

Physiological Importance of Quinoenzymes and the O-Quinone Family of Cofactors

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ABSTRACT O-quinone cofactors derived from tyrosine and tryptophan are involved in novel biological reactions that range from oxidative deaminations to free-radical redox reactions. The formation of each of these cofactors appears to involve post-translational modifications of either tyrosine or tryptophan residues. The modifications result in cofactors, such as topaquinone (TPQ), tryptophan tryptophylquinone (TTQ), lysine tyrosylquinone (LTQ) or the copper-complexed cysteinyl-tyrosyl radical from metal-catalyzed reactions. Pyrroloquinoline quinone (PQQ) appears to be formed from the annulation of peptidyl glutamic acid and tyrosine residues stemming from their modification as components of a precursor peptide substrate. PQQ, a primary focus of this review, has invoked considerable interest because of its presence in foods, antioxidant properties and role as a growth-promoting factor. Although no enzymes in animals have been identified that exclusively utilize PQQ, oral supplementation of PQQ in nanomolar amounts increases the responsiveness of B- and T-cells to mitogens and improves neurologic function and reproductive outcome in rodents. Regarding TPQ and LTQ, a case may be made that the formation of TPQ and LTQ is also influenced by nutritional status, specifically dietary copper. For at least one of the amine oxidases, lysyl oxidase, enzymatic activity correlates directly with copper intake. TPQ and LTQ are generated following the incorporation of copper by a process that involves the two-step oxidation of a specified tyrosyl residue to first peptidyl dopa and then peptidyl topaquinone to generate active enzymes, generally classed as "quinoenzymes." Limited attention is also paid to TTQ and the copper-complexed cysteinyl-tyrosyl radical, cofactors important to fungal and bacterial redox processes. *J. Nutr.* 130: 719–727, 2000.

KEY WORDS: • *pyrroloquinoline quinone* • *topaquinone* • *tryptophan tryptophylquinone* • *lysine tyrosylquinone* • *quinoproteins*

A novel family of redox enzymes, the quinoenzymes, utilizes o-quinones derived from tyrosine or tryptophan as coenzymes or cofactors. Quinoenzymes exhibit unusual fluorescence properties and catalyze redox reactions by free-radical mechanisms (McIntire 1998). Pyrroloquinoline quinone (PQQ)³ was the first of the ortho-(O)-quinone cofactors to be identified (Salisbury et al. 1979). In many prokaryotic organisms, PQQ serves as a noncovalent cofactor for various simple sugar and alcohol dehydrogenases (Goodwin and Anthony 1998). Following the discovery of PQQ, (Fig. 1), subsequent observations led to the identification of other cofactors in the o-quinone cofactor family, e.g., tryptophan tryptophylquinone (TTQ), trihydroxyphenylalanine quinone (topaquinone or TPQ), lysine tyrosylquinone (LTQ) and the copper-complexed cysteinyltyrosyl radical. In contrast to PQQ, these cofactors are covalently linked to the enzymes that they serve (Fig. 2).

In the first part of this review, observations related to the

nutritional importance of PQQ will be addressed. The observations supporting nutritional importance of PQQ are interesting and at some levels even compelling, although far from complete. Following the discovery of PQQ, the compound was reported to be present as an active-site cofactor in over 20 enzymes derived from plant and animal tissues. This is now known not to be the case. PQQ was incorrectly identified as the cofactor for enzymes that contained TPQ or LPQ, owing in part to chemical similarities between the compounds and the acceptance of descriptive and equivocal chemical evidence.

In the second part of this review, functional relationships involving TPQ and LPQ will be described. It is now well established that TPQ and LTQ are found at the active sites of copper-containing amine oxidases, such as diamine oxidase (TPQ) and lysyl oxidase (LTQ). The formation and activity of TPQ- and LTQ-requiring enzymes are dependent on dietary copper intake. Mention will also be made of TTQ and the copper-complexed cysteinyltyrosyl radical, although these cofactors appear to be utilized primarily in bacterial and fungal quinoenzymes.

PQQ

Chemistry. PQQ, (4,5-dihydro-4, 5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) or methoxatin, as it is desig-

¹ This review was funded in part by grants NIH-HD 26777, NIH-AM 25358 and USDA-NRI 94-372-00-0696, and a generous gift from Mars, Incorporated.

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³ Abbreviations used: ECM, extracellular matrix; GDH, glucose dehydrogenase; GSH, glutathione; IPQ, imidazolopyrroloquinoline quinone; LTQ, lysine tyrosylquinone; NGF, nerve growth factor; NMDA, N-methyl-D-aspartic acid; PAQ, phenanthrene quinone; PL, pyridoxal; PQQ, pyrroloquinoline quinone; TME, trimethyl ester; TOPA, trihydroxyphenylalanine; TPQ, topaquinone; TTQ, tryptophan tryptophylquinone.

