S-6-4 The Quinoid Cofactors, Pyrroloquinoline Quinone(PQQ), Topaquinone (TPQ) and Tryptophan Tryptophylquinone (TTQ)

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I. INTRODUCTION

About 30 years ago, interest developed in the industrial production of proteins by microbes (single cell protein) and the conversion of waste materials into biogas. Related to that, several research groups started to isolate methylotrophs, microbes carrying out the dissimilation of C(1)-compounds (methane, methanol, methylamine, etc.) or the formation of methane (methanogenic bacteria). Subsequently, the pathways involved in these processes were elucidated and the enzymes detected catalyzing the steps. The spin-off of this work was the discovery of several cofactors, some really novel, others merely variants on already known ones. As far is known today, these cofactors play a role restricted to these specialistic microbes, except 2,4,7-tricarboxy-1*H*-pyrrolo[2,3-*f*]quinoline-4,5-dione, indicated as pyrroloquinoline quinone or with the abbreviation PQQ. This compound (Fig. 1) was originally identified as the cofactor of methanol dehydrogenase. Surprisingly, shortly thereafter several other bacterial dehydrogenases, appeared to contain PQQ, as demonstrated by simple denaturation of the homogeneous protein preparation or by reconstitution of the apo-enzyme to the active holo-form (see reference [1] for a review).

Given the clear significance of PQQ for bacteria, the question arose whether this would also be the case for eukaryotes. Although in the first instance a positive answer was obtained, it appears now that the storey is more complicated. Initially these studies concentrated on structure elucidation of the covalently-bound cofactor of mammalian copper-containing amine oxidases. Upon derivatization the cofactor in the enzyme with a hydrazine, the isolated product was claimed to be the hydrazone of PQQ, so that it was concluded that the amine oxidase contained covalently bound PQQ. Moreover, PQQ was found in a hydrolyzate of such an enzyme. Subsequently, the presence of covalently bound PQQ was detected with several physical techniques and in many other enzymes. However, it appears now that these techniques are unreliable since some of these enzymes do not contain PQQ and others contain a different quinone cofactor, namely topaquinone (TPQ) or tryptophan-tryptophyl quinone (TTQ) (in contrast to PQQ, these cofactors form part of the protein chain, as indicated in Fig. 1). Therefore, the existence of covalently-bound PQQ is questionable, the more as no evidence exist for the occurrence of free PQQ in eukaryotes (*vide supra*). Since TPQ occurs in amine oxidases distributed from man to microbes, quinoproteins (enzymes with a quinone cofactor) occur in eukaryotes as well as prokaryotes. More details and references to the original papers can be found in reviews [1, 2].

II. OCCURRENCE

1. PQQ. The presence of PQQ has been well established for a number of bacterial dehydrogenases and for the spent culture liquid of the Gram-negative bacteria producing these enzymes. The latter may be related to the fact that those dehydrogenases investigated, occur in the periplasm of these bacteria in which most probably assembly of these enzymes will occur, leading to escape of PQQ through the outer membrane. In this context it should be mentioned that although PQQ is very stable to heat and light, it easily reacts with electrophilic compounds. As a consequence, PQQ will condense with several amino acids to practically undetectable products. This may form the reason that PQQ has only been detected in mineral media with simple carbon substrates, but not in rich media containing for instance peptone. Since quinoxaemoprotein alcohol dehydrogenase (type II) is the crucial enzyme in ethanol conversion by Acetic acid bacteria, vinegar contains substantial amounts of PQQ [2].

Reports on the presence of PQQ in Gram-positive bacteria are scarce: the facultative methylotroph, *Amycolatopsis methanolica* (formerly known as *Nocardia spec. 239*), produces PQQ only when grown on methanol and it has been stated that methanol dehydrogenase from *Clostridium thermoautotrophicum* contains PQQ.

Fig. 1. Structures of quinone cofactors.

It is highly unlikely that PQQ occurs in eukaryotes since: there is no real evidence for the existence of covalently-bound PQQ in enzymes from prokaryotic as well as eukaryotic origin; screening of samples from plants and animals for free PQQ or its condensation products, gave negative results [3]. The latter statement needs some further comment since it is controversial. During the past few years, several reports by others mentioned the presence of PQQ in body fluids and tissues (e.g. milk, blood plasma, egg yolk) from animals as well as plant materials (especially citrus fruits). In all these cases detection was based on a chemical assay in which the oxidative decarboxylation of glycine by PQQ leads to reduction of a dye. However, the outcomes appear to be unreliable since other biochemicals can mimic the behaviour of PQQ in this assay. Moreover, biological assays with at least the same sensitivity were unable to detect any PQQ in samples from these sources, though they showed a positive result in dye reduction. Recently, much lower amounts of PQQ were reported to occur in milk, as demonstrated by h.p.l.c.

and electrochemical detection. However, such tiny amounts have also been detected in beer and could well result from PQQ production by bacterial contaminations of the samples. Moreover, since PQQ ingested by rats is taken up by the body, in the case of milk the PQQ could originate from that present in the ingested food or from the bacteria in the digestive apparatus of the cow (although the latter seems unlikely since anaerobic conditions prevent PQQ formation in some bacteria). In conclusion, there exists no evidence for the production of PQQ by eukaryotes or a biochemical rationale for the effects observed by administration of PQQ.

