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Original Article

Pyrroquinoline Quinone as an *In-vivo* Biofactor in Polyol Pathway

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ABSTRACT

Pyrroquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) of acetic acid bacteria is a membrane-bound enzyme involved in the acetic acid fermentation by oxidizing ethanol to acetaldehyde coupling with reduction of membranous ubiquinone (Q), which is, in turn, re-oxidized by ubiquinol oxidase, reducing oxygen to water. PQQ-ADHs seem to have co-evolved with the organisms fitting to their own habitats. The enzyme consists of three subunits and has a pyrroloquinoline quinone, 4 heme c moieties and a tightly bound Q as the electron transfer mediators. Biochemical, genetic and electrochemical studies have revealed the unique properties of PQQ-ADH since it was purified in 1978. The enzyme is unique to have ubiquinol oxidation activity in addition to Q reduction. This focuses on the molecular properties of PQQ-ADH, such as the roles of the subunits and the cofactors, particularly in intramolecular electron transport of the enzyme from ethanol to Q. Biotechnological applications of PQQ-ADH as to enantiospecific oxidations for production of the valuable chemicals and bioelectrocatalysis for sensors and fuel cells using indirect and direct electron transfer technologies and discuss unsolved issues and future prospects related to this elaborate enzyme. PQQ with ABCDE gene cluster from Gluconobacter oxydans, which is involved in pyrroloquinoline quinone (PQQ) biosynthesis, in Escherichia coli, resulting in PQQ accumulation in the medium. Since the gene cluster does not include the tldD gene needed for PQQ production, this result suggests that the *E. coli* tldD gene, which shows high homology to the G. oxydans tldD gene, carries out that function. The synthesis of PQQ activated d-glucose dehydrogenase in *E. coli* and the growth of the recombinant DNA has been improved. In an attempt to increase the production of PQQ, which acts as a vitamin or growth factor, *E. coli* has been transferred with various recombinant plasmids, resulting in the overproduction of the PQQ synthesis enzymes and, consequently, PQQ accumulation--up to 6 mM--in the medium.

Keywords: PQQ, ADH, Q10, Polyol, NADPH, NAD^+, *E. coli*.

INTRODUCTION

Pyrroloquinoline quinone (PQQ) was discovered by J.G. Hauge as the third redox cofactor after nicotinamide and flavin in bacteria (although he hypothesized that it was naphthoquinone).[1] Anthony and Zatman also found the unknown redox cofactor in alcohol dehydrogenase and named it methoxatin. In 1979, Salisbury and colleagues as well as Duine and colleagues extracted this prosthetic group from methanol dehydrogenase of methylotrophs and identified its molecular structure. Adachi and colleagues identified that PQQ was also found in Acetobacter.[2] (See figure 1.)

The previous publication of Q10 was on cholesterol lowering device and present article focuses on glucose lowering activity *in-vivo*. [Piyush A. Gediya, Nadim M. Chhipa, Viraj P. Jatakiya, Sachin M. Patel and Prof. Dr. Dhrubo Jyoti Sen; Antioxidant coenzyme Q10 as free radical scavengers in hypercholesterolemia: *Internationale Pharmaceutica Sciencia*: 2(4), 18-25, 2012.][3]

These enzymes containing PQQ are called quinoproteins. Glucose dehydrogenase, one of the quinoproteins, is used as a glucose sensor. Subsequently, PQQ was found to stimulate growth in bacteria.[4] In addition, antioxidant and neuro-protective effects were also found. Also called the sorbitol-aldose reductase pathway, the polyol pathway appears to be implicated in diabetic complications, especially in microvascular damage to the retina, kidney and nerves. Sorbitol cannot cross cell membranes and when it accumulates, it produces osmotic stresses on cells by drawing water into the insulin-independent tissues.[5] (See table 1.)

Cells use glucose for energy; however, unused glucose enters the polyol pathway when aldose reductase reduces it to sorbitol.[6] This reaction oxidizes NADPH to $NADP^+$. Sorbitol dehydrogenase can then oxidize sorbitol to fructose, which produces NADH from NAD^+. Hexokinase can return the molecule to the glycolysis pathway by phosphorylating fructose to form fructose-6-phosphate. However, in uncontrolled diabetics that have high blood glucose - more than the glycolysis pathway can handle - the reaction's mass balance ultimately favors the production of sorbitol.[7] (See figure 2.)