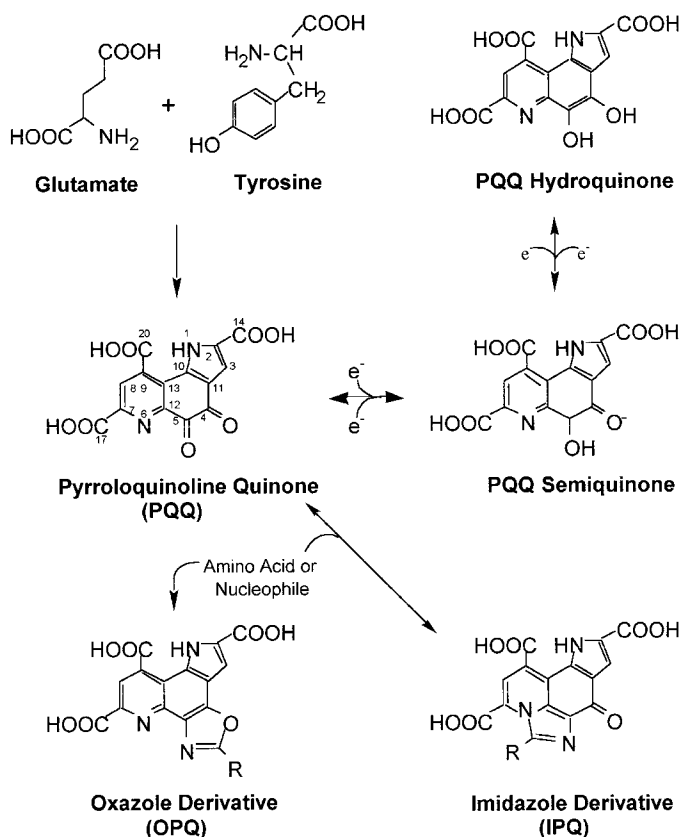


FIGURE 1 Pyrroloquinoline quinone (PQQ) and related derivatives.

nated in earlier literature, is highly soluble, heat-stable and capable of continuous redox cycling (Fig. 1). In the presence of amino acids, PQQ readily forms numerous imidazole derivatives and, with time, oxazole adducts (Mitchell et al. 1999).

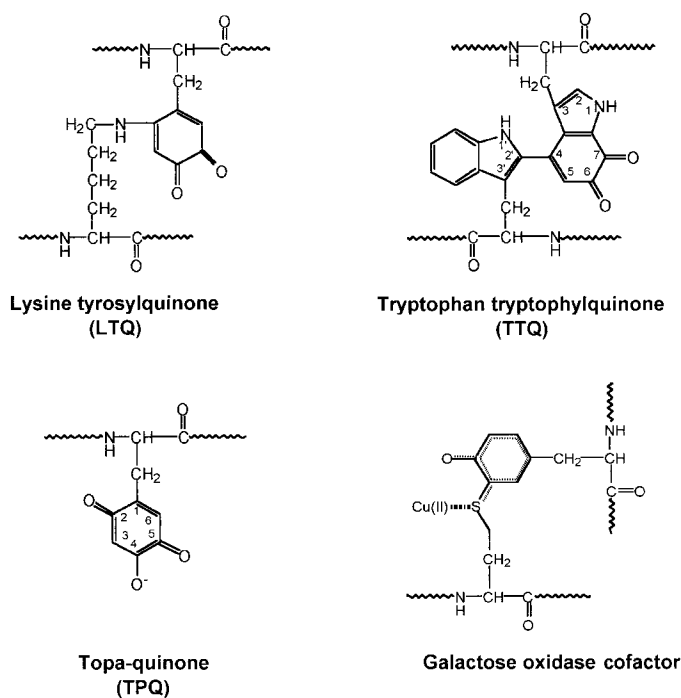


FIGURE 2 Tyrosine and tryptophan-derived prosthetic groups of quinoenzymes. Lysine tyrosylquinone (LTQ), tryptophylquinone tryptophan (TTQ), topaquinone (TPQ) and the copper-complexed cysteinyl-tyrosyl radical, often designated as the galactose oxidase cofactor.

The chemical properties of PQQ are analogous to combining some of the best chemical features of ascorbic acid (reducing potential), riboflavin (redox reactions), and pyridoxal (PL) (carbonyl reactivity) cofactors into one molecule. PQQ has the ability to carry out redox cycling so that picomole amounts of PQQ are capable of generating micromolar amounts of product (Fluckiger et al. 1993, 1995). On a molar basis, PQQ is at least 100 times more efficient than ascorbic acid, menadione and all of the isoflavinoids and polyphenolic compounds tested to date in assays that assess redox cycling potential (Fig. 3).

PQQ can also substitute for riboflavin in certain reductases (Xu et al. 1993). For example, PQQ is a high-affinity substrate for erythrocyte flavin reductase. For bovine erythrocyte flavin reductase, the apparent K_m for PQQ is $2 \mu\text{mol/L}$, which is less than the values observed for riboflavin, FMN, or FAD, e.g., $\sim 10\text{--}25 \mu\text{mol/L}$ (Hultquist et al. 1993). The pyrroloquinoline quinol generated by this reaction also interacts rapidly with ferryl myoglobin radical and can protect isolated rabbit heart preparations from re-oxygenation injury (Xu et al. 1993).

PQQ catalyzes the continuous oxidation of primary amines. For example, PQQ can carry out the oxidation of pyridoxamine 5'-phosphate to PL 5'-phosphate at rates that even exceed those of pyridoxamine 5'-phosphate oxidase (Churchich 1989). PQQ also catalyzes the nonspecific oxidation of peptidyl lysine in elastin and collagen substrates (Shah et al. 1992). The optimal rate of elastin oxidation occurs at a 2:1 PQQ:Cu²⁺ ratio. Elastin oxidation under aerobic conditions results in the formation of aldehydes and eventually interchain crosslinks.

