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Review Article

PYRROLOQUINOLINE QUINONE A REDOX COFACTOR AND ITS INVOLVEMENT IN BIOLOGICAL SYSTEM

Satyabrata Ghosh, Runu Chakraborty & *Utpal Raychaudhuri
Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata 700032, India.
*Author for correspondence: utpal31@hotmail.com

ABSTRACT
This paper highlights some features, which describe the characterization and physiological importance of Pyrroloquinoline quinone (PQQ), discovered more than forty years ago. Mechanism of biosynthetic pathway of PQQ is described based on sequence analysis and homology models. But still now all activities of PQQ are not discovered. PQQ is not only a bacterial redox cofactor in alcohol oxidation catalysed by alcohol dehydrogenase enzyme, it is also considered as a redox cofactor for mammals also. Among physiological importance of PQQ, it shows antioxidant property, cardioprotection, neuro protection, improvement of neonatal growth and reproductive performance. It acts as a microbial stimulant and reduces the lag phase of long generation time strains. PQQ takes part in the oxidation process of glucose metabolism also. In mitochondriogenesis cell signaling pathway PQQ play an important role. The controversy, that whether it should be considered as a vitamin or not still remains. But it has various physiological roles in biological system.

KEY WORDS: PQQ, redox cofactor, PQQ-vitamin, neurotoxic agent.

INTRODUCTION

Pyrroloquinoline quinone (PQQ) is a magical prosthetic group of several bacterial dehydrogenase enzymes (Anthony 1993; Matushita and Adachi 1993). Hauge (1964) discovered it in bacteria and after a few years later Anthony and Zatman (1967) also found the unknown redox cofactor in alcohol dehydrogenase and named Methoxatin. It was the first of the ortho-(O)-quinone cofactors to be extracted from methanol dehydrogenase of methylotrophs and identified its molecular structure (Salisbury *et al.,* 1979; Westerling 1979). Other quinine cofactors and their precursor molecules are tyrosine and tryptophan residues whereas PQQ are derived from tyrosine and glutamate residue and it is biosynthesized independent of its site of action which differ from other quinine synthetic group (Mure 2004; Houck *et al*., 1991). In biosynthesis of PQQ, several bacterial genes are involved but one of the most important is pqqC gene. PQQ is synthesized by PqqC protein, which facilitates eight electron oxidation of substrate-3aα (2-amino-2-carboxyethyl) - 4, 5dioxo–4,5,6,7,8,9-hexahydroquinoline-7,9-dicarboxylic acid to PQQ (Magnusson *et al*., 2004). PQQ acts as a redox cofactor such as NAD, FAD in oxidation reaction along with alcohol dehydrogenase enzyme (Anthony 2001; Chen *et al*., 2002). The PQQ dependent alcohol dehydrogenase of *Pseudomonas aeruginosa* is involved in oxidoreductase activity of utilization of acyclic terpene derivative as a substrate and it also helps in ethanol metabolism (Chattopadhyay *et al*., 2010). Trace amount of PQQ stimulates bacterial auxotrophic growth by the reduction of lag phase without affecting the growth rate in the exponential phase and the total cell yield at the stationary phase (Ameyama *et al*., 1984a). Oxidative glucose metabolism takes place through Entner–Doudoroff pathway in presence of the enzymes whose one of the cofactor is also PQQ (Adamowicz *et al*., 19991). In the stressed condition, generation of the reactive oxygen species (ROS) induce oxidative damage to biological molecules like protein, lipid, nucleic acid (Ohwada *et al*., 2008; Nishio *et al*., 2006). PQQ has antioxidant properties it protects mice against acute liver damage, and decreases glucocorticoid induced cataract formation in fertilized chicken egg and also decrease in carrageenin induced edema (Ouchi *et al*., 2009). PQQ is an inhibitor of CCl_4 enhanced chemiluminescence in isolated hepatocytes (Urakami *et al*., 1997). PQQ inhibits zymosan, carrageenin or N-formyl–leucyl-phenylal-anine initiated chemiluminescence in mouse peritoneal cells better than α-tocopherol or ascorbic acid (Hamagishi *et al*., 1990). But now it is arguably considered as a new vitamin of B complex group for mammals (Kasahara and Kato 2003).