Mode of action

Activation of the polyol pathway results in a decrease of reduced NADPH and oxidized NAD^+; these are necessary cofactors in redox reactions throughout the body and under normal conditions they are not interchangeable.[8] The decreased concentration of these NADPH leads to decreased synthesis of reduced glutathione, nitric oxide, myo- inositol, and taurine. Myo-inositol is particularly required for the normal function of nerves. Sorbitol may also glycate nitrogens on proteins, such as collagen and the products of these glycations are referred-to as AGEs - advanced glycation end products.[9] (See figure 3)

AGEs are thought to cause disease in the human body, one effect of which is mediated by RAGE (Receptor for Advanced Glycation End products) and the ensuing inflammatory responses induced.[10] They are seen in the hemoglobin A1C tests performed on known diabetics to assess their levels of glucose control. Aldose reductase inhibitors are a class of drugs being studied as a way to prevent eye and nerve damage in people with diabetes. Their target, aldose reductase, is an enzyme that is normally present in many other parts of the body and catalyzes one of the steps in the sorbitol (polyol) pathway that is responsible for fructose formation from glucose.[11] Aldose reductase activity increases as the glucose concentration rises in diabetes in those tissues that are not insulin sensitive, which include the lenses, peripheral nerves and glomerulus. Sorbitol does not diffuse

through cell membranes easily and therefore accumulates, causing osmotic damage which leads to retinopathy and neuropathy. While most cells require the action of insulin for glucose to gain entry into the cell, the cells of the retina, kidney and nervous tissues are insulin-independent, so glucose moves freely across the cell membrane, regardless of the action of insulin.[12]

The cells will use glucose for energy as normal and any glucose not used for energy will enter the polyol pathway. When blood glucose is normal (about 100 mg/dl or 5.5 mmol/l), this interchange causes no problems, as aldose reductase has a low affinity for glucose at normal concentrations.[13] In a hyperglycemic state, the affinity of aldose reductase for glucose rises, causing much sorbitol to accumulate, and using much more NADPH, leaving less NADPH for other processes of cellular metabolism. This change of affinity is what is meant by activation of the pathway. The amount of sorbitol that accumulates, however, may not be sufficient to cause osmotic influx of water.[14] NADPH acts to promote nitric oxide and glutathione production, and its deficiency will cause glutathione deficiency as well. A glutathione deficiency, congenital or acquired, can lead to hemolysis caused by oxidative stress.[15] Nitric oxide is one of the important vasodilators in blood vessels.[16] Therefore NADPH prevents reactive oxygen species from accumulating and damaging cells. Excessive activation of the polyol pathway increases intracellular and extracellular sorbitol concentrations, increased concentrations of reactive oxygen species and decreased concentrations of nitric oxide and glutathione. Each of these imbalances can damage cells; in diabetes there are several acting together. It has not been conclusively determined that activating the polyol pathway damages microvasculature.[17]

CONCLUSION

Pyrroloquinoline quinone (PQQ) is a novel biofactor for which a proposition can be made for physiological importance. PQQ was first recognized as an enzyme cofactor in bacteria. It has recently been tentatively identified as a component of interstellar dust. Thus, PQQ may have been present throughout early biological conception and evolution. PQQ is also a potent plant growth factor. Consequently, for animals and humans, there has been constant exposure to PQQ. In animals, PQQ is reported to participate in a range of biological functions with apparent survival benefits (e.g., improved neonatal growth and reproductive performance). There are also benefits from PQQ supplementation related to cognitive, immune, and antioxidant functions, as well as protection from cardiac and neurological ischemic events. Although PQQ is not currently viewed as a vitamin, its involvement in cell signaling pathways, particularly those important to mitochondriogenesis in experimental animal models, may eventually provide a rationale for defining PQQ as vital to life. For humans, such evidence suggests there may be similar parallels or benefits from improving PQQ status. (See figure 4 & 5.)