Prokaryotic production and utilization of PQQ. Numerous bacteria including methylotrophic bacteria, *Pseudomonas*

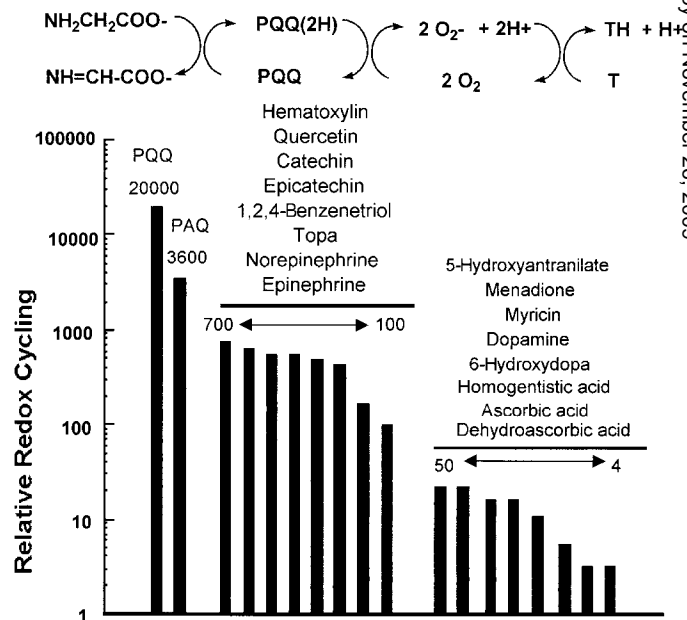


FIGURE 3 Pyrroloquinoline quinone (PQQ) and redox cycling. Glycine at alkaline pH will drive reactions involving quinones or dioxo derivatives to corresponding hydroquinols or enediols. When coupled to nitroblue tetrazolium (T) the rates of reaction and amounts of products may be followed by measuring formazan production (TH). Shown here are data from various sources (cf. Gallop et al. 1990 and Rucker R. B., unpublished) expressed as relative formazan production (absorbance at 530 nm) per hour per μmol of quinone or oxo-related derivative. Note that PQQ is 30 to 5000 times more efficient in sustaining redox cycling initiated by glycine than other common quinones, e.g., catechin, or enediols, e.g., ascorbic acid. PAQ = phenanthrene quinone.

Acetobacter and *Gluconobacter* species are capable of PQQ synthesis (Duine et al. 1990). Currently, the best evidence indicates that PQQ is derived from an annulation between glutamyl (or glutaminyl) and tyrosyl residues (Houck et al. 1988). Although the pathway for PQQ synthesis remains to be resolved, all of the requisite genes for PQQ synthesis have been cloned. *Acinetobacter calcoaceticus* requires at least four genes for PQQ synthesis (Goosen et al. 1989), whereas seven genes are required in *Methylobacterium extorquens* (Morris et al. 1994). In *A. calcoaceticus*, three of the four genes encode proteins of molecular weights of 29,700, 10,800 and 43,600 Da. The fourth gene encodes a polypeptide of only 24 amino acids. In the absence or modification of this gene, optimal PQQ synthesis does not occur. Moreover, the small peptide is relatively conserved, although size of the peptide varies depending upon the organism. The peptide is 23 amino acids in *Klebsiella pneumoniae* (Meulenberg et al. 1992), 29 amino acids in *Methylobacterium extorquens* (Morris et al. 1994), 24 amino acids in *Methylobacillus flagellatum* (Gomelsky et al. 1996) and 24 or 39 amino acids in *Pseudomonas fluorescens* (Schnider et al. 1995). The site of synthesis is thought to involve a conserved glu-X-X-X-tyr motif in the small peptide. In *A. calcoaceticus*, when glutamate is replaced by aspartate or tyrosine is replaced by phenylalanine, PQQ is not synthesized (Goosen et al. 1992). In *K. pneumoniae* a shift in the reading frame of the small peptide also abolishes PQQ synthesis (Meulenberg et al. 1992). These observations imply that the peptide serves as a complex substrate for PQQ synthesis. However, a recent study by Toyama and Lidstrom (1998) suggests that an alternative path for PQQ synthesis may exist in *M. extorquens*. In mutants lacking the gene for the small peptide, PQQ is synthesized, but at a reduced rate, i.e., 10–20% of that for the corresponding *M. extorquens* wild type.

It is interesting to note that there are bacterial organisms that do not make PQQ even though they contain proteins that require PQQ as a cofactor. In the closely related enteric bacterial family, *K. pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*, all synthesize a PQQ-dependent enzyme glucose dehydrogenase (GDH), but only *K. pneumoniae* synthesizes an active holo-GDH enzyme (Matsushita et al. 1997). *E. coli* and *S. typhimurium* are not capable of PQQ synthesis, but are capable of utilizing PQQ as a "vitamin" under certain nutrient-limiting conditions. The apo-GDH from *E. coli*, *S. typhimurium* and similar organisms becomes functional (holo-GDH) when PQQ is added. The reconstitution of GDH and other PQQ requiring enzymes with PQQ has become the basis for sensitive enzyme assays for PQQ quantitation (van der Meer et al. 1990). *E. coli* mutants that are dysfunctional in glucokinase and phosphotransferase are able to grow similarly to wild type when supplemented with PQQ (Adamowicz et al. 1991). In addition, PQQ is a chemotactic attractant in *E. coli* organisms (Dejonge et al. 1996). By using PQQ, *E. coli* retains an alternate glucose-metabolizing pathway, which could provide selective advantage, particularly in aerobic- and phosphate-limiting environments (Fliege et al. 1992).

