems.

BIOLOGICAL ASSAYS In a biological assay, reconstitution of a quinoprotein dehydrogenase apoenzyme occurs with the PQQ in the sample. The presence of nucleophilic compounds can lead to serious underestimation or even to a false negative result, because PQQ adducts or condensation products with modified *o*-quinone group are inactive in biological assays. For this reason, a pretreatment step to remove the nucleophiles is advisable. The amplification achieved by forming a holoenzyme implies that biological assays are very sensitive and that precautions must be taken to avoid contamination with adventitious PQQ (see section on stability).

Quinoprotein dehydrogenase apoenzymes form the base of several biological assays. The apoenzyme can be used in the crude form from whole bacteria and in cell-free extracts, or it can be used in its purified form. Reconstitution frequently requires Ca or Mg ions, so one of these cations should be present in amounts sufficient to overcome the effect of possible chelators in the sample. Soluble glucose dehydrogenase from *Acinetobacter calcoaceticus* strain LMD 79.41 has a number of attractive properties for a bioassay (63). Its gene has been cloned and expressed at high levels in *Escherichia coli* (this bacterium is unable to produce free PQQ), and the enzyme can be purified rather easily. Consequently, 0.01 pmoles of PQQ are routinely determined in a 100-$\mu$l sample in our laboratory (the enzyme has a high turnover number and background activity is absent). Even much higher sensitivities can be achieved by applying a preconcentration step (63).

[1]For reasons of brevity, the term *covalently bound PQQ* is used throughout this review.

LIQUID CHROMATOGRAPHY Excellent high-pressure liquid chromatography (HPLC) procedures with reversed phase columns are available to determine PQQ and its derivatives. A reaction with nucleophiles can be disastrous for the successful outcome of this approach. If samples are prepared at low pH and a chromatographic solvent is used with low pH, one should be aware of the possibility that the cofactor is present in the form of $PQQH_2$ (reoxidation scarcely occurs at low pH). When crude samples are investigated, it is advisable, for selectivity, to use fluorescence instead of absorbance detection. To be sure that a peak really belongs to PQQ, at least one of the following checks should be made: application of different chromatography systems; monitoring the fluorescence as well as the absorbance of the eluate, and comparison of the ratio of the detector signals of the presumed peak with that of authentic PQQ; measuring the absorption spectrum of the peak (which is most easily performed with photodiode array detection); converting the presumed PQQ in the sample to a relatively stable adduct (e.g. the acetone adduct, see Figure 1), leading to a novel peak and the disappearance of the PQQ peak upon subsequent chromatography.

GAS-LIQUID CHROMATOGRAPHY Recently (58) a procedure was developed in which PQQ is derivatized to a volatile compound suited for gas-liquid chromatography (GLC) analysis. Spiking of natural samples with PQQ showed that this is a very sensitive and quantitative method for the determination of free PQQ (as was also confirmed in our laboratory, M. A. G van Kleef, unpublished results).

A COLORIMETRIC REDOX-CYCLING ASSAY PQQ reacts with certain amino acids in a catalytic fashion during oxidative decarboxylation of the amino acids and concomitant $PQQH_2$ production (however, a competing reaction also occurs, leading to noncatalytically active PQQ-oxazoles) (73). The system can be reoxidized with oxygen, or with a tetrazolium dye, which becomes reduced to the corresponding formazan, as demonstrated in a recently proposed colorimetric assay (50). The assay is said to be specific for PQQ since the color change occurs with glycine (good decarboxylation with PQQ) but not with valine (no decarboxylation with PQQ, so that interfering reducing substances can be traced). The method has also been used to determine PQQ in biological samples (20). PQQ has been reported to be present in certain foods and mammalian body fluids (20, 39, 49, 50). We checked some of these samples with biological methods and methods designed to detect derivatized PQQ, but we were unable to detect the presence of PQQ (11, 15). Moreover, we found that the assay is not specific and that the response of the assay in the aforementioned materials can be fully ascribed to interfering substances (e.g. dehydroascorbic and ascorbic acid, riboflavin) or to other

compounds catalyzing decarboxylation of glycine (but not valine). For these reasons, the assay is totally unsuited for biological samples, and the claims based on it concerning the presence of PQQ in foods and mammalian samples mentioned in the studies are incorrect.

AN IMMUNOLOGICAL ASSAY Recently it was reported that antibodies raised against a PQQ-gelatin complex react with covalently bound PQQ in lentil seedling amine oxidase (8). Because it was also possible to inhibit the activity of the native enzyme with the antibody preparation, and the inhibition disappeared on PQQ addition, the assay could be used to determine free PQQ.

## *Methods for Covalently Bound and Derivatized Forms*

The reactivity of PQQ with nucleophilic amino acids precludes straightforward hydrolysis of enzymes in cases where the cofactor occurs in covalently bound form. Although it has been claimed that the colorimetric assay is suited for determination of PQQ in this type of quinoproteins (20, 49, 50), apart from the problems of interfering compounds, this method is not quantitative. The immunological method (8) is able to detect covalently bound PQQ in lentil seedling amine oxidase, and we have found (R. A. van der Meer, B. W. Groen, F. Huitema, and J. Frank, unpublished results) that other quinoprotein amine oxidases are also inhibited, but not bacterial dehydrogenases (glucose dehydrogenase, methanol dehydrogenase, methylamine dehydrogenase). However, it is unknown whether the inability to react with the antibodies results from differences between cofactor present in the antigen and that present in the dehydrogenases or from insufficient exposure of the cofactor in the latter enzymes. Therefore, further investigations are necessary to see whether this method has general applicability in the detection of eukaryotic quinoproteins. The two methods discussed below, the so-called hydrazine method and the hexanol extraction procedure, give consistent results. However, they lack specificity concerning discrimination between different forms of the cofactor because they failed to reveal that the deviating structure of the cofactor in methylamine dehydrogenase was pro-PQQ (79).