The observation that increased mitochondriogenesis and antioxidant functions may be healthful features of PQQ supplementation opens the doors for both therapeutic applications and possible use as an ergogenic aid. Having normal mitochondrial function is essential to a broad range of health and disease relationships; thus, the need for continuing research that examines the efficacy and use of PQQ is compelling. PQQ derivatives are widely distributed in tissues and biological fluids at concentrations that may be sustained by typical dietary exposures. Given the range of functions and apparent survival benefits (e.g., improved reproductive performance), it is reasonable to suggest that PQQ may play a

fundamental role in metabolism. Diabetic cataract formation follows an increase in sugars in the lens. The excess sugar within the lens is reduced by aldose reductase to its alcohol, but the lens capsule is relatively impermeable to sugar alcohols. Because of the excess sugar alcohol (polyol), the lens imbibes water, causing osmotic imbalance. Eventually, increased sodium and decreased potassium levels and decreased glutathione levels lead to cataract formation. Topical administration of aldose reductase inhibitors prevents the cataract in rats. Many bacteria can synthesize the cofactor pyrroloquinoline quinone (PQQ), a cofactor of several dehydrogenases, including glucose dehydrogenase (GCD). Among the enteric bacteria, Klebsiella pneumoniae has been shown to contain the genes required for PQQ biosynthesis. Escherichia coli and Salmonella typhimurium were thought to be unable to synthesize PQQ but it has been reported that strain EF260, a derivative of *E. coli* FB8, can synthesize PQQ after mutation and can oxidize glucose to gluconate via the GCD/PQQ pathway (F. Biville, E. Turlin & F. Gasser, 1991, J Gen Microbiol 137, 1775-1782). We have re-investigated this claim and conclude that it is most likely erroneous. (i) Strain EF260, isolated originally by Biville and coworkers, was unable to synthesize a holo-enzyme GCD unless PQQ was supplied to the growth medium. No GCD activity could be detected in membrane fractions. (ii) The amount of PQQ detected in the growth medium of EF260 was very low and not very different from that found in a medium with its parent strain or in a medium containing no cells. (iii) EF260 cells were unable to produce gluconate from glucose via the PQQ/GCD pathway. (iv) Introduction of a gcd:: Cm deletion in EF260, eliminating GCD, did not affect glucose metabolism. This suggested a pathway for glucose metabolism other than the PQQ/GCD pathway. (v) Glucose uptake and metabolism in EF260 involved a low-affinity transport system of unknown identity, followed most likely by phosphorylation via glucokinase.

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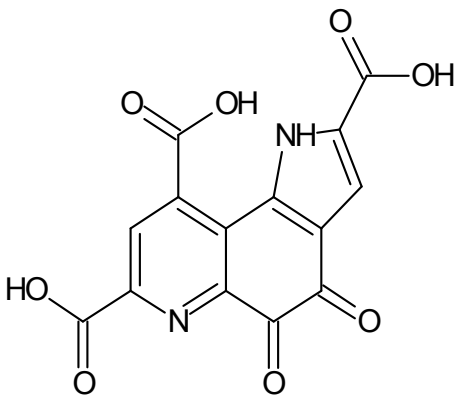
Table 1. Physical properties of PQQ[2]

Properties	Value	Properties	Value
LogP	-0.65	Parachor	573.1 ± 6.0 cm^3
Molecular Formula	$C_{14}H_6N_2O_8$	Index of Refraction	1.801 ± 0.02
Formula Weight	330.20604	Surface Tension	134.8 ± 3.0 dyne/cm
Composition	C(50.92%) H(1.83%) N(8.48%) O(38.76%)	Density	1.963 ± 0.06 g/cm^3
Molar Refractivity	71.98 ± 0.3 cm^3	Polarizability	28.53 ± 0.5 $10^{-24} cm^3$
Molar Volume	168.2 ± 3.0 cm^3	Monoisotopic Mass	330.012415 Da



4,5-dioxo-4,5-dihydro-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid

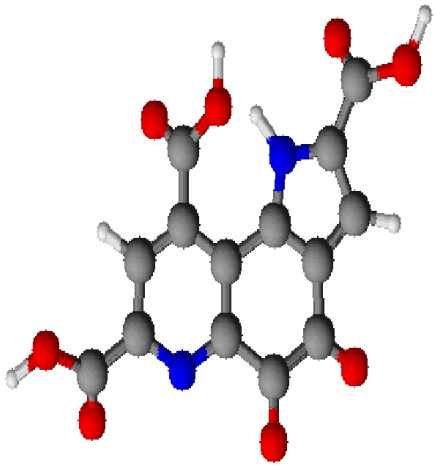


Figure 1. Pyrroquinoline quinone[1,2]