PQQ in foods. In addition to bacteria and fermented products, PQQ has been detected in animal and plant tissues (Bishop et al. 1994, Kumazawa et al. 1995, Mitchell et al. 1999). Several approaches for detection and quantitation of PQQ have been described. However, problems associated with specificity or exclusion of PQQ-like compounds have led to both over- and underestimation of PQQ and PQQ-like compounds. For example, the methods based on redox cycling are sensitive (Fig. 3), but may overestimate PQQ concentrations owing to lack of specificity (Fluckiger et al. 1995, Paz et al. 1996). Other methods based on enzymatic (Geiger and

Gorisch 1987, Olsthoorn and Duine 1996), immunologic (Marini et al. 1993, Narita and Morishita 1995), or chemical derivation and chromatographic separation (Bergethon 1990, Buffoni et al. 1992, Esaka et al. 1993, Kano et al. 1991, Kumazawa et al. 1992, 1995, Zhang et al. 1995) are more specific, but often exclude PQQ derivatives. These methods therefore may underestimate the total amounts of PQQ and PQQ derivatives.

PQQ easily forms imidazole derivatives in the presence of amino acids. Owing to the high reactivity of PQQ and the complex nature of tissues and biological fluids, PQQ most likely exists in foods as either imidazole or oxazole adducts. Mitchell et al. (1999) determined that human milk contains 140–180 ng/mL of PQQ and IPQ of which ~ 80–90% is present as IPQ. On a dry weight basis, this amounts to 1–2 µg of PQQ/g of solid, which is also similar to the PQQ concentrations reported for bovine milk (Fluckiger et al. 1993). Table 1 contains concentrations of PQQ and selected vitamins in foods. The reported values for PQQ in the table were from studies in which chromatographic separation and validation of PQQ and PQQ-like compounds were achieved by either chemical derivation or by mass spectrometry. PQQ is present at the same order of magnitude as biotin and 5–10% of values typically reported for folic acid. It is also important to note that PQQ appears readily absorbed. Smidt et al. (1991a) determined that the apparent absorption of an oral dose of ¹⁴C-PQQ (80 nmol or ~30 µg) ranges from 20 to 80%. The ¹⁴C-PQQ was administered to adult mice in the fed state. The percentages were estimated from the amount of radioactivity present in urine and tissues at 24 h after administration.

Importance of PQQ to animal growth and development.

Although the function of PQQ in animals remains unclear, the ability to carry out continuous redox cycling suggests a role for PQQ as a cofactor or antioxidant. Mice fed chemically defined diets devoid of PQQ that are otherwise nutritionally adequate have impaired neonatal growth and abnormal features. Maximal growth is achieved when as little as 1 nmol or 300 ng of PQQ is added per g of diet or per 4–5 kcal (16–17 KJ) (Steinberg et al. 1994). Signs of PQQ deprivation include friable skin, evidence of hemorrhage and diverticuli, reduction in general fitness, and a hunched posture. Decreased fertility (fewer successful pregnancies and smaller litter size) and defects in immune function (reduction in interleukin-2 levels and loss of sensitivity of B- and T-cells to mitogens) also occurs with PQQ deficiency (Steinberg et al. 1994).

Further, the number and size of mitochondria are affected by PQQ deficiency. PQQ-deficient mice have a 30–40% reduction in the numbers of mitochondria compared to supplemented mice. The mitochondrial area per cell is also reduced (~30%) in PQQ-deficient mice compared to corresponding supplemented mice. Further, mitochondrial preparations from PQQ-deficient mice respond poorly in assays designed to assess the respiratory control (Stites et al. 1996). For example, the values for the respiratory control rate and P/O ratio are observed to be within normal ranges for PQQ-supplemented and -deficient mice. However, less than half of the mitochondrial preparations from PQQ-deficient mice remained viable or patent in assays, whereas all of the preparations from PQQ-supplemented mice could be used.

The response to PQQ supplementation is most obvious in neonates (F₁ generation) derived from PQQ-deprived dams (F₀ generation). However, at the onset of sexual maturation (6–8 wk), F₁ generation mice fed PQQ-deficient diets exhibit "catch-up" growth (Steinberg et al. 1994). At this time, PQQ is detected in fecal samples. Note that studies by Smidt et al. (1991b) suggest that PQQ is not synthesized by microorgan-

TABLE 1

Concentration of pyrroloquinoline quinone (PQQ), biotin, folate, vitamin B-12 and vitamin B-6 in selected foods¹

Food	PQQ	Biotin	Folate	Vitamin B-12	Riboflavin	Vitamin B-6
	$\mu\text{g}/\text{kg}$ or L					
Fruits ²						
Apple	6	10	28	0	140	480
Banana	13	40	191	0	1000	5780
Kiwi fruit	27	—	0.27	0	280	670
Oranges	7	10	303	0	400	600
Papaya	27	—	380	0	320	190
Tomatoes	9	—	150	0	480	800
Vegetables ²						
Cabbage	16	1	430	0	400	960
Carrot	17	30	140	0	590	1470
Celery	6	1	280	0	450	870
Green pepper	28	—	220	0	300	2480
Parsley	34	—	1520	0	980	900
Legumes ²						
Broad bean	18	—	1041	0	890	720
Tofu (bean curd)	24	—	330	0	1030	610
Fermented products						
Cocoa powder ³	800	—	148	19	3320	3170
Fermented soybeans (natto) ²	61	—	80	0	1900	1300
Vinegar, white ⁴	0.3–2.0	0	0	0	0	10
Animal products						
Milk, human ³	140–180	5–9	80–130	0.16–0.97	400–600	90–310
Milk, cow ²	3.4	32	52	3.7	1670	430
Egg yolk ²	7	469	1500	32	6560	4020
Egg white ²	4.1	72	31	2	4640	40
Guinea-pig neutrophils ⁵	>10 $\mu\text{g}/10^8$ cells					

¹ Vitamin values are from the Handbook of Milk Composition (Jensen 1995) and the First Data Bank, Nutritionist Five (Version 1.7) San Bruno, CA.