THE HYDRAZINE METHOD The hydrazine method (63, 64, 66) is based on derivatization of the cofactor in the enzyme to prevent conversion into condensation products with the nucleophilic amino acids liberated during subsequent pronase-catalyzed hydrolysis of the enzyme. Depending on the type of enzyme and conditions applied during derivatization, the hydrazone of PQQ or an isomeric form of it is produced (33). The product is purified and is identified either by converting it into PQQ (and quantifying it with a biological assay) or by comparing it with an authentic model compound (with respect to chromatographic behavior and absorption spectra). The method has been

shown to be quantitative. It seems unsuited, however, in cases where the cofactor is already derivatized or is in a reduced form and for enzymes that do not accept the rather bulky aromatic hydrazines.

A similar approach has been used for lysyl oxidase: It is derived with a diamine reagent to the stable quinoxaline form of the cofactor (19). It is presently unknown, however, whether other quinoproteins will accept the derivatizing agent.

*IN SITU* MEASUREMENTS BY RAMAN SPECTROSCOPY Dooley and coworkers (45) have used Raman spectroscopy to identify the cofactor in hydrazine-derivatized enzymes. Their reports, however, indicate that the spectra were not always compared with those of the relevant model compound. In principle, this method should be very useful for discriminating between quinoproteins with covalently bound PQQ and with pro-PQQ, although in practice the effect of the protein moiety of the enzyme on the derivatized cofactor could make interpretation impossible.

THE HEXANOL EXTRACTION PROCEDURE The hexanol extraction procedure consists (15, 63, 66, 67) of the following elements: The sample is mixed with hexanol and hydrochloric acid, and the mixture is refluxed for several hours; hexanol reacts with the cofactor to the ketal form so that unwanted attack by nucleophiles is prevented; the hydrochloric acid hydrolyzes the proteins at the refluxing temperature; the ketal compound is isolated and compared with authentic material or is hydrolyzed to PQQ; under somewhat different conditions, the more stable 4-hydroxy-5-hexoylpyrroloquinoline is formed, which can also be compared with the synthetic compound or converted into PQQ by oxidation with cerium ammonium nitrate. The hexanol extraction method has some unique features: It does not depend on enzyme-mediated derivatization; it is able to determine derivatized PQQ, because these compounds are reconverted under the applied conditions.

## OCCURRENCE

### *In Prokaryotes*

Free PQQ (1 to 10 mg per liter) has been found (72) in the culture medium of Gram-negative methylotrophic bacteria [and Gram-positive *Nocardia* spec. 239 (29)] and *Pseudomonas, Gluconobacter,* and *Acetobacter* species growing on ethanol. In all these cases, a quinoprotein dehydrogenase constituting a large part of the cellular protein is induced. When the amount of quinoprotein is less (e.g. in the case of glucose dehydrogenase in *A. calcoaceticus*), there is also a much lower PQQ level in the culture medium. Similarly, if no

induction of a quinoprotein dehydrogenase occurs, no PQQ is found. However, no systematic studies have been performed, and only in some cases is it mentioned in the literature that the cultured organism did not produce a holoquinoprotein enzyme (and thus PQQ). Thus, insight into the distribution of PQQ is mainly based on work showing the occurrence of a quinoprotein dehydrogenase in a selected number of bacteria. In this respect, quinoprotein glucose dehydrogenase is an interesting example: its distribution demonstrates that although many bacteria are able to produce the protein part of the quinoprotein, they are unable (under the described growth conditions) to produce PQQ (76). This is illustrated by an apparently random distribution of the holo- and apo-forms among Enterobacteriacea (7). The genus *Escherichia coli* belongs to the category producing apo-enzymes (30), despite the fact that one of the strains produces glutamate decarboxylase, which contains covalently bound PQQ (62). The latter compound is easily converted into PQQ with the analytical procedures, and one may wonder what is lacking in the organism to catalyze such a conversion. In this respect it is interesting that four PQQ-genes derived from *Acinetobacter calcoaceticus* were required to produce glucose dehydrogenase holoenzyme in an *E. coli* strain (23). In summary, no systematic studies on the distribution of free PQQ in prokaryotes have been performed to date. Many bacteria produce PQQ, but others do not despite the fact that some of them have quinoprotein apoenzymes and/or enzymes with covalently bound PQQ.

## *In Eukaryotes*

The presence of free PQQ in eurkaryotes has not yet been reported (see next paragraph). On the other hand, several enzymes have been discovered that contain covalently bound PQQ (Table 2). The mechanism for biosynthesis of the cofactor in these enzymes is unknown. It is possible that biosynthesis

**Table 2** Quinoproteins in mammals and plants

| Enzyme | Source | Reference |
|---|---|---|
| Plasma amine oxidase | Bovine blood | 41 |
| Diamine oxidase | Pig kidney | 68 |
| Lysyl oxidase | Human placenta | 60 |
| | Chicken cartilage | 40, 81 |
| Dopamine β-hydroxylase | Bovine medulla | 65 |
| Dopa decarboxylase | Pig kidney | 24 |
| Lipoxygenase-1 | Soybean | 61 |
| Amine oxidase | Lentil seedling | 53 |
| | Pea seedling | 22 |
| *N*-Methylputrescine oxidase | Tobacco roots | 10 |
| Tryptophan decarboxylase | *Catharantus roseus* | 51 |

occurs *in situ,* as has been suggested in the case of glutamate decarboxylase from *E. coli* (62). Furthermore, even if it is assumed that the compound is produced, one cannot expect that the free form would survive the reactive internal environment prevailing in these organisms without the assistance of special carriers. With respect to the latter, serum albumin binds PQQ (1), although glucose dehydrogenase apoenzyme is able to remove it (J. A. Duine et al, unpublished results). However, in contrast to what has been reported (1, 49), we were unable with the apoenzyme assay to detect any PQQ in samples of serum albumin (J. A. Duine et al, unpublished results).

