Chris Anthony

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Full accounts of my research	My publications [in WORD]	My publications with pdf links	Outline CV

Presentations used in research and teaching: These are PowerPoint presentations and all include links to download the original presentations if required. These were prepared for use in the University of East Anglia and Sri Venkateswara University in Tirupati, India. Topics include Introduction to Microbial metabolism, Bioenergetics, Methylotrophy

Brief summary of my research career with list and pictures of my research students

Recent lecture on my 40 years of research on methylotophs: "Methylotroph Stories"

Summary of some of my research topics as Powerpoint presentations

Websites I use [to data bases, literature, protein structure manipulations]

The Quinoprotein Symposium I organisised in 2002 in Southampton

Bits and pieces (for my use)

Summaries of my research

Topics on this page	Topics on other my pages
The Biochemistry of Methylotrophs	Growth of methylotrophs on C2 and C3 compounds
The PQQ-containing quinoprotein dehydrogenases	Completion of the Serine cycle in methylotrophs; a his
The structure and function of methanol dehydrogenase	A History of Methanotrophy
Electron transport during methanol oxidation	PQQ page including its nutritional aspects
Structure of cytochrome cH	Electron transport during methylamine oxidation
Structure of Cytochrome c_L	

Glucose dehydrogenase (GDH)	
Alcohol dehydrogenase (ADH)	
Electron transport with GDH and ADH	
The proposed role of PQQ as a vitamin etc	

The Biochemistry of Methylotrophs. Microbes that are able to grow on compounds with only one carbon atom [C1-compounds] such as methane, methanol, methylamine etc are called Methylotrophs. Those that grow on methane are also called Methanotrophs.All the earlier work on these microbes [up to 1981] is described in Anthony (1982): **The Biochemistry of Methylotrophs.**

My main interests are their carbon assimilation pathways and their energy transduction mechanisms.
The Serine Cycle has at last been completed; for the work completing the cycle click here.

The first step in the oxidation of methanol is catalysed by the enzyme methanol dehydrogenase [MDH]. We first described this in 1964 and later showed it to be a new type of enzyme having a completely novel prosthetic group called PyrroloQuinoline Quinone [PQQ]; it was the first of a whole new family of enzymes called quinoproteins. We then had to work out how this was linked to electron trasport systems to make ATP.
We also worked on energy transduction (electron transport systems) in bacteria growing on methylamine.

TOP

The PQQ-containing quinoproteins. These are the equivalent of flavoproteins that have the riboflavin derivatives FMN or FAD as their prosthetic groups.
The quinoproteins on which we have worked are:
The Methanol Dehydrogenases [MDH] of methylotrophs;
The membrane Glucose Dehydrogenase of enteric bacteria, acetic acid bacteria and pseudomonads;
The membrane Alcohol Dehydrogenase of acetic acid bacteria.

Link to the pictorial celebration of the quinoprotein Symposium I organised in 2002 in Southampton
A major part of my work has been working out how energy [ATP] is obtained by linking the methanol dehydrogenase in an electron transport chain to oxygen. After our discovery of PQQ [whose structure was later determined by others] it was found to be the prosthetic group of other bacterial enzymes such as glucose dehydrogenase [GDH] and we have also been involved in studies of this quinoprotein. The structure of PQQ.