² Kumazawa et al. (1992, 1995).

³ Mitchell et al. 1999.

⁴ Determined by the glucose dehydrogenase enzyme assay (see text).

⁵ Bishop et al. 1994.

isms common to the mouse intestinal tract. This suggests that PQQ may be synthesized sufficiently at the onset of sexual maturation or is only of utility to the neonate. Work in several laboratories is currently attempting to resolve this issue.

In cell culture experiments, PQQ and IPQ enhance cell growth and proliferation (Naito et al. 1993). PQQ and IPQ enhance the incorporation of [³H]-thymidine into human fibroblast cells. PQQ causes stimulation at concentrations as low as 3 nmol/L, whereas IPQ causes stimulation at ~15 nmol/L IPQ. What is remarkable about these observations, both in vivo and in vitro, are the concentrations at which PQQ produces effects, i.e., nmol PQQ/g of diet or nmol/L of cell culture medium. Such concentrations are usually characteristic of potent cytokines and related growth factors.

Antioxidant properties. At concentrations of 15–30 $\mu\text{mol}/\text{kg}$ body weight, PQQ and IPQ function as antioxidants. Tsuchida et al. (1993) and Urakami et al. (1997) have reported that PQQ and IPQ protect mice against acute liver damage, induced by agents, such as CCl_4 . PQQ treatment also decreases glucocorticoid (hydrocortisone)-induced cataract formation in fertilized chicken eggs (Nishigori et al. 1989, 1993, Urakami et al. 1997). A possible mechanism for the decrease in cataract formation is restoration of glutathione levels, which are reduced in response to hydrocortisone.

As additional examples, using a carrageenin model to produce inflammation and paw edema in rats, Hamagishi et al. 1990 observed that PQQ administered (i.p.) at 10 or 30 mg PQQ/kg body weight caused a decrease in carrageenin-induced

edema by 39 and 76%, respectively. Both PQQ and IPQ decreased CCl_4 -enhanced chemiluminescence in isolated hepatocytes (Urakami et al. 1997). It is also noteworthy that PQQ is a better inhibitor of chemiluminescence (initiated by zymosan, carrageenin or *N*-formyl-methionyl-leucyl-phenylalanine) in mouse peritoneal cells than α -tocopherol and ascorbic acid (Hamagishi et al. 1990). On a molar basis, PQQ was fifteen times more effective than ascorbic acid in decreasing chemiluminescence generated by the xanthine-xanthine oxidase reaction and seven times more effective than α -tocopherol at preventing lipid peroxidation in rat brain preparations (Hamagishi et al. 1990).

PQQ toxicity. It is important to point out that compounds that engage in redox cycling can also be effective free radical initiators. Although toxicity studies are limited, nephrotoxicity and oxidative damage have been reported when 35 μmol or more per kg body weight (~10 mg PQQ/kg) was administered daily by injections (i.p) to rats over a 4–5-d period (Watanabe et al. 1989). PQQ has also been demonstrated to initiate DNA damage in vitro (Hiraku and Kawanishi 1996). However, relatively high concentrations of PQQ (2 $\mu\text{mol}/\text{L}$) are required for DNA damage relative to the concentrations of PQQ needed to cause cell proliferation (nmol/L amounts; Naito et al. 1993). In addition, an electron regeneration system is needed to initiate DNA damage. For example, an NADH/ Cu^{2+} /PQQ-dependent redox system is very efficient in generating superoxide and hydrogen peroxide. Interestingly DNA

cleavage does not occur when Fe^{2+} , Fe^{3+} or Mn^{2+} is substituted for Cu^{2+} .

PQQ and neural cells and tissue. Nerve cells become excited upon stimulation of NMDA glutamate-receptors. In cell culture experiments, PQQ protects neuronal cells from NMDA toxicity by directly oxidizing the receptor's NMDA redox site (Aizenman et al. 1992, 1994; Scanlon et al. 1997). Jensen et al. (1994) have extended these observations by showing in vivo that PQQ protects against the likelihood of severe stroke in an experimental animal model for stroke and brain hypoxia. PQQ administration reduced the infarct size when the animals were pretreated with PQQ prior to induction of hypoxia and ischemia.

PQQ also increases production of nerve growth factor (NGF) in some cell lines. NGF is required for peripheral sympathetic and sensory neuron function and aids in protecting the magnocellular cholinergic neurons in the basal forebrain nuclei. These areas are involved in memory and learning and are often significantly damaged in Alzheimer's disease.

In L-M cells (a nerve growth factor-productive cell line), PQQ enhances NGF synthesis 50-fold and PQQ-TME increases NGF synthesis 70-fold. However, IPQ-TME has no NGF-enhancing effects (Urakami et al. 1996, Yamaguchi et al. 1993). PQQ also enhances NGF synthesis and secretion in mouse astroglial cells (17-fold) and human WS-1 cells (6-fold), but has no effect in mouse BALB c/3T3 cells (Murase et al. 1993). The mechanism for enhancement is currently under investigation. Features of this response have also been observed in vivo. Yamaguchi et al. (1993) have reported that PQQ in the form of the trimethyl ester imidazole derivative (IPQ-TME) causes a nearly 2-fold increase in NGF in the neocortex of rat brain.