2. TPQ and TTQ. TPQ was first detected in amine oxidase from bovine blood serum. In view of the similar inhibition sensitivity towards carbonyl group reagents and the spectral similarity, copper-containing amine oxidases (EC 1.4.3.6) present in bacteria (Gram-positives as well as -negatives), yeasts, fungi, plants, and animals, including man, will contain TPQ. TTQ was first detected in methylamine dehydrogenase from *Methylobacterium extorquens*. It is also present in the enzymes from *Paracoccus denitrificans* and from *Thiobacillus versutus*, and in view of the many similarities, in all (methyl)amine dehydrogenases (found so far only in Gram-negative bacteria). Assays to detect the presence of these quinones in free form are not available. However, since they are chemically very reactive and most likely their biosynthesis occurs *in situ* in the protein chain (*vide infra*), the occurrence of these compounds in free form seems very unlikely.

II. FUNCTION

1. PQQ. The bacterial PQQ-containing dehydrogenases are involved in the oxidation of hydroxy- or oxo-groups of non-phosphorylated substrates (e.g. several types of alcohol dehydrogenases, quinate dehydrogenase, glucose dehydrogenases). Therefore, PQQ plays an important role in incomplete microbial oxidations, such as production of vinegar from ethanol and gluconic acid from glucose. Since the cofactor is synthesized by the bacteria catalyzing these processes, addition of PQQ is not required, except under circumstances where it has been detached from the enzymes by manipulation of the bacteria. In such cases, addition of PQQ shortens the lag phase of growth.

Curiously, many bacteria produce quinoproteins in their apo-form, as judged from the fact that they require external PQQ to bring these enzymes to activity. Apparently, these organisms are unable to produce PQQ required for holo-enzyme formation. However, the latter conclusion may be premature since very recent work shows that some of these bacteria contain cryptic genes for PQQ biosynthesis which become deblocked by mutation (F. Gasser, personal communication) or PQQ is synthesized but apparently in a form unsuited to convert the apo-enzyme into holo-enzyme (B.W. Groen and J.A. Duine, unpublished results). The reasons for these uncoordinated behaviour are presently unclear, although PQQ could be supplied in the ecological niche by PQQ producers. Since PQQ auxotrophs (natural as well as man-made) grow normal on substrates not requiring the involvement of a quinoprotein, PQQ is not a crucial compound for common metabolic pathways. An interesting hypothesis has been put forward in which it is assumed that quinoprotein glucose dehydrogenase plays a role in *Escherichia coli* when it occurs in an aquatic environment outside the mammalian body.

Administration of PQQ to animals is able to prevent or to cure

many diseases, as is apparent from the patent literature. For instance, PQQ has been incorporated in hair tonics, eye lotions, preparations to treat cerebral disorders, etc. Since PQQ will react in the body with electrophilic compounds and will be easily reduced by compounds like reduced glutathione and NADH (leading to reduced PQQ which becomes oxidized with oxygen, leading to reduced oxygen species), the effects observed may be related to these reactivities.

2. TPQ and TTQ. Overwhelming evidence exists that TPQ and TTQ act as organic cofactors in the enzymes where they have been detected. TPQ is a special case since the amine oxidases in which it occurs have also inorganic copper as a cofactor, providing opportunities for an interplay and internal electron transfer.

III. BIOSYNTHESIS

1. PQQ. Tyrosine and glutamic acid are the building blocks for biosynthesis of PQQ in methylotrophic bacteria. All searches for intermediates from the pathway have given negative results so far. From cloning and sequencing of DNA from several organisms and transfer of it to *E. coli*, it appears that six genes are required for biosynthesis. One of the genes consistently found in these studies, codes for a small peptide of about 20 amino acids. Sequencing of the terminal gene in a piece of DNA coding for PQQ biosynthesis in *Klebsiella aerogenes*, revealed homology with a recently detected class of metallo-proteases (P.W Postma and J. Meulenbergh, personal communication). Both findings fit very well in a hypothesis put forward some time ago [2] in which it was assumed that the whole process occurs on a protein matrix, the final step being the excision of PQQ. However, to achieve functional quinoprotein holo-enzyme, more steps may be required since in the case of methanol dehydrogenase, genes have been detected which play a role in assembly of this enzyme. A consensus sequence has been found in all quinoprotein dehydrogenases known so far which could be involved in binding of PQQ.

2. TPQ and TTQ. Since these cofactors form part of the protein chain, it seems logical to assume biosynthesis starting with a pro-enzyme form. In view of the complicated structure of TTQ, this will require a post-translational modification process consisting of oxidation and dimerization steps of the two tryptophans. However, for TPQ it could be imagined that redox changes of the copper ion in the pro-enzyme form may convert a specific tyrosyl residue into the TPQ. These processes may be related to those converting specific tyrosyl or tryptophan residues in enzymes into their free radical form, acting as cofactors in a number of recently detected cases.

REFERENCES

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