Structure and chemical nature of PQQ

Pyrroloquinoline quinone (Fig 1) is an aromatic tricyclic ortho-quinone that serves as the redox cofactor for several bacterial dehydrogenase enzymes. Among the best known examples are methanol dehydrogenase, ethanol dehydrogenase, and glucose dehydrogenase (Flores *et al*., 2004). PQQ has been recognized as the third class of redox cofactors following pyridine nucleotide and flavin-dependent cofactors (Duine 2001). The methanol dehydrogenase (MDH) is a heterotetramer of two large and two small subunits ($\alpha_2\beta_2$) (Kay *et al*., 2006). The large subunits contain the main catalytic center for oxidation of methanol. PQQ is bound to Ca^{2+} ion and buried in the interior of the super barrel of α-subunit of MDH and

sandwiched between the indole ring of a tryptophan residue and along with adjacent cystine residue (Cys-103-Cys-104), formed eight member disulfide ring. In quinoprotein ethanol dehydrogenase (QEDH) these are Cys-105- Cys-106 (Kay *et al*., 2006). Ca^{2+} has a major role in maintaining PQQ in the correct configuration, as predicted from spectroscopic and reconstitution studies of MDH that lacks Ca^{2+} and is unable to oxidize the substrate (Richardson and Anthony, 1992).

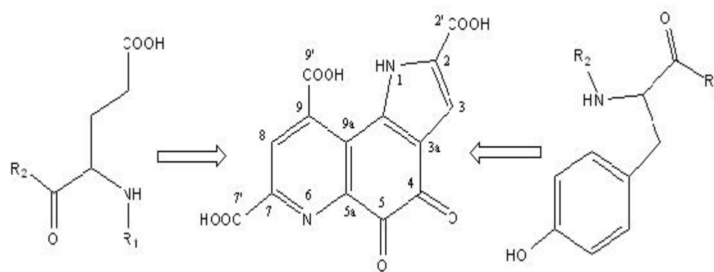

FIGURE 1: Reference: Puehringer *et al*. (2008)

Sequence of gene in bacteria that is responsible for biosynthesis of PQQ

PQQ synthesis gene was found in various bacteria such as *A. calcoaceticus*, *K. pneumoniae etc*. In *A. calcoaceticus*, five genes were identified and designated as IV, V, I, II and III (Goosen *et al*., 1989). In *K. pneumoniae*, genes designated pqqABCDE and a sixth gene pqqF was found immediately downstream of pqqE (Meulenberg *et al*., 1992). In *Methylobacterium*, a five gene cluster (designated pqqDGCBA) was identified. Complementation analysis and sequence data showed all three of these genes (pqqDGC) from *M. extorquens* AM1, to be analogous to pqqABC of *K. pneumoniae*. In *Pseudomonas fluorescens*, gene analogous to pqqFBA of *K. pneumoniae* (Biville *et al*., 1989; Morris *et al*., 1994). *Gluconobacter oxydans* 621H the complete gene cluster is pqqABCDE (Holscher T and Gorisch H 2006) [Table 1].

Table 1 Correlation of pqq gene in different organisms

K. Pneumonia	*A. calcoaceticus*	Gluconobacter oxidants	*M. extorquens* AM1
1. pqqA	IV	pqqA	pqqA
2. pqqB	V	pqqB	pqqB
3. pqqC	I	pqqC	pqqC/D
4. pqqD	II	pqqD	pqqC/D
5. pqqE	III	pqqE	pqqE
6. pqqF	-	-	pqqF
7. -	-	-	pqqG

Reference: Holscher *et al*., 2006 (Gluconobacter oxidants), Toyama *et al*., 1997 (K. pneumonia, *A. calcoaceticus*, *M. extorquens* AM1)

Function of the gene in all organisms' gene sequence, a small gene is present that encodes PqqA peptide consisting of a 22-29 amino acid, which contains conserved tyrosine and glutame residues (Fig 2). Therefore, tyrosine and glutamate are the most probable precursor for PQQ synthesis (Goosen *et al*., 1992; Houck *et al*., 1988). The PqqB protein might be involved in transport of PQQ into the periplasm and PqqC catalyzes the final step in PQQ formation (Velterop *et al*., 1995). The function of PqqD is to release the PQQ from PqqC and it helps in binding of PqqB to PqqC. Other activity of PqqD, is involved in dioxygenase pathway. PqqE are still unknown. The PqqF and PqqG are family of divalent cation–containing endopeptidase that cleaves small peptides (Springer *et al*., 1996). Han et. al., 2008 reported that *Enterobacter intermedium* 60-2G has the ability of phosphate solubilization and induce systemic resistance in plants against soft rot pathogen (*Erwinia carotovora*) in tobacco, by the pqqA and pqqB gene. They have also said that PQQ has antifungal activity.