The aftermath of quinoprotein degradation is unknown, and thus the occurrence of PQQ-like compounds cannot be excluded. However, if these compounds occur, they do not appear to have biological activity, as we were unable to detect any activity with the apoenzyme assay in blood or urine (J. A. Duine et al, unpublished results).



## *In Food and Drinks*

No systematic investigations on the occurrence of free PQQ in food and drinks have been reported. However, in view of the composition of these materials, the same remarks on the occurrence of PQQ can be made as in the case of eukaryotes. Recent claims (20, 39, 49, 50) about the presence of free PQQ in certain foods are incorrect (11, 15). There are, however, a number of cases where its occurrence has been proven: vinegar (values vary according to brand and type investigated; the highest value was 20 nM, J. A. Duine et al, unpublished results), and therefore most probably products to which it has been added as an acidifier; single-cell protein, consisting of bacteria grown on methanol, used as a component or additive of cattle feed; gluconic acid prepared by bacterial fermentation (J. A. Duine et al, unpublished results), used as an additive in the food manufacturing industry. Although not yet investigated, it can be expected that certain supplies of drinking water will contain PQQ: for example, *Hyphomicrobium* species are frequently found in water, and methane and methanol (as a hydrolysis product of plant materials like lignins, pectins) are omnipresent in the biosphere (26). This bacterium is used for denitrification of water (with methanol as a carbon and energy source), so that even higher amounts of free PQQ can be expected in such cases.

In screening a number of products (J. A. Duine et al, unpublished results) from local food stores and slaughter houses, PQQ appeared to be absent (below a detection level of 1 pM, as determined with a biological assay using glucose dehydrogenase apoenzyme) in most of them [milk, egg yolk, citrus fruits, sauerkraut, wine (a red and a white variety were tested), pig heart, and pig blood]. Low amounts (50 pM) were detected in beer (several brands were investigated). The origin of PQQ in this beverage is unknown, but its pres-

ence may be due to contamination by acetic acid bacteria during the fermentation process.

The wide distribution of enzymes with covalently bound PQQ in animals and plants means that this form of the cofactor will be present in the diet. What happens to it (and also to free PQQ) during the digestion of the food is presently unknown. It has been claimed that components of microbial culture media contain PQQ and are (partly) responsible for stimulating the growth of certain microbes (3). However, data on quantification or detection with an appropriate method were not presented, and in a study of yeast extract, we could not confirm this claim either with the apoenzyme assay or with assays for derivatized PQQ (J. A. Duine et al, unpublished results).



## PRODUCTION

### *By Bacteria*

Methylotrophic bacteria grown on methanol excrete substantial amounts of PQQ into their growth media. Several of them have been patented and some of them form the basis of commercial processes (Mitsubishi Gas Chemical Company and Ube Industries Ltd., Japan). In principle, quinoprotein alcohol dehydrogenase-containing bacteria, like certain pseudomonads or acetic acid bacteria, could also be used. Factors relevant to PQQ production have been investigated (72): The organism should produce a quinoprotein dehydrogenase, and conditions should be set to induce this enzyme [although asynchronous induction may occur (77)]; highest levels of PQQ are attained in organisms with a quinoprotein dehydrogenase that is produced in large amounts [and, accordingly, probably has a low turnover number]. Thus, simple inorganic media with alcohols as a carbon and energy source are adequate for PQQ production, whereas rich media containing peptone, nutrient broth, etc, are not (only constitutive quinoprotein dehydrogenases and PQQ amenable to nucleophilic attack are produced in this latter case). A compilation of PQQ-producing bacteria is given in Ref. 72. The recent insight obtained from the biosynthesis of PQQ by methylotrophic bacteria (see below) allows specific labelling of the molecule by manipulation of the building blocks tyrosine and glutamic acid.

### *By Chemical Synthesis*

Several different routes have been described for the chemical synthesis of PQQ. The method developed by Corey & Tramontano (9) has been modified by us and is routinely used in our laboratory (63) as well as in commercial production (Fluka A. G., Switzerland). Labelled forms and analogues of PQQ have been made via this synthesis route and by other procedures (33, 63).

## COFACTOR ACTIVITY OF ANALOGUES

As has been shown for a number of analogues, modification of the *o*-quinone moiety of PQQ completely destroys the ability to reconstitute quinohemoprotein alcohol dehydrogenase apoenzyme from *Comamonas testosteroni* (34). It also appears that the analogues are bound rather well, so that their inactivity may be related to their inability to catalyze the reaction. Modification of the structure at other positions ($C_{(3)}$,$C_{(8)}$,$N_{(1)}$ is allowed, although activity drops significantly in some cases. The decrease in activity may be due to inadequate binding as PQQ easily displaces the bound analogues [inadequate binding may also explain the absence of activity with PQQ-esters and decarboxy-PQQ (56)]. On the other hand, the change in hydration behavior of the $N_{(1)}$-alkylated analogues could indicate that the electrophilic character of the $C_{(5)}$-carbonyl group has changed, resulting in a lower catalytic activity. Of special note is the activity of the PQQ-acetone adduct. Extraction of the reconstituted apoenzyme showed that only PQQ was present, which indicates an enzyme-mediated conversion of the adduct into PQQ. This process occurs in a stereo-selective way, since only one of the enantiomers is converted. To date, the effect of the analogues on whole organisms has not been studied.

## BIOSYNTHESIS

### *Genes Involved*

Studies of *A. calcoaceticus* have revealed that four complementation groups of mutants are unable to synthesize PQQ. The four genes have been cloned and sequenced, and when they were all transferred to *A. lwoffii* or *E. coli* (both organisms seem unable to produce free PQQ because they contain quinoprotein glucose dehydrogenase apoenzyme), a slight activity of quinoprotein glucose dehydrogenase was observed (23). Similar genes and two others have been found for PQQ biosynthesis in the methylotroph *Methylobacterium organophilum* (6). In addition, genes in methylotrophs may be involved in the assembly of methanol dehydrogenase (48). Because this is a periplasmic enzyme, the process may occur outside the cytoplasmic membrane and allow the escape of PQQ, which is then found in the spent medium.