It is of interest to note that TOPA in the form of 6-hydroxy-dopa or TPQ acts as a potent neurotoxin. TPQ interacts with NMDA glutamatergic receptors and produces neuronal death in contrast to the neurotrophic effects of PQQ (Newcomer et al. 1995). Using a pheochromocytoma catecholaminergic cell line, (Newcomer et al. 1995) have identified TOPA-related products. DOPA secretion from pheochromocytoma cells coincides with detectable generation of TOPA-related compounds. Inhibition of TOPA formation occurs upon addition of GSH. This suggests that TOPA and related products probably occur by the extracellular auto-oxidation of DOPA, i.e., TOPA and TPQ are selective non-NMDA agonists that can be synthesized by brain tissue.

GSH decreases the neurotoxicity of TOPA or TPQ through the formation of glutathionyl complexes. These complexes switch the pathway away from the formation of toxic factors to less toxic pheomelanins (Nappi and Vass 1994). In the absence of GSH, TPQ is a component of catecholamine toxicity that is generally underestimated (Newcomer et al. 1995). As noted above, the toxic effects of TPQ and TOPA are in marked contrast to the protective interactions of PQQ with NMDA receptors.

TTQ and the copper-complexed cysteinyltyrosyl radical

There are no known dietary relationships involving TTQ or the copper-complexed cysteinyltyrosyl radical. McIntire (1998) has provided a recent review describing the role of both cofactors. The copper-complexed cysteinyltyrosyl radical is found in fungal galactose oxidase (Baron et al. 1994). TTQ is a cofactor for methylamine dehydrogenase and aromatic amine dehydrogenase.

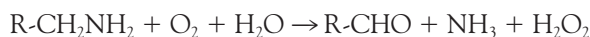
The activation of galactose oxidase by copper is novel, because of catalysis of a protein-derived radical as an active

site cofactor, i.e., a cysteinyltyrosyl radical (Baron et al. 1994). The activation of certain bacterial amine oxidases is novel because of the use of TTQ as a cofactor. McIntire and his colleagues studying methylamine dehydrogenase provided the first unequivocal proof that TTQ was the redox prosthetic group in an alkylamine dehydrogenase. Methylamine redox reactions involve methylamine dehydrogenase (MADH), the type I copper protein amicyanin and cytochrome C. MADH is commonly found in methylotrophic soil bacteria. Bishop et al. (1998) recently isolated an aromatic amine dehydrogenase that contains TTQ, which broadens the specificity of TTQ-containing dehydrogenases to include both alkyl and aromatic amines.

TPQ and lysyl tyrosine quinone

Amine oxidases and TPQ. TPQ is the principal cofactor in copper containing amine oxidases. Copper aids in initiating the oxidation of a tyrosyl residue to form TPQ (Cai et al. 1997). TPQ is derived from peptidyl tyrosine in the sequence, -Asn-X-Glu-, where X represents the modified tyrosine. The tyrosyl residue is first converted to peptidyl dopa and then to TPQ. The unsaturated quinone structure of dopaquinone promotes the reaction at the C-2 position to produce TPQ (Fig. 4). Recent evidence suggests that copper is required for the initiation of the reaction at the C-2 position.

Amine oxidases are oxidoreductases that carry out oxidative deaminations (EC 1.4.3.6).



The classical example of an amine oxidase enzyme is benzylamine or polyamine oxidase, which was first fully characterized from bovine plasma (McIntire 1998 and references cited). Lysyl oxidase and histaminase are other important examples. Generally, such enzymes are semicarbazide-sensitive and when reacted with hydrazines, e.g., *p*-nitrophenyl hydrazine, form covalent complexes with the cofactor TPQ. Another common feature is a catalytic base at the active site, usually an aspartic acid residue (Hevel et al. 1999).

Amine oxidases carry out reactions in two steps in the manner of a so-called ping-pong reaction (Shah et al. 1993). The first part of the reaction initiates reduction of the cofactor, and in the second phase an oxidation occurs.

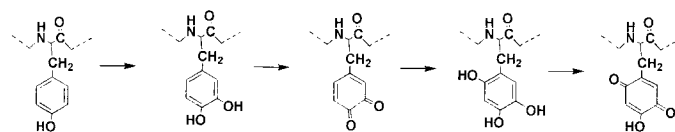
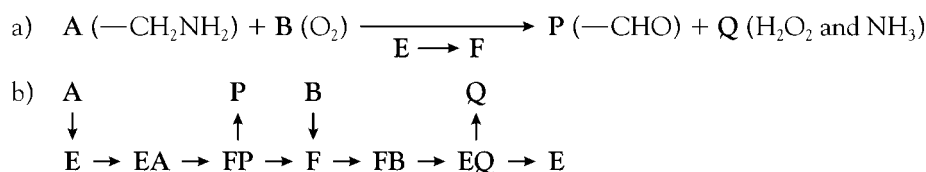


FIGURE 4 Topaquinone formation. The generation of topaquinone from tyrosine is in part based on observations by Cai et al. 1997, Matsuaki et al. 1994, Mu et al. 1994, Rinaldi et al. 1998 and 1999, Ruggiero and Dooley 1999 and Ruggiero et al. 1997, Wang et al. 1997. The reaction involves the six-electron oxidation of tyrosine to topaquinone. Two moles of O_2 are consumed in the reaction, and 1 mole of H_2O_2 is produced. Copper acts as the catalyst and must first be incorporated into the enzyme's active center before the topaquinone is formed. From left to right, the reaction sequence is the hydroxylation of a tyrosyl residue to a 3,4-dihydroxyphenylalanyl residue, followed by oxidation to peptidyl dopaquinone. Rotation of the dopaquinone intermediate has also been proposed (Mu et al. 1994), which would allow hydroxylation (at the 2-position) to eventually result in a trihydroxyphenylalanyl residue and (following oxidation) peptidyl topaquinone. Rinaldi et al. 1999 have provided an excellent review on the complexities of this process.