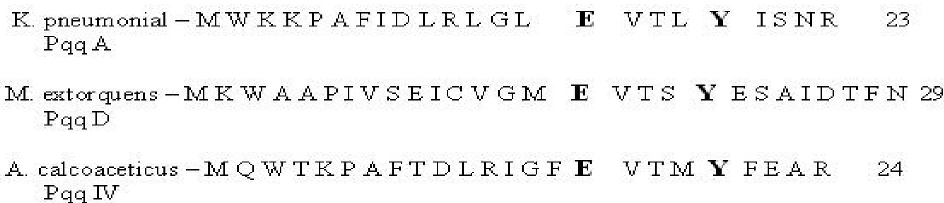

FIGURE 2: Reference: Morris *et al*., 1994

Further experimental report of sequence comparisons study showed that PqqC/D of AM1 to be identical to PqqC of *K. pneumoniae* (44%) and PqqI of *A. calcoaceticus* (43%) in its N terminus residues 11-241. PqqD of *K.*

pneumoniae (29%) and PqqII of *A. calcoaceticus* (30%) in its C terminus residues 285-366 identical with PqqC/D of AM1. PqqE of AM1 consists of 384 amino acid, molecular mass and isoelectric point were 41714Da and 6.42 respectively, its identity (45-46%) in residues 17-358 to pqq gene products from other bacteria such as AM1, *A. calcoaceticus* etc. PqqE of AM1 amino acid sequence of 28-45 and 109-135 showed identity to NifB from *Enterobacter agglomerans* and *K. pneumoniae*. PqqE contains a conserve sequence CxxxCxxxYC that is proposed to anchor an iron-sulphur cluster or some type of metal binding site (Menendez *et al*., 1995). The C terminal region PqqE consists of cysteine rich residue and almost all of these cysteine residues were conserved in all the corresponding Pqq protein.

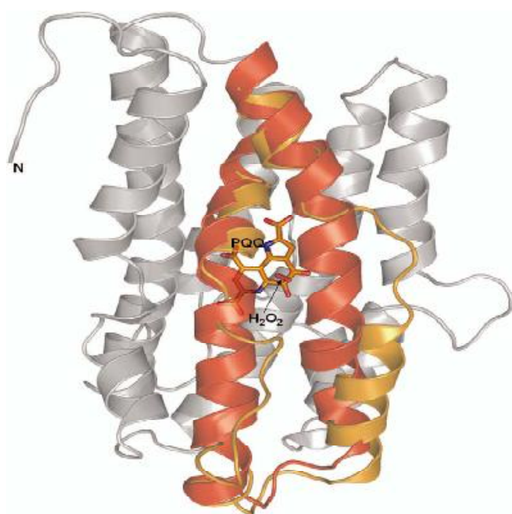

FIGURE 3: Reference: Puehringer *et al*. (2008)

Holscher reported that pqqABCDE gene cluster of *Glucolnobacter oxydans* 621H show high homology to the *E. coli* tldD gene. This *Glucolnobacter oxydans* 621H tdlD gene has same function as the pqqF gene found in other PQQ producing bacteria (Holscher and Gorisch 2006). This statement proved by Yang *et al*., 2010, showed that pqqABCDE gene of *Glucolnobacter oxydans* 621H transferred to *E. coli* cell without tldD gene but pqqABCDE gene is expressed in *E. coli* cell and resulting in PQQ production in media. It was means that *E. coli* tldD gene shows high homology and carried out same function as *Glucolnobacter oxydans* 621H tdlD gene (Yang *et al*., 2010). All pqq genes except pqqC are involved to produce an intermediate PQQ complex. The pqqC is the main gene for PQQ biosynthesis from PQQ intermediate. The PqqC catalyzes the last step in PQQ synthesis (Magnusson *et al*., 2004). Analysis of the PqqC structure shows that the seven α-helices provide the scaffold for an active site cavity. The cavity is lined with 42 mostly hydrophilic and aromatic residues that are highly conserved within PqqC proteins from different bacteria. The cavity shows a distinct overall positive charge, measures $9A^0$ X 13 A^0 X 23 A^0 and embraces a molecular surface volume of 2,200 A^{03}. The two openings connect it to the outside. PqqC change its conformation after binding with substrate. During this change, the active site of residues rearrangement and it moves from a solvent exposed location to a position directly in the center of the molecules (Liang *et al*., 1998) (Fig 3). PqqC facilitates ring closure and an unprecedented eight electrons oxidation of the substrate, which raises a number of important questions regarding the mechanism of this enzyme (Magnusson *et al*., 2004).

The mechanism of PQQ Biosynthesis

New structural data of some PQQ biosynthesis proteins and their homologues provide new insights and functional assignments of the protein in the pathway, based on sequence analysis and homology models that proposed the role and catalytic function for each enzyme involved in this intriguing biosynthesis pathway (Puehringer *et al*., 2008).