### *Precursors and Intermediates*

Labelling experiments combined with $^{13}$C-NMR spectroscopy have shown that tyrosine and glutamic acid act as precursors of biosynthesis of free PQQ in methylotrophic bacteria (31, 70). Despite an intensive search for cross feeding between groups of $PQQ^-$-mutants, no positive results were obtained (71). This has led to the suggestion that the whole process occurs on a protein matrix in steps as indicated in Figure 7 (16). If this view is correct, one may

ask, what are the differences between biosynthesis of the covalently bound form and of the free form? In this context, we note that free PQQ is not required for the synthesis of the covalently bound form in glutamate decarboxylase of *E. coli* (62), which suggests that biosynthesis of the cofactor in the enzyme occurs *in situ*. Thus, the four genes required for production of active quinoprotein glucose dehydrogenase may be involved in processing a covalently bound form to free PQQ, which implies that the genes for the route to the covalently bound form are still unknown.

## PHYSIOLOGICAL EFFECTS

### *On Bacteria*

Bacteria producing a quinoprotein dehydrogenase apoenzyme will not grow when the particular substrate for that enzyme is the sole carbon (nitrogen) and energy source in the medium (provided that no other types of dehydrogenase can replace the enzyme). This situation is obviously the case for one of the organisms in a mixed culture degrading polyvinylalcohol (55). One of the *Pseudomonas* species produces the polyvinylalcohol dehydrogenase apoenzyme, but does not produce free PQQ. Therefore, it can only grow on this substrate when PQQ is added to the medium or is provided by another *Pseudomonas* species in the mixed culture.

A somewhat different situation exists for bacteria that are not adversely affected by the absence of active quinoprotein dehydrogenase because: (*a*) the step catalyzed by the enzyme is not essential or (*b*) it can be circumvented by producing another type of dehydrogenase for that step. The first case can be illustrated by strains of *A. calcoaceticus* in which quinoprotein glucose

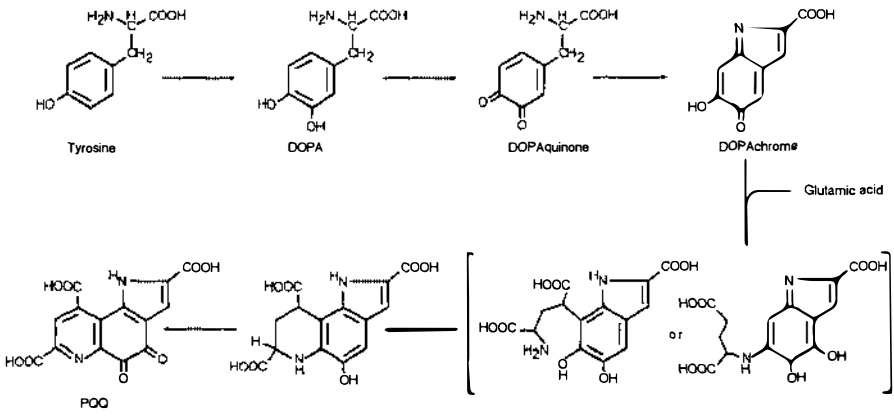


*Figure 7* Proposed steps for the biosynthesis of PQQ (bonds to the protein matrix are not indicated).

dehydrogenase catalyzes the conversion of glucose into gluconic acid. However, most strains lack other routes for glucose conversion and are unable to consume the gluconic acid. It has been shown, however, that glucose can be used as an auxiliary energy source, which illustrates the functionality of the glucose dehydrogenase. Some of these strains are unable to produce free PQQ. Thus when glucose is present in the medium, these strains have a distinct advantage when supplementation occurs with PQQ. A comparable example is provided by *Comamonas testosteroni:* Addition of PQQ shortens the lag time when it is grown on ethanol [most likely, the indication of NAD-dependent alcohol dehydrogenase is rather slow, and the conversion of ethanol via the quinohemoprotein alcohol dehydrogenase initiates growth (25)]. The second case can be illustrated with *E. coli*. Normally, growth on glucose proceeds via the phosphotransferase system (pts), and the route via glucose dehydrogenase will not operate, not even in the presence of PQQ [except under conditions of growth limitation by certain nutrients (47)]. Thus although it seems at first sight that the apoenzyme is superfluous in this organism, it may be essential under certain conditions, as illustrated by a $pts^-$ mutant that shows growth on glucose when PQQ is present (30).

A different physiological effect has been reported in certain bacteria when supplementation of the media with PQQ shortened the lag phase (2). Because several washings of the cells during transfer from the preculture to the medium were necessary to observe the effect (4), stimulation could be explained by the assumption that PQQ had been removed from the (periplasmic) quinoprotein dehydrogenases. However, this may not be the only explanation, because the effect was reportedly observed not only in acetic acid bacteria, but also in yeast (2), a eukaryotic organism in which the presence of enzymes with free PQQ as cofactor has not been reported. Because these are the only reports of this phenomena and stimulation could be due to a secondary effect, further investigations are required before conclusions can be drawn about the existence and causes of this type of physiological effect.

## *On Plants and Animals*

One reported effect of PQQ on plants is the stimulation of *Lilium* pollen germination (82). Quinoprotein enzymes occur in plants (Table 2), but all contain covalently bound PQQ so that stimulation of these enzymes by free PQQ is not obvious.

Mice injected with *E. coli* toxin show a shock effect, but when PQQ is also injected, the effect is less (43). Nutritional studies on these animals were recently reported by Rucker and co-workers (39). Mice fed a chemically defined diet supplemented with 2% succinyl sulfathiozole grew poorly, failed to reproduce, and became osteolathyritic. Those heavily affected had a friable

skin, skin collagen that was readily extractable into neutral salt solutions, and decreased lysyl oxidase. Rats fed with the diet supplemented with PQQ were healthy and behaved normally. Covalently bound PQQ has been found in lysyl oxidase from human placenta (60) and from chicken cartilage (81) [although pyridoxal phosphate has also been reported as cofactor for the latter (40)]. Also in this case, it is difficult to understand how the free PQQ can stimulate the production of active enzyme if it is assumed that biosynthesis of the cofactor of the enzyme occurs *in situ*.