In the scheme above, *E* represents the *o*-quinone form of TPQ and *F* represents the aminoquinol formed from the half reaction (also see Fig. 5).

A wide range of substrates including aliphatic diamines, many polyamines, and in the case of lysyl oxidase, the ϵ -amine function of lysyl residues in a variety of proteins may be oxidatively deaminated.

From a functional perspective, more information is known about lysyl oxidase than other amine oxidases. Lysyl oxidase serves as a key enzyme in the growth and development of all higher animals (Rucker et al. 1998). Both intracellular and extracellular forms of the enzyme exist. In malignant cells, it is currently speculated that the intracellular form(s) of lysyl oxidase target as substrates oncogenic and growth-promoting transcription factors. Two examples are RAS oncogene and IRF-1 transcription factor (Kenyon et al. 1993, Ren et al. 1998, Tan et al. 1996). Within the extracellular matrix (ECM), lysyl oxidase catalyzes the crosslinking of the collagens or elastin. Developmental processes beyond gastrulation are blocked when lysyl oxidase is inhibited. (Rucker et al. 1998 and references cited). In addition, at least one form of lysyl oxidase localizes in the nucleus (Li et al. 1997).

The cofactor for lysyl oxidase is LTQ. The formation of

LTQ requires an additional step (Fig. 5), resulting in the formation of the lysyl adduct of TPQ (Wang et al. 1997). This cofactor was identified following derivation of lysyl oxidase with *p*-nitrophenylhydrazine followed by treatment with thermolysin and finally separation of the resulting peptides. Edman degradation of the protein indicates that the quinone-containing peptide is crosslinked with another peptide. Extensive mass spectral analysis and Raman spectroscopy eventually led to the identification of LTQ. LTQ exists as a neutral 79-quinone, due to the nitrogen associated with lysine. In contrast, TPQ exists as a resonance-stabilized hybrid with a net negative charge (Wang et al. 1997).

Role of copper in TPQ formation. Copper has two functions: the single-turnover and modification of peptidyl tyrosine to form TPQ, and the subsequent involvement in the oxidative deamination of the primary amine substrates. Copper-catalyzed generation of TPQ is based on observations by (Cai et al. 1997, Matsuzaki et al. 1994, Rinaldi et al. 1998, Ruggiero and Dooley, 1999, Ruggiero et al. 1997, Wang et al. 1997). The reaction involves a six-electron oxidation of tyrosine to form TPQ in which 2 mol of O_2 are consumed in the reaction and one mole of H_2O_2 is produced (Ruggiero and Dooley, 1999). Copper must first be incorporated into the enzyme's active center and then TPQ is formed (Fig. 4). In related studies, Mure and Tanizawa (1997) have utilized 4-tertiary-butyl-derived compounds to study TPQ generation. The researchers examined the effects of Cu^{+2} and Zn^{+2} additions. They concluded that the role of the active site copper ion in TPQ biogenesis is limited to the catalysis of the two quinolone steps. Interestingly, the activity of a fully processed copper-depleted enzyme can be regenerated with the addition of cobalt. Further, activation by cobalt has mechanistic implications. A semiquinone radical is not formed as an intermediate in the enzymatic reaction, e.g., an oxidative deamination (Agostinelli et al. 1998).

As an additional mechanistic feature of TPQ formation, Nakamura et al. (1996) have observed (based on changes in Raman spectra) that when apo-enzyme is reacted with Cu^{+2} in the presence of H_2^{18}O , the oxygen at the C-2 position of TPQ most likely comes from the solvent and not O_2 . Due to the electron destabilization between the oxygen atoms associated with C-2 and C-4, the oxygen at the C-5 position has the predominant carbonyl character (Nakamura et al. 1996).

LTQ formation and dietary copper. A relationship between the expression of amine and lysyl oxidase activity and copper intake has been established since the 1960s. For example, intakes of copper over the range of 0.5 to 16 μg copper/g of diet causes a progressive increase in lysyl oxidase activity in tendon and bone (Rucker et al. 1999). These observations, in addition to a number of early observations, are the basis for using amine and lysyl oxidase activities for assessment of

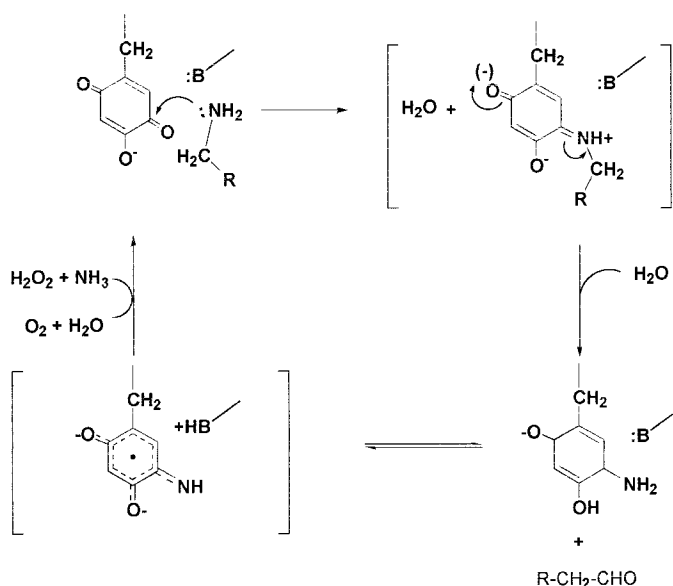


FIGURE 5 Proposed mechanism for the oxidation of a primary amine by an amine oxidase. The first part of the reaction initiates reduction of the cofactor and conversion of the amine substrate to an aldehyde product. In the second part of the reaction, the ammonia and hydrogen peroxide are released with re-oxidation of the cofactor.