Tyr and Glu are part of the peptide precursor PqqA. These two amino acids form C-C bond at atoms C9 and C9a (Fig 4, step1) is most probably one of the first reaction steps in order to link the two amino acids before the peptide bonds are cut. PqqE is catalyzing this reaction because it is the only enzyme in the pathway with significant sequence similarly (score-64, 16% sequence identity to Molybdenum cofactor biosynthesis protein A) to radical SAM protein capable of catalyzing C-C bond formation. The mechanism is not cleared but the analogy to radical SAM protein implies that the mechanism of the reduced 4Fe-4S cluster transfers an electron to the sulfur of SAM. The $C5'-S^+$ bond of SAM is cleaved, producing methionine and highly oxidizing 5'-deoxyadenosyl radical (Wecksler *et al*., 2009). The radical abstracts a hydrogen atom from the tyrosine in PqqA, creating a tyrosine radical at position C9a. This tyrosine radical (C9a) reacts with atom C9 of glutamate leading to cyclization (Fig.4, step1). After this reaction, the new covalently linked amino acids Glu and Tyr are still linked to the PqqA- peptide backbone. PqqF is responsible for cleaving the four peptide bonds at R1 and R2 of Glu and R2 and R3 of Tyr (Fig 4, step 2). After the linkage and proteolytic cleavage of the four-peptide bonds the amino group N6 Glu and the OH (C5a) of Tyr are primed to spontaneously form a Schiff-base reaction (Fig 4, step 3). As a next possible reaction step, two OH groups are added to atoms C4 and C5 in the Tyr ring, which requires most likely a dioxygenase (Fig 4, step 4). Yet, there is no protein with apparent sequence similar to a dioxygenase in the PQQ operon. Candidates could be PqqB and PqqD, which however do not feature oxygenase similarities, or another oxygenase from the bacterium not exclusively used for PQQ –biosynthesis. The final step in the reaction has been elucidated and is catalyzed by PqqC. The multi step reaction includes a ring closure at N1 and the removal of eight electrons and eight protons from the intermediate to form PQQ (Fig 4, step5) (Puehringer *et al*., 2008).