Medical aspects have been disclosed in a number of patents: PQQ has been proposed as a component of an ophthalmic solution used as an anticataract agent (38). In the same report it is noted that PQQ is an inhibitor of tyrosinase ($IC_{50}$ of $7.10^{-4}$M) and aldose reductase ($IC_{50}$ of $5.10^{-6}$M). In another report (46) it is mentioned that PQQ inhibits human placental aldose reductase ($IC_{50}$ of $4.10^{-7}$M) and reverse transcriptase from porcine leukemia retrovirus ($IC_{50}$ of $6.10^{-5}$). PQQ has been reported to be effective in prophylaxis or treatment of bronchial asthma, allergic rhinitis, etc (42). Injection of rats with PQQ (intraperitoneal, 30 mg/kg) completely inhibited ovalbumin-induced anaphylaxis. Harmful effects have also been reported: Nephrotoxicity was observed in rats (injection of 11.5 mg/kg for 4 days) (80). Functional and morphological changes of the proximal tubular epithelium as well as hematuria and elevation of serum creatinine concentration were observed; although not mentioned in the literature cardiotoxic effects may be caused by PQQ in combination with reactive oxygen species formation. *o*-Quinones become reduced to the semiquinone form and produce superoxide and hydroxyl radicals from oxygen (37). Production of these reactive species has indeed been observed when PQQ is incubated with NAD(P)H under aerobic conditions (57). Finally, it should be mentioned that in nutritional experiments with chickens, feed supplemented with Pruteen (single cell protein from methanol-grown bacteria, manufactured by ICI, Agricultural Division, UK) gave excellent results, which might be related to the substantial levels of PQQ in this material (12). A possible relationship between pregnancy in humans and the excessive consumption of vinegar-acidified products (and thus of PQQ) has been suggested (35).

## CONCLUSIONS, IMPLICATIONS, AND PROSPECTS

Free PQQ is only produced by bacteria and is found in their cells, culture media, and in samples containing certain bacterial quinoprotein dehydrogenases after denaturation. Quinoprotein enzymes also occur in plants and animals, but so far all appear to have covalently bound PQQ. Glutamate decarboxylase from *E. coli* also contains this form of the cofactor, and because the organism does not produce free PQQ, free PQQ is not required

for the synthesis of the covalently bound form, which would suggest that the latter might be formed *in situ*. It is unknown whether this observation also applies to the mammalian and plant quinoprotein enzymes.

The presence of uptake systems for PQQ in mammals has not been studied. Vinegar is a source of free PQQ. Food and drinks may be contaminated with PQQ if they come in contact with bacteria. Moreover, it is conceivable that degradation of quinoproteins with covalently bound PQQ in the digestive tract leads to formation of free (derivatized) PQQ and that the microbial flora in the gut could also produce the compound (although *E. coli* does not). On the other hand, in these complex environments it is likely that PQQ is transformed into condensation products with nucleophiles, e.g. into PQQ-oxazoles and PQQ-tryptophan products. However, transport systems (serum albumin) or "oxazolases" (enzymes that hydrolyze the condensation products) might facilitate the availability of PQQ and the expression of its physiological effects. Although no evidence for the presence of free or derivatized PQQ is available in eukaryotes (except in their quinoproteins), all the possibilities mentioned here should be kept in mind when devising nutritional experiments with PQQ (and its condensation products).

Nutritional studies with mice on a synthetic diet led to deleterious effects when no supplemention with PQQ occurred. Although lower levels of lysyl oxidase were found, which would explain the inadequate collagen biosynthesis and the bad condition of the skin, it is difficult to understand how PQQ administration was able to increase the enzyme level. To answer that question, more insight is needed concerning the production of free PQQ by mammals and the presence of uptake systems for the compound, and the biosynthesis of covalently bound PQQ in the quinoproteins. Therefore, the effects of PQQ observed in the nutritional experiments can also be explained by assuming the exertion of noncofactor effects [for instance, PQQ may act as a general dicarbonyl compound and shift the balance of acceptor/donor compounds involved in regulation of cell division in living systems, or it may remove pyridoxal phosphate (PLP) from serum albumin by competing for the same binding site (59)]. Thus, although several reports mention the positive effects of PQQ on administration to plants and animals, the physiological role of the compound in these organisms has not been determined at the present time.

The carbonyl groups of PQQ are similar in reaction to the one in the nonnucleotide cofactor PLP. Thus drugs and inhibitors previously thought to act on the aldehyde function of PLP (carbonyl group reactive compounds, lathyrogenic agents) may in fact (also) act on PQQ. Conceivably, PLP and PQQ may replace each other to a certain extent when the same transport or storage systems are used. Therefore, to discriminate between the physiological effects of PLP and PQQ, more selective inhibitors and drugs should be developed.

Recently, it was proposed that a whole class of enzymes contains the free radical of tyrosine as cofactor (52). Pro-PQQ and PQQ can be viewed as extensions of tyrosine-based cofactors (an evolutionary relationship might exist between these nonphosphorylated amino acid cofactors). At present, insight is scant with respect to biosynthesis and the effect of deficiencies on mammalian organisms of these cofactors. Since phenylalanine (and thus tyrosine) is an essential amino acid, several effects ascribed in the past to its absence could in fact be related to inadequate levels of these cofactors.