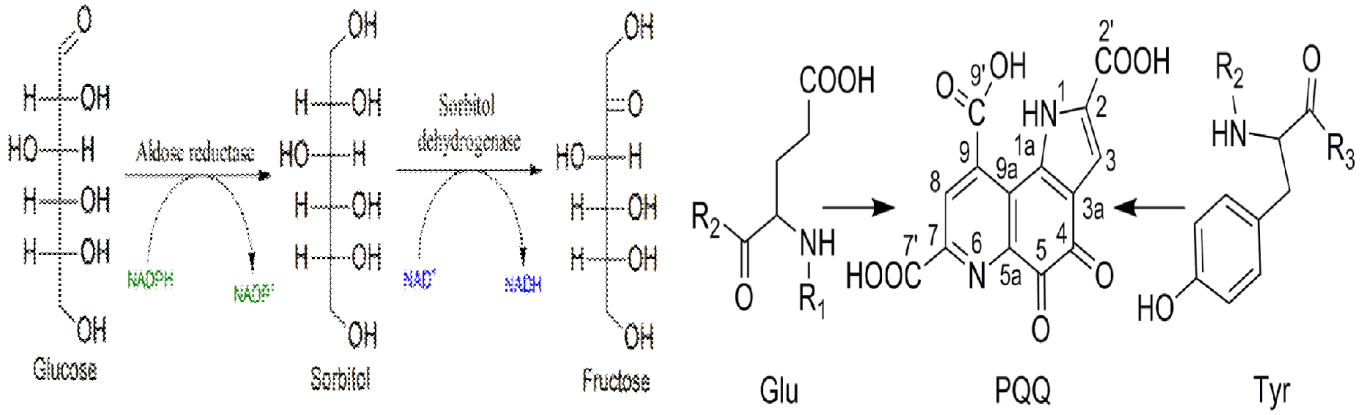
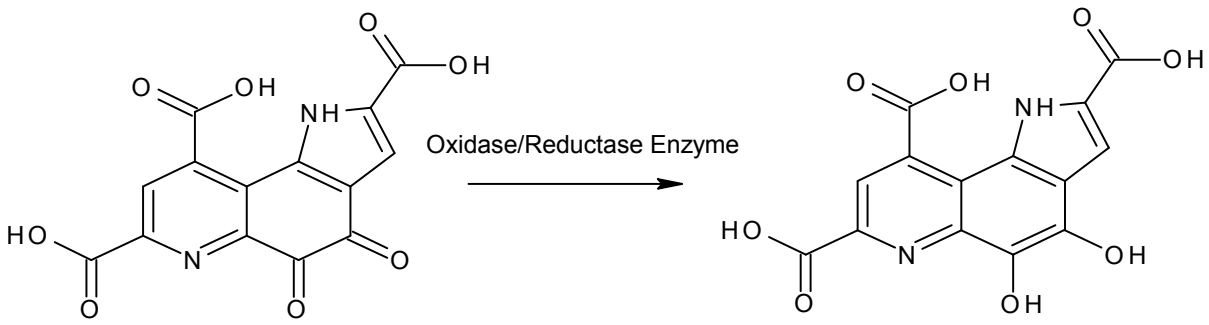


Figure 2. Polyol pathway[3,4]

4,5-dihydroxy-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid



4,5-dioxo-4,5-dihydro-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid

Figure 3. PQQ as cofactor[5,6]

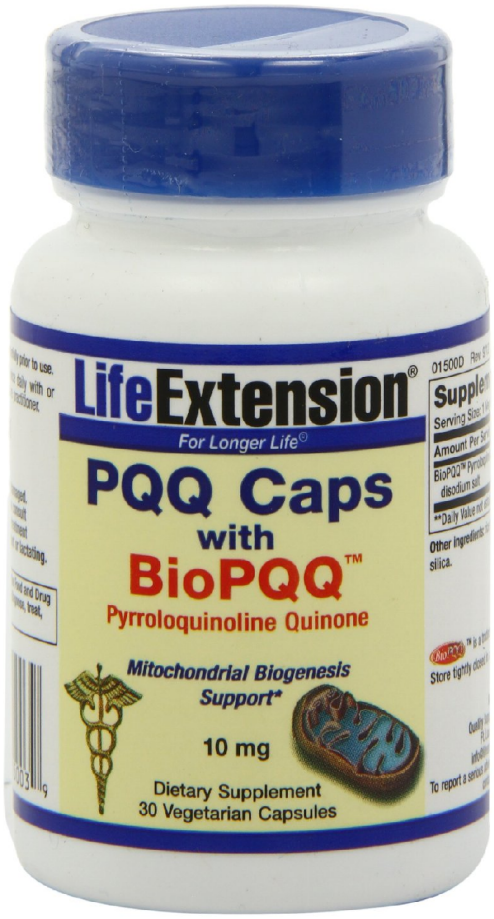


Figure 4. PQQ formulation[16]