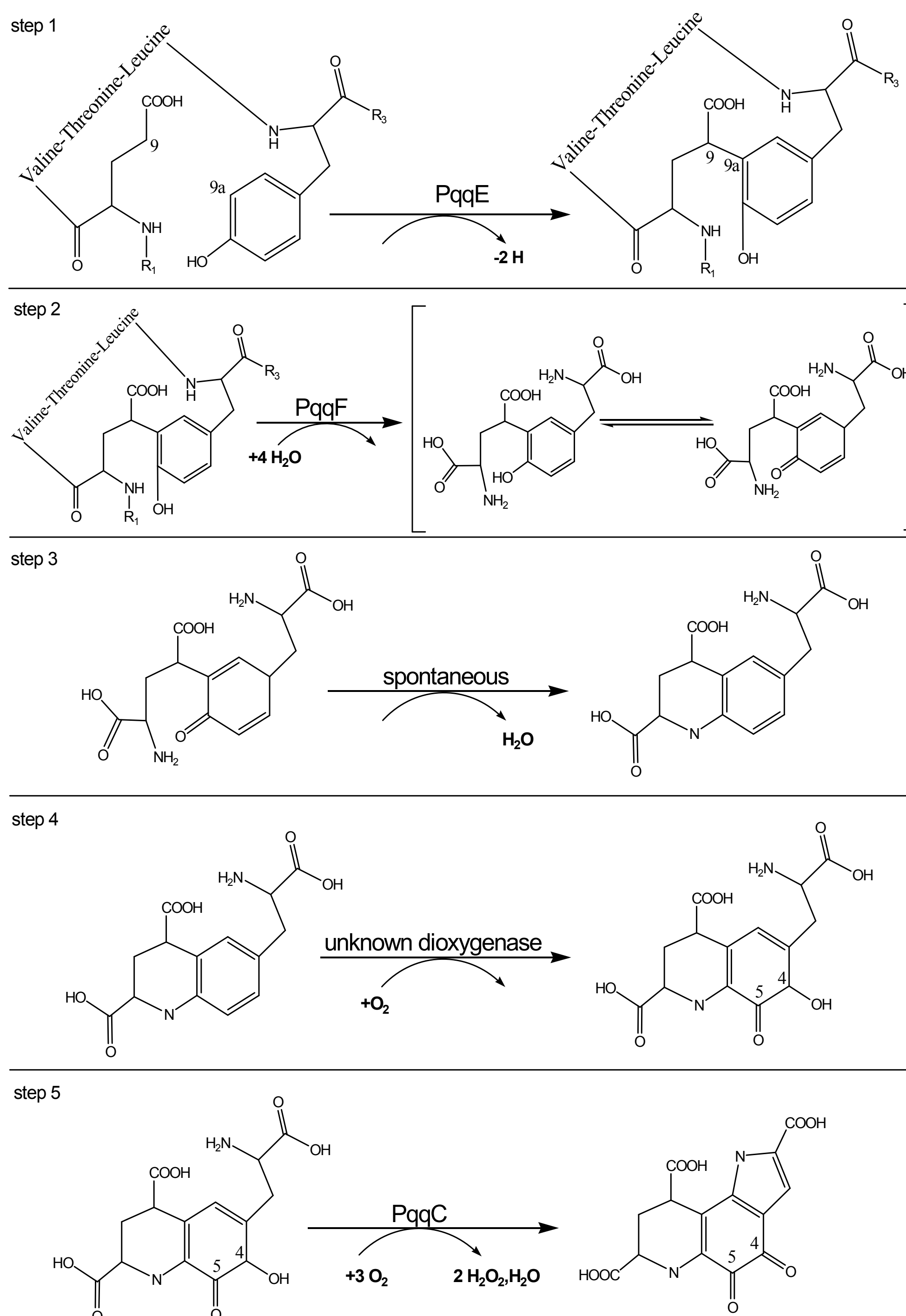

FIGURE 4: Reference: Puehringer *et al*. (2008)

PQQ stimulate bacterial growth by reduction of lag phase

PQQ is more important for the bacteria, which grows on alcohol (Lidstrom *et al*., 1991). It stimulates auxotrophic and heterotrophic growth by reduction of lag phase (Ameyama *et al*., 1984b). Trace amount of PQQ marked reduction of the lag phase without affecting the growth rate of exponential phase as well as the total cell yield at the stationary phase (Ameyama *et al*., 1985). PQQ uptake is facilitated by PhoE porin. The PhoE porin protein is induced by phosphate limitation but resulting pores are more efficient for the transport of negatively charged compounds in genera. It was experimentally confirmed that PQQ dependent lag periods were reduced when inoculums contain low phosphate (50µM) gluconate [Table 2] (Adamowicz *et al*., 1991).

TABLE 2. Phosphate limitation and the PQQ dependent lag period in *E.coli* ZSC113

Inoculum [a]	Glucose + PQQ Growth medium	Lag period (h)
Nutrient agar	High PO_4	≥ 16
Nutrient agar	Low PO_4	8
Tryptone-salts	High PO_4	30
Tryptone-salts	Low PO_4	8
High PO_4-Gluconate	High PO_4	7
High PO_4- Gluconate	Low PO_4	5
Low PO_4 – Gluconate	High PO_4	1

[a] High PO_4 = 78mM ; low PO_4 = 50 μM . The low PO_4 –gluconate medium and all glucose plus PQQ media are supplemented with 50 mM morpholinepropanesulfonic acid (MOPS) to maintain adequate buffering.
[b] Tryptone (10g/L) and NaCl (10g/L) ,pH 7.5.
Reference: Adamowicz *et al*.(1991)

The microbial strains such as *Acetobacter* and *Yeast,* which grow on minimal media, contain various nutrients such as glucose, glycerol, various amino acids or inorganic ammonium salt, which is essential for particular strain. In medium where the essential nutrients are lacking, trace amount of PQQ act as a stimulating factor for microbial growth. Most of the organisms produce PQQ and such PQQ can be used for another batch of culture as a growth starter. The PQQ also affects the starter predominance of long generation time strains by the help of reduction of lag phase (Ameyama *et al*., 1984a). It was detected during chromatographic purification, some microbial contaminants specifically present only in presence of fractions of PQQ. Shida *et al*., 1975 investigated the reduction of the lag phase in bacterial growth and concluded that the inoculums sizes greatly affected the length of the lag phase but not growth rate (Ameyama *et al*., 1984b). The extremely short generation time of wild strains such as *Pseudomonas*, show no appreciable effect from exogenous PQQ. With a sufficient amount of PQQ, they can produce endogeneously at an early stage of growth (Ameyama *et al*., 1984b). So PQQ is an interesting growth stimulating factor of microbes.