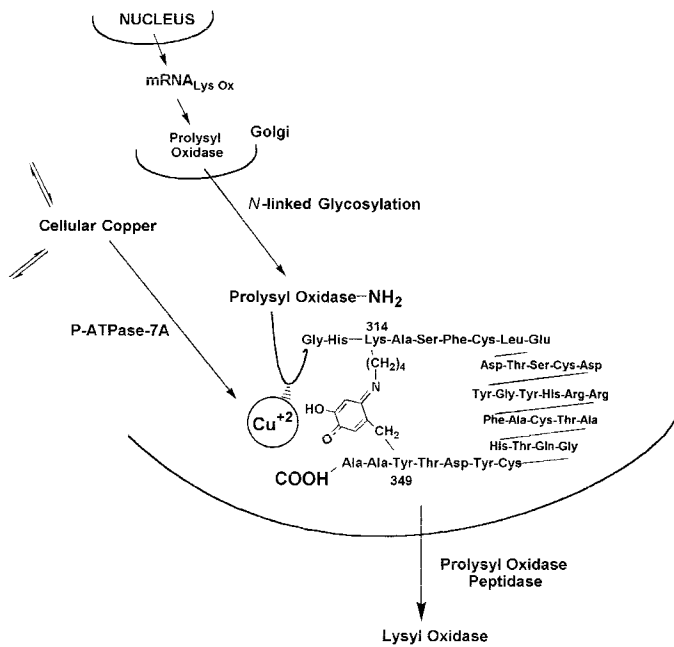


FIGURE 6 Lysyl oxidase processing. Lysyl oxidase is directed to the Golgi as a 46 kDa proenzyme (prolylsyl oxidase). *N*-linked glycosylation occurs and in postgolgi steps, prolylsyl oxidase is packaged into vesicles, wherein copper binding and quinone cofactor formation occur. The copper transporting ATPase (P-ATPase-7A) is linked to this process (Pena et al. 1999). This metal transporter is a principal component in the movement of copper into vesicles and eventual egress from cells. The positions corresponding to the lysine and tyrosine residues involved in topaquinone formation correspond to those in rat lysyl oxidase (Trackman et al. 1990, 1991). At the cell surface, prolylsyl oxidase is cleaved to lysyl oxidase (~30 kDa).

dietary copper (Rucker et al. 1998). The relationship between copper intake and lysyl oxidase activity appears to be due to enzyme activation and not to changes in lysyl oxidase mRNA or content within the extracellular matrix (Rucker et al. 1998).

What are the processes that link lysyl tyrosine quinone formation to copper intake and eventually lysyl oxidase activation? For lysyl oxidase, some clues come from the recognition that two genetic diseases, Menkes disease and occipital horn syndrome (possibly a form of X-linked cutis laxa), are related to defects in Cu-ATPase genes, specifically P-ATPase-7A, the gene that is involved in copper transport. Cellular copper homeostasis appears to be regulated mostly by cellular copper efflux pathways. Cu-ATPase activity is an important component of the copper efflux pathway. An excellent recent review on this topic has been provided by (Pena et al. 1999).

The exit of copper from connective tissue cells occurs by transport involving secretory vesicles with the eventual release of copper into extracellular space. Lysyl oxidase obviously interacts with copper at some point during the egress pathways designed to transport either copper or lysyl oxidase out of cells. More detailed examination of this process should eventually lead to an explanation as to why there is such remarkable linkage between lysyl oxidase activation, vesicular copper concentration and dietary copper intake (Fig. 6).

CONCLUSION

Data generated over the past two decades provide a basis for adding PQQ, TPQ, LPQ and TTQ, and the copper-complexed cysteinyltyrosyl radical to the list of cofactors that engage in

novel deaminations and redox reactions. Nutritional relationships, albeit indirect, have been established for PQQ, TPQ and LPQ. Whether PQQ is nutritionally important as a conditional vitamin requires further clarification. Nevertheless, PQQ can function as a growth-promoting agent and oxidant scavenger. Relatively small amounts of PQQ (nmol to $\mu\text{mol/L}$ or kg concentrations) are needed to elicit given responses. Moreover, PQQ is present in a wide variety of foods and appears to be readily absorbed.

TPQ and LPQ are not required as nutrients, but their formation is dependent on dietary copper intake. In this regard, it may be inferred that TPQ- and LPQ-containing enzymes may be indicators of copper status.

Further studies that focus on the action of these quinone compounds should have broad impact. Specifically, mechanistic studies that aid in defining the function of PQQ in higher animals could provide a more solid basis for explaining the general systemic effects of other quinone compounds including numerous flavinoids and flavinols that are common in diets.

ACKNOWLEDGMENT

The authors wish to acknowledge the advice and contributions of a colleague, Paul Gallop (now deceased), Laboratory of Human Biochemistry, Harvard University Schools of Medicine and Dental Medicine. Paul Gallop's near-four-decade career provided information that ranged from our current understanding of pyrroloquinoline quinone to the chemistry of elastin and collagen crosslinking amino acids and the detection and quantification of novel amino acids, such as γ -carboxyglutamic acid. There are many who miss his advice, humor and enthusiastic support.

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