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*Literature Cited*

1. Adachi, O., Shinagawa, E., Matsushita, K., Nakashima, K., Takimoto, K., Ameyama, M. 1989. See Ref. 18, pp. 145–47
2. Ameyama, M., Shinagawa, E., Matsushita, K., Adachi, O. 1984. Growth stimulating substance for microorganisms produced by *Escherichia coli* causing the reduction of the lag phase in microbial growth and identity of the substance with pyrroloquinoline quinone. *Agric. Biol. Chem.* 48:3099–3107
3. Ameyama, M., Shinagawa, E., Matsushita, K., Adachi, O. 1985. Growth stimulating activity for microorganisms in naturally occurring substances and partial characterization of the substances for the activity as pyrroloquinoline quinone. *Agric. Biol. Chem.* 49:699–709
4. Ameyama, M., Shinagawa, E., Matsushita, K., Adachi, O. 1985. How many times should the inoculum cells be rinsed before inoculation in the assay for the growth stimulating activity of pyrroloquinoline quinone? *Agric. Biol. Chem.* 49:853–54
5. Anthony, C., Zatman, L. J. 1967. The microbial oxidation of methanol. The prosthetic group of the alcohol dehydrogenase of *Pseudomonas* sp. M27. *Biochem. J.* 104:960–69
6. Biville, F., Turlin, E., Gasser, F. 1989. Cloning and genetic analysis of six pyrroloquinoline quinone biosynthesis genes in *Methylobacterium organophilum* DSM760. *J. Gen. Microbiol.* 135: 2917–29
7. Bouvet, O. M. M., Lenormand, P., Grimont, P. A. D. 1989. Taxonomic diversity of the D-glucose oxidation pathway in the *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* 39:61–67
8. Citro, G., Verdina, A., Galati, R., Floris, G., Sabatini, S., Finazzi-Agro, A. 1989. Production of antibodies against the coenzyme pyrroloquinoline quinone. *FEBS Lett.* 247:201–4
9. Corey, E. J., Tramontano, A. 1981. Total synthesis of the quinonoid alcohol dehydrogenase coenzyme (1) of methylotrophic bacteria. *J. Am. Chem. Soc.* 103:5599–5600
10. Davies, H. M., Hawkins, D. J., Smith, L. A. 1989. Quinoprotein characteristics of *N*-methylputrescine oxidase from tobacco roots. *Phytochemistry* 28:1573–78
11. Duine, J. A. 1990. PQQ, an elusive coenzyme? *Trends Biochem. Sci.* 15: 96
12. Duine, J. A., Frank, J. 1980. The prosthetic group of methanol dehydrogenase. Purification and some of its properties. *Biochem. J.* 187:221–26
13. Duine, J. A., Frank, J., Jongejan, J. A. 1987. Enzymology of quinoproteins. *Adv. Enzymol.* 59:169–212
14. Duine, J. A., Frank, J., Verwiel, P. E. J. 1980. Structure and activity of the prosthetic group of methanol dehydrogenase. *Eur. J. Biochem.* 108:187–92
15. Duine, J. A., Groen, B. W., Jongejan, J. A., van der Meer, R. A. 1990. The redox-cycling assay is not suited for the detection of PQQ in biological samples. *FEBS Lett.* 261:131–34
16. Duine, J. A., Jongejan, J. A. 1989. Pyrroloquinoline quinone (PQQ): a novel redox cofactor. *Vitam. Horm.* 45:223–62

17. Duine, J. A., Jongejan, J. A. 1989. Enzymes with pyrroloquinoline quinone as cofactor. *Annu. Rev. Biochem.* 58:403–26
18. Duine, J. A., Jongejan, J. A., eds. 1989. *PQQ and Quinoproteins. Proc. 1st Int. Symp. PQQ and Quinoproteins, Delft, The Netherlands, 1988.* Dordrecht: Kluwer Academic. 379 pp.
19. Gacheru, S. N., Trackman, P. C., Calaman, S. D., Greenaway, F. T., Kagan, H. M. 1989. Vicinal diamines as pyrroloquinoline quinone-directed irreversible inhibitors of lysyl oxidase. *J. Biol. Chem.* 264:12963–69
20. Gallop, P. M., Paz, M. A., Flueckiger, R., Kagan, H. M. 1989. PQQ, the elusive coenzyme. *Trends Biochem. Sci.* 14:343–46
21. Geiger, O., Goerisch, H. 1989. Reversible thermal inactivation of the quinoprotein glucose dehydrogenase. *Biochem. J.* 261:415–21
22. Glatz, Z., Kovar, J., Macholan, L., Pec, P. 1987. Pea *(Pisum sativum)* diamine oxidase contains pyrroloquinoline quinone as a cofactor. *Biochem. J.* 242:603–6
23. Goosen, N., Horsman, H. P. A., Huinen, R. G. M., van de Putte, P. 1989. *Acinetobacter calcoaceticus* genes involved in biosynthesis of the coenzyme pyrroloquinoline quinone: nucleotide sequence and expression in *Escherichia coli* K-12. *J. Bacteriol.* 171:447–55
24. Groen, B. W., van der Meer, R. A., Duine, J. A. 1988. Evidence for PQQ as cofactor in 3,4-dihydroxyphenylalanine (dopa) decarboxylase of pig kidney. *FEBS Lett.* 237:98–102
25. Groen, B. W., van Kleef, M. A. G., Duine, J. A. 1986. Quinohaemoprotein alcohol dehydrogenase apoenzyme from *Pseudomonas testosteroni. Biochem. J.* 234:611–15
26. Harder, W., Attwood, M. M. 1978. Biology, physiology and biochemistry of *Hyphomicrobia. Adv. Microbiol. Physiol.* 17:303–59
27. Hartmann, C., Klinman, J. P. 1988. Pyrroloquinoline quinone: a new redox cofactor in eukaryotic enzymes. *Biofactors* 1:41–49
28. Hauge, J. G. 1964. Glucose dehydrogenase of *Bacterium anitratum:* an enzyme with a novel prosthetic group. *J. Biol. Chem.* 239:3630–36
29. Hazeu, W., de Bruyn, J. C., van Dijken, J. P. 1983. *Nocardia* sp. 239, a facultative methanol utilizer with the ribulose monophosphate pathway of formaldehyde fixation. *Arch. Microbiol.* 135:205–10
30. Hommes, R. W. J., Postma, P. W., Neijssel, O. M., Tempest, D. W., Dokter, P., Duine, J. A. 1984. Evidence of a quinoprotein glucose dehydrogenase apo-enzyme in several strains of *Escherichia coli. FEMS Microbiol. Lett.* 24:329–33
31. Houck, D. R., Hanners, J. L., Unkefer, C. J. 1988. Biosynthesis of pyrroloquinoline quinone. Identification of biosynthetic precursors using $^{13}C$ labeling and NMR spectroscopy. *J. Am. Chem. Soc.* 110:6920–21
32. Ishida, T., Doi, M., Tomita, K., Hayashi, H., Inone, M., Urakami, T. 1989. Molecular and crystal structure of PQQ (methoxatin), a novel coenzyme of quinoproteins: extensive stacking character and metal ion interaction. *J. Am. Chem. Soc.* 111:6822–28
33. Jongejan, J. A. 1989. *Chemistry of PQQ*. PhD thesis. Delft Univ. Technol., Delft, The Netherlands. 143 pp.
34. Jongejan, J. A., Groen, B. W., Duine, J. A. 1989. See Ref. 18, pp. 205–16
35. Jongejan, J. A., van der Meer, R. A., Duine, J. A. 1986. Biochemical explanations for folk tales. A vitamin in disguise? *Trends Biochem. Sci.* 11:511
36. Jongejan, J. A., van der Meer, R. A., van Zuylen, G. A., Duine, J. A. 1987. Spectrophotometric studies on pyrroloquinoline quinone-copper(II) complexes as possible models for copper-quinoprotein amine oxidases. *Recl. Trav. Chim. Pays-Bas* 106:365
37. Kalyanaraman, B., Korytowski, W., Pilas, B., Sarna, T., Land, E. J., Truscott, T. G. 1988. Reaction between *ortho*-semiquinones and oxygen: pulse radiolysis, electron spin resonance, and oxygen uptake studies. *Arch. Biochem. Biophys.* 266:277–84
38. Katsumata, M., Osawa, Y., Nakagiri, T., Uchikuga, S. 1988. Pyrroloquinolinedione derivatives as anticataract agents. *Chem. Abstr.* 110:29104
39. Killgore, J., Smidt, C., Duich, L., Romero-Chapman, N., Tinker, D., et al. 1989. Nutritional importance of pyrroloquinoline quinone. *Science* 245: 850–52
40. Levene, C. I., O'Shea, M. P., Carrington, M. J. 1988. Protein lysine 6-oxidase (lysyl oxidase) cofactor: methoxatin (PQQ) or pyridoxal? *Int. J. Biochem.* 20:1451–56
41. Lobenstein-Verbeek, C. L., Jongejan, J. A., Frank, J., Duine, J. A. 1984. Bovine serum amine oxidase: a mamma-