PQQ induced oxidative glucose metabolic pathway (E.D)

Enter–Doudoroff (E.D) pathway (Conway 1992) is a branch metabolic pathway of oxidative glucose metabolism, where oxidative glucose converted to gluconate catalyzed by PQQ dependent enzyme (Holscher etal., 2009). But in many bacteria including *Zymomonas mobilis*, *Pseudomonas aeruginosa*, it is a core metabolic pathway of this organism. In mutant *E. coli,* E.D pathway is an alternative route for glucose catabolism, oxidation of glucose to gluconic acid in the periplasm, which also occurrs in the presence of PQQ dependent glucose dehydrogenase enzyme (Fliege *et al*., 1992). The wild type *E.coli* does not synthesize PQQ because *E. coli* and *S. typhimurium* lack the genes to synthesize PQQ (Phibbs 1988). The wild type *E. coli* would be capable of utilizing the E.D pathway for oxidative glucose metabolism in the presence of PQQ. Growth of wild type *E. coli* on medium containing glucose and PQQ caused induction of 6-phosphogluconate dehydratase activities equivalent to induction of mutant *E. coli.*

Table 3: Effect of phosphate concentration on 6-phosphogluconate dehydratase activity in *E.coli* W3110.

Medium	carbonsource(s)	Mean enzyme activity(SD)[a] 6-phosphategluconate Dehydratase
Minimal	Glucose	4.3 (1.0)
Minimal	Gluconate	43.8(5.7)
Minimal	Glucuronate	2.8 (1.4)
Minimal	Glucose + Gluconate	30.7(3.7)
Minimal	Glucose +PQQ	14.8(0.3)
Low PO_4[b]	Glucose	5.5(0.4)
Low PO_4	Gluconate	31.4(0.9)
Low PO_4	Glucuronate	3.8(0.3)
Low PO_4	Glucose +Gluconate	24.0(1.3)
Low PO_4	Glucose + PQQ	12.9(0.5)

[a] Enzyme activities are expressed as nano moles per minute per milligram 216 of total cell protein.
[b] Minimal MOPS medium containing 10 μM potassium phosphate.
Reference: Ronda *et al.* (1992)

The mutant *E. coli* ZSC113 grown on medium containing glucose and PQQ resulted in a nine fold induction of 6-phosphogluconate dehydratase compared with same strain in luria broth in the absence of carbohydrate, and this rule compares with a 25 fold induction of 6-phosphogluconate dehydratase on gluconate [Table 3]. Thus, the inducing activity of glucose plus PQQ is one third of the gluconate containing media. By effect of limiting phosphate

concentration in the regulation of E.D pathway, it can be concluded that for *E. coli*, a low phosphate concentration promotes use of the E.D pathway indirectly by providing access of PQQ into the periplasm rather than directly by derepressing edd and eda (Biville *et al*. 1991; Kupar *et al*., 1972; Tyrrell *et al*., 1990). Experimental report showed that limiting phosphate concentration does not affect the E.D pathway because 6-phosphogluconate dehydratase is not induced by phosphate limiting glucose containing media. So limiting phosphate does not induce E.D pathway. Inductions of 6-phosphogluconate dehydratase were similar in the presence of high and low phosphate concentration with all other carbon sources [Table 3].

The low phosphate concentration in growth medium contains PQQ plus glucose, which greatly reduce the lag phase of the organism because of induction of the PhoE porin facilitated PQQ uptake in the periplasm (Fliege *et al*., 1992). Low phosphate concentration in aquatic environment would result in the uptake of PQQ and is induced to glucose dehydrogenase for the oxidation of glucose to gluconate. So E.D pathway is indirectly controlled by limiting phosphate concentration. It seems reasonable that *E. coli* and dehydrogenase bacteria should be able to harness the energy of electron transfer via PQQ dependent glucose dehydrogenase, therefore higher yield of cell growth occurs in medium containing glucose plus PQQ other than glucose alone in medium (Sashidhar *et al.,* 2010).

PQQ considered as a Vitamin for mammals

Thirteen substances have been recognized as Vitamin, the first is B1 and latest one is vitamin B12. Kasahara and Kato (2003) claimed that PQQ could be considered as a new vitamin, because they identified a PQQ- dependent dehydrogenase enzyme, which was crucial for lysine metabolism in mice. PQQ acted as a mammalian redox cofactor in this reaction and therefore, they concluded that PQQ to be a newcomer to the B group of the vitamins (Kasahara and Kato (2003). Rucker *et al* contradicted the statement of Kasahara & Kato (Rucker *et al*., 2005). They found that activity of α- aminoadipic acid δ-semialdehyde dehydrogenase (AASDH) in liver and plasma levels of α-amino adipic acid (AAA), both of which act as indicators of lysine degradation in mammals are not effected by changes in PQQ in dietary status (Steinberg *et al*., 1994). Killgor *et al*. (1989) reported that PQQ deficiency was associated with a decrease in lysyl oxidase, which was thought at that time to require PQQ (Killgore *et al*., 1989). But they now know that their observation of decrease lysyl oxidase activity in PQQ-deprived mice is due mostly to diminished expression of lysyl oxidase as a result of impaired growth (Rucker *et al*. 2005). From this fact, Rucker *et al*. (2005) concluded that PQQ might be an important biological factor for which nutritional and pharmacological exposure can elicit positive and presumed healthy out comes. They also suggested that sufficient information is not available so far to state the PQQ uniquely performs an essential vitamin function in animal.