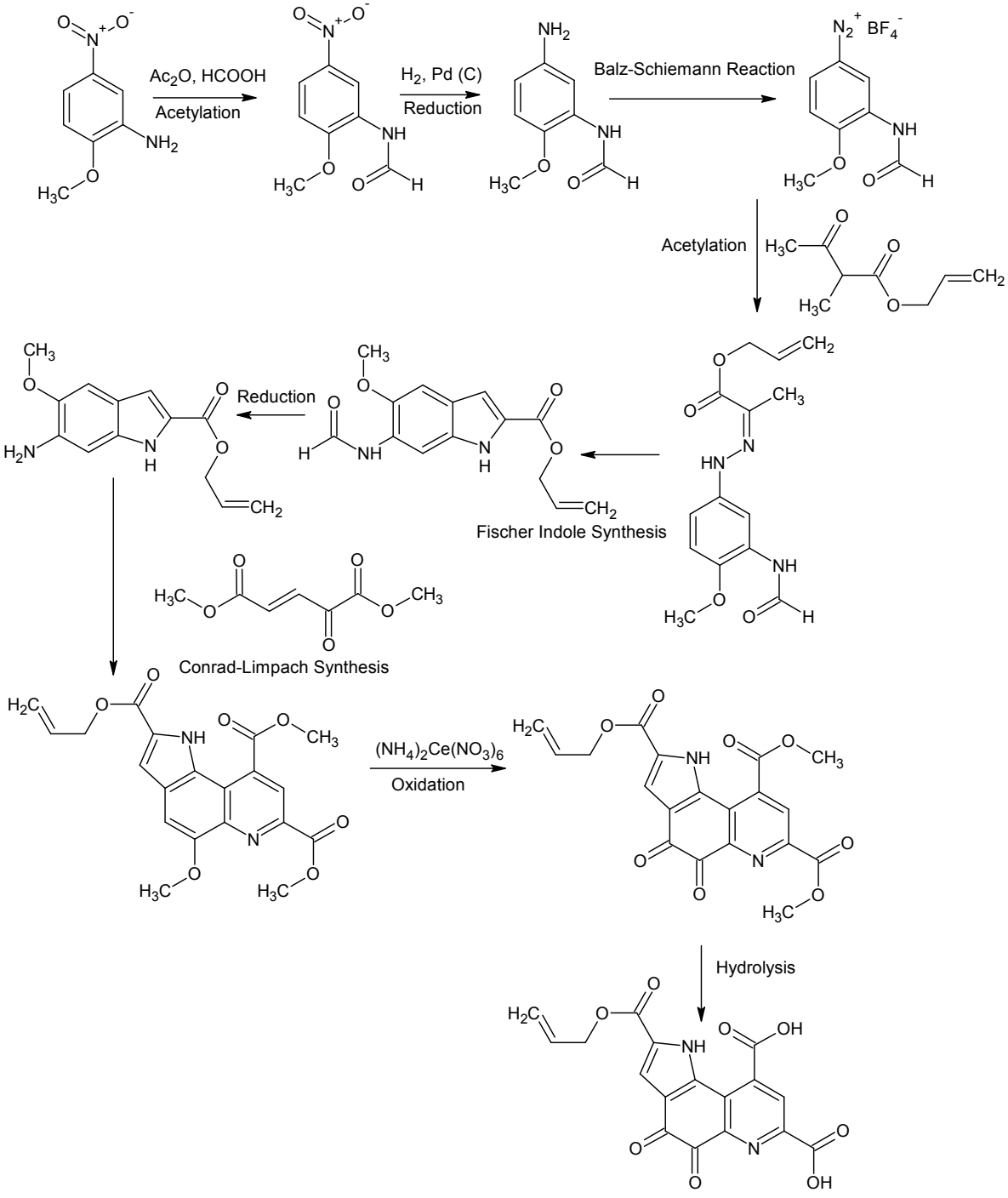


Figure 5. PQQ Synthetic route[7-10]