lian enzyme having covalently bound PQQ as prosthetic group. *FEBS Lett.* 170:305–9

42. Maruyama, Y., Iwayama, A., Satoshi, O. 1988. Allergy inhibitors containing 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid or its salts. *Chem. Abstr.* 109:222472
43. Matsumoto, T., Suzuki, O., Hayakawa, H., Ogiso, S., Hayakawa, N., et al. 1989. See Ref. 18, pp. 162–64
44. McIntire, W. S., Stults, J. T. 1986. On the structure and linkage of the covalent cofactor of methylamine dehydrogenase from the methylotrophic bacterium W3A1. *Biochem. Biophys. Res. Commun.* 141:562–68
45. Moog, R. S., McGuirl, M. A., Cote, C. E., Dooley, D. M. 1986. Evidence for methoxatin (pyrroloquinoline quinone) as the cofactor in bovine plasma amine oxidase from resonance Raman spectroscopy. *Proc. Natl. Acad. Sci. USA* 83:8435–39
46. Narutomi, Y., Katsumata, M., Osawa, Y., Uchikuga, S. 1988. Pyrroloquinoline quinones as enzyme inhibitors of aldose reductase, glyoxalase I, and reverse transcriptase. *Chem. Abstr.* 109:222458
47. Neijssel, O. M., Hommes, R. W. J., Postma, P. W., Tempest, D. W. 1989. See Ref. 18, pp. 57–67
48. Nunn, D. N., Lidstrom, M. E. 1986. Phenotypic characterization of 10 methanol oxidation mutant classes in *Methylobacterium* sp. strain AM1 *J. Bacteriol.* 166:591–97
49. Paz, M. A., Flueckiger, R., Henson, E., Gallop, P. M. 1989. See Ref. 18, pp. 131–43
50. Paz, M. A., Gallop, P. M., Torrelio, B. M., Flueckiger, R. 1988. The amplified detection of free and bound methoxatin (PQQ) with nitroblue tetrazolium redox reactions: insights into the PQQ locus. *Biochem. Biophys. Res. Commun.* 154: 1330–37
51. Pennings, E. J. M ., Groen, B. W., Duine, J. A., Verpoorte, R. 1989. Tryptophan decarboxylase from *Catharantus roseus* is a pyridoxo-quinoprotein. *FEBS Lett.* 255:97–100
52. Prince, R. C. 1988. Tyrosine radicals. *Trends Biochem. Sci.* 13:286–88
53. Rinaldi, A., Giartosio, A., Floris, G., Medda, R., Finazzi-Agro, A. 1984. Lentil seedling amine oxidase: preparation and properties of the copper-free enzyme. *Biochem. Biophys. Res. Commun.* 120:242–49
54. Salisbury, S. A., Forrest, H. S., Cruse, W. B. T., Kennard, O. 1979. A novel coenzyme from bacterial primary dehydrogenases. *Nature* 280:843–44
55. Shimao, M., Yamamoto, H., Ninomiya, K., Kato, N., Adachi, O., et al. 1984. Pyrroloquinoline quinone as an essential growth factor for a poly(vinyl alcohol)-degrading symbiont, *Pseudomonas* ap. VM15c. *Agric. Biol. Chem.* 48:2873–76
56. Shinagawa, E., Matsushita, K., Nonobe, M., Adachi, O., Ameyama, M., Ohshiro, Y. 1986. The 9-carboxyl group of pyrroloquinoline quinone, a novel prosthetic group, is essential in the formation of holoenzyme of D-glucose dehydrogenase. *Biochem. Biophys. Res. Commun.* 139:1279–84
57. Sugioka, K., Nakano, M., Naito, I., Tero-Kubota, S., Ikegami, Y. 1988. Properties of a coenzyme, pyrroloquinoline quinone: generation of an active oxygen species during a reduction-oxidation cycle in the presence of NAD(P)H and $O_2$. *Biochim. Biophys. Acta* 964:175–82
58. Suzuki, O., Kumazawa, T., Seno, H., Matsumoto, T., Urakami, T. 1989. See Ref. 18, pp. 123–29
59. Szent-Györgyi, A. 1976. *Electronic Biology and Cancer.* New York: Dekker
60. van der Meer, R. A., Duine, J. A. 1986. Covalently-bound pyrroloquinoline quinone is the organic prosthetic group in human placental lysyl oxidase. *Biochem. J.* 239:789–91
61. van der Meer, R. A., Duine, J. A. 1988. Pyrroloquinoline quinone is the organic cofactor in soybean lipoxygenase-1. *FEBS Lett.* 235:194–200
62. van der Meer, R. A., Groen, B. W., Duine, J. A. 1989. On the biosynthesis of free and covalently bound PQQ: glutamic acid decarboxylase from *Escherichia coli* is a pyridoxo-quinoprotein. *FEBS Lett.* 246:109–12
63. van der Meer, R. A., Groen, B. W., van Kleef, M. A. G., Frank, J., Jongejan, J. A., Duine, J. A. 1990. Isolation, preparation, and assay of pyrroloquinoline quinone. *Methods Enzymol.* 100:In press
64. van der Meer, R. A., Jongejan, J. A., Duine, J. A. 1987. Phenylhydrazine as probe for cofactor identification in amine oxidoreductases. Evidence for PQQ as the cofactor in methylamine dehydrogenase. *FEBS Lett.* 221:299–304
65. van der Meer, R. A., Jongejan, J. A., Duine, J. A. 1988. Dopamine β-hydroxylase from bovine adrenal medulla contains covalently bound PQQ. *FEBS Lett.* 231:303–7
66. van der Meer, R. A., Jongejan, J. A.,