Felton *et al* (2005) reported that Kasahara & Kato (2003) claimed their experiment on the basis of sequence analysis using databases that inappropriately label β –propeller sequence as a PQQ binding motifs and evidence actually suggested that the enzyme was a interesting novel protein and it has a seven- bladed β propeller structure, but there is nothing to indicate that it is a PQQ dependent dehydrogenase (Felton and Anthony 2005). PQQ could not be accepted as a vitamin until it could be proved that it would be required by an enzyme as an essential cofactor.

But Kasahara & Kato (2005) replied all controversial questions. According to them, Rucker overlooked the possibility that there might be two isoenzymes catalysing oxidation of (α- aminoadipic acid δ-semialdehyde) AAS to AAA (Kasahara and Kato 2005). There are two pathways for lysine degradation in mammals and the isoenzyme function in each pathway might require a different redox cofactor (Carson *et al*., 1968; Gatfield *et al*., 1968). Because their experiments were based on earlier work in which the formation of NADH from NAD^+ was measured spectroscopically to determine enzyme activity, it might be impossible to detect PQQ-dependent enzyme activity (Chang *et al*., 1990). Rucker *et al* (2005) did not measure plasma AAA concentrations under the same condition because of technological limitations, and the data presentations were undermined in the absence of appropriate statistical analysis (Kasahara and Kato 2005). Secondly, Kasahara & Kato replied to the question of Felton and Anthony (2005) that their identified mU26 matches these profiles although any amino acid residue in the PQQ enzyme repeat cannot easily bind to a PQQ molecule directly (Anthony and Ghosh 1998), the β-propeller structure formed by the PQQ enzyme repeats does provide a space for PQQ. Also the repeated sequences of mU26 are more similar to those of bacterial PQQ dependent enzymes than to those of other β-propeller protein such as G-protein β-subunits Kelch or RCC1 proteins.

Kasahara & Kato concluded that PQQ is an important nutrient for experimental rodents (Rucker *et al*., 2005; Steinberg *et al*., 1994; Killgore 1989). Whether or not PQQ provides nutritional benefit for humans has yet to be determined. The dietary requirements of PQQ may vary between species in the same way as ascorbic acid (vitamin C), which is essential for humans but not for mice. Developing a reliable method to micro assay, PQQ in human samples should help to resolve this issue (Kasahara and Kato 2005).

Physiological importance of PQQ

PQQ has a diverse role in biological system (Rucker *et al*., 2009). It is important for animal growth and development (Killgore 1989). It is present in mammalian tissue and milk and also common fruits and vegetables (He *et al*., 2003; Kumazawa *et al*., 1995; Mitchell *et al*., 1999; Noji *et al*., 2007) [Table 4]. Mice fed with PQQ devoid diet therefore showed decreased fertility and slower rates of neonatal growth and also displayed effects of friable skin, reduction in general fitness, and defect in immune function and loss of sensitivity of B and T cell to mitogens (Killgore 1989). PQQ may also be acting as a pre-or probiotic, given its known role as a bacterial chemotactic agent and co-factor for prokaryotic organism (Tannock 2001).

TABLE 4 Concentration of PQQ in various foods

sample PQQ	(ng/g fw or ng/mL)
field mustard	5.54 ± 1.50
spinach	7.02 ± 2.17
green pepper	2.12 ± 0.40
broccli sprout	1.55 ± 0.37
Japanese radish	0.70 ± 0.42
rape blossoms	5.44 ± 0.8
fermented soybeans	1.42 ± 0.32
green tea	0.16 ± 0.05
beer	1.66 ± 0.82

Reference: Noji *et al.,* 2007

PQQ is an effective cardioprotective agent. PQQ act as a free radical scanvenger in ischemic myocardium. It is given either as a pretreatment before ischemia or as a treatment at the onset of reperfusion following ischemia. This is highly effective in reducing myocardial infract size and improving cardiac function in a dose related manner in intact rats. The optimal dose which exhibited neither renal nor hepatic toxicity is 15mg/ kg, but lower dose may also be efficacious (Bo-qing *et al*., 2004). A protective effect on brain injury in a rodent of cerebral hypoxia / ischemia suggested that PQQ may have potential use in the therapy of stroke (Jensen *et al*., 1994).