The structure and mechanism of the quinoprotein methanol dehydrogenase

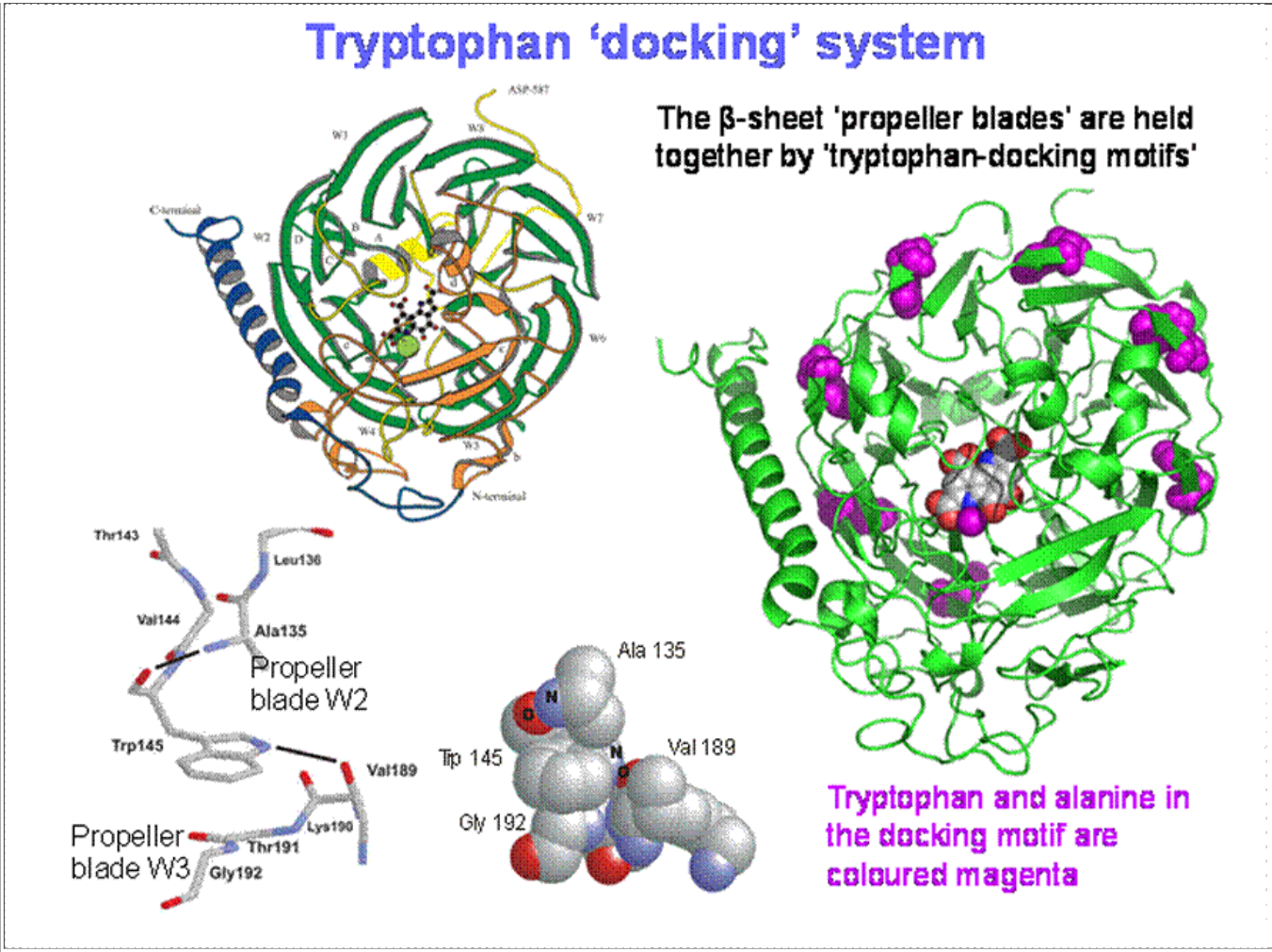
Our X-ray structure of methanol dehydrogenase [MDH] was the first high resolution structure of a quinoprotein. It has a tetrameric structure. The α2β2-subunit (600 amino acids) is a superbarrel made up of eight radially-arranged β-sheets (the 'propeller fold'), held together by 'tryptophan-docking motifs. PQQ, intimately bonded to a Ca2+ ion, is buried in the interior of the superbarrel. The floor of the active site chamber is formed by a tryptophan residue and the ceiling formed by a ring structure arising from a disulphide bridge between adjacent cysteine residues joined by a novel non-planar peptide bond. This is the only example of such a structure in an active enzyme; reduction inactivates (reversibly) the enzyme but its function is unknown. It has been proposed that a catalytic base (possibly Asp303) initiates the reaction by abstraction of a proton from the alcohol substrate. The Ca2+ ion is coordinated to The C-5 carbonyl oxygen of PQQ thus facilitating polarisation of the electrophilic C-5 for subsequent attack by an oxyanion or hydride.
The remarkable 'propeller' superbarrel structure with the PQQ prosthetic group and calcium ion at its active site is best appreciated in the movie [produced with help of Stuart Findlow].

Protein Data Base [pdb] files: A2B2; A1B1 [for pqq select pqq; for calcium ion select ca]





The 'propeller' structure of MDH



The 'Tryptophan docking' system that holds together the propeller blades

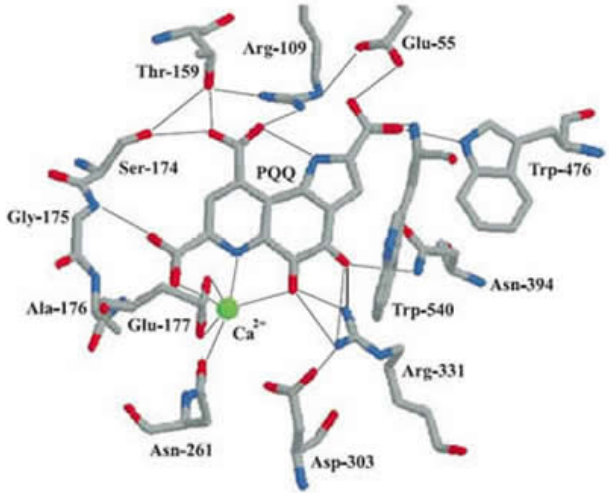


Fig. 2. The equatorial interactions of PQQ and the coordination of Ca^{2+} in the active site of MDH [10]. The figure also shows the proposed active site base (Asp^{303}) and Arg^{331} which may also be involved in the mechanism.

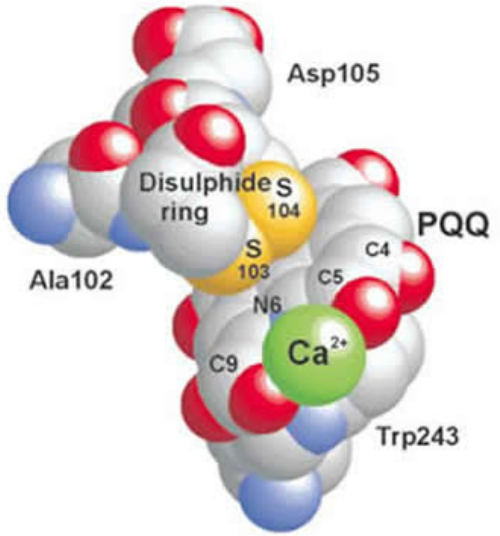