Duine, J. A. 1989. See Ref. 18, pp. 111–22
67. van der Meer, R. A., Mulder, A. C., Jongejan, J. A., Duine, J. A. 1989. Determination of pyrroloquinoline quinone in quinoproteins with covalently bound cofactor and in PQQ derivatives. *FEBS Lett.* 254:99–105
68. van der Meer, R. A., van Wassenaar, P. D., van Brouwershaven, J. H., Duine, J. A. 1989. Primary structure of pyrroloquinoline quinone containing peptide isolated from porcine kidney diamine oxidase. *Biochem. Biophys. Res. Commun.* 159:726–33
69. van Kleef, M. A. G., Dokter, P., Mulder, A. C., Duine, J. A. 1987. Detection of PQQ. *Anal. Biochem.* 162:143–49
70. van Kleef, M. A. G., Duine, J. A. 1988. L-tyrosine is the precursor of PQQ biosynthesis in *Hyphomicrobium* X. *FEBS Lett.* 237:91–97
71. van Kleef, M. A. G., Duine, J. A. 1989. A search for intermediates in the biosynthesis of PQQ. *BioFactors* 1:297–302
72. van Kleef, M. A. G., Duine, J. A. 1989. Factors relevant in bacterial PQQ production. *Appl. Environ. Microbiol.* 55:1209–13
73. van Kleef, M. A. G., Jongejan, J. A., Duine, J. A. 1989. Factors relevant in the reaction of PQQ with amino acids. Analytical and mechanistic implications. *Eur. J. Biochem.* 183:41–47
74. van Kleef, M. A. G., Jongejan, J. A., Duine, J. A. 1989. See Ref. 18, pp. 217–26
75. van Koningsveld, H., Jongejan, J. A., Duine, J. A. 1989. See Ref. 18, pp. 243–51
76. van Schie, B. J., de Mooy, O. H., Linton, J. D., van Dijken, J. P., Kuenen, J. G. 1987. PQQ-dependent production of gluconic acid by *Acinetobacter, Agrobacterium* and *Rhizobium* species. *J. Gen. Microbiol.* 133:867–75
77. van Schie, B. J., van Dijken, J. P., Kuenen, J. G. 1984. Noncoordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. *FEMS Microbiol. Lett.* 24:133–38
78. Vellieux, F. M. D., Hol, W. G. J. 1989. A new model for the pro-PQQ cofactor of quinoprotein methylamine dehydrogenase. *FEBS Lett.* 255:460–64
79. Vellieux, F. M. D., Huitema, F., Groendijk, H., Kalk, K. H., Frank, J., et al. 1989. Structure of quinoprotein methylamine dehydrogenase at 2.25 Å resolution. *EMBO J.* 8:2171–78
80. Watanabe, A., Hobara, N., Ohsawa, T., Higashi, T., Tsuji, T. 1989. Nephrotoxicity of pyrroloquinoline quinone in rats. *Chem. Abstr.* 111:53070
81. Williamson, P. R., Moog, R. S., Dooley, D. M., Kagan, H. M. 1986. Evidence for pyrroloquinoline quinone as the carbonyl cofactor in lysyl oxidase by absorption and resonance Raman spectroscopy. *J. Biol. Chem.* 261:9477–82
82. Xiong, L. B., Sekiya, J., Shimose, N. 1988. Stimulation of *Lilium* pollen germination by pyrroloquinoline quinone. *Agric. Biol. Chem.* 52:1065–66