Clinical application of PQQ suggested that either prophylactic administration of PQQ in high risk patient or treatment at the time of an active ischemic episode could be of benefit by reducing infract size and ventricular arrhythmias. It also suggested that PQQ could also be effective at the time of reperfusion which occurs with chemical thrombolysis or ballon angioplasty/ stenting when these procedures are employed as early therapy of acute myocardial infraction (Bo-qing *et al*., 2004).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 (Stites *et al*., 1996). Function of mitochondria also depends on the PQQ. The lack of PQQ leads to an apparent decrease in mitochondrial content or perturbation in function (Stites *et al*., 2006). PQQ also has an effect on protein synthesis. It inhibits protein synthesis in hemin-supplemented rabbit by increased phophorylation of eIF-2α and by diminished guanine nucleotide exchange activity of eIF-2α. This study reported that low and high concentration of PQQ stimulate eIF 2B activity. These findings further emphasize the importance of the redox state in the regulation of inhibition of protein synthesis (Ramaiah *et al*., 1997).

Reactive oxygen species (ROS) are thought to be responsible for neurotoxicity caused by the neurotoxin 6-hydroxydopamine (6-OHDA). PQQ has to protect against 6-OHDA- induced neurotoxicity using human neuroblastoma SH-SYSY cell by the help of 6-OHDA-induced cell death and DNA fragmentation (Hirokazu *et al*., 2007). It has a potent superoxide –scavenging activity compared to both antioxidant and vitamins (Hirokazu *et al*., 2007). Methylmercury (MeHg) induced neurotoxicity in undifferentiated PC12 cells is protected by PQQ. The protection mechanism of PQQ is exerted by maintaining the plasma membrane integrity, inhibiting DNA fragmentation, preventing the disruption of mitochondrial membrane potential, up- regulating the level of Bcl-2 and consequently inhibiting the activation of caspase-3, suppressing the overproduction of the intracellular ROS and stabilizing the GSH level may also contribute to the protective effect of PQQ on MeHg –induced apoptosis and oxidative stress (Peng *et al*., 2008).

The brain is more affected by oxidative stress than other organs and ROS attack brain neurons causing neuron degenerative disease like Alzheimer's and Parkinson. Actually hyperbaric oxygen significantly induced deficits of cognative performance and as a result reduce learning ability and memory retentions. It has been reported that PQQ has potentiality to prevent neurodegeneration, its effect is independently or along with other antioxidant (Zhang *et al*., 2009). The nerve growth factor (NFG) production is also increased by PQQ and protects N-methyl-D-aspartate (NMDA) receptor by a direct oxidation of the receptor redox site. The neuron protection is made by suppressing peroxynitrite and it act as a redox modulation of NMDA receptor (Ohwada *et al*., 2008).

PQQ is also a toxic compound, which damages the DNA in vitro (Hiraku *et al*., 1996). However 2μmol/l of PQQ is needed to damage the DNA and nano mol amount of PQQ is required for cell proliferation (Naito *et al*., 1993). Potentiality of the toxic effect of PQQ depends upon the divalent metal ion, for example NADH/Cu^{2+}/PQQ dependent redox system is more efficient in generating superoxide and hydrogen peroxide but if Cu^{2+} is replaced by Fe^{2+},$Fe^{3+}$and Mn^{2+}, DNA cleavage does not occur. Nephrotoxicity and oxidative damage have been reported when 35μmol or more per kg body weight (10mg PQQ/kg) was administered daily by injections (i.p.) to rats over a period of 4-5 days (Watanabe *et al*., 1989).

CONCLUSION

This paper shortly describes the characterization and involvement of PQQ in biological system .This new cofactor can act as general base catalyst and involved in electron transport chain and alcohol oxidation along with alcohol dehydrogenase enzyme. The main gene for PQQ synthesis is pqqC in bacteria and it is a multi step reaction pathway. The physiological importance of PQQ is involved in antioxidant as well as clinical treatment also. Recently, one question has been raised, whether PQQ is to be considered as a vitamin or not, but this answer is not explained properly due to lack of information for all biochemical roles of PQQ. Scientists have found a PQQ

dependent enzyme gene in humans but the symptoms as a result of deficiency of PQQ in human body is not known yet. This might be due to the reason that common fruits and vegetables containing PQQ, are taken up daily by humans. So we need to study further the phenomena of biogenesis of PQQ that focuses on the mechanism of metabolism in biological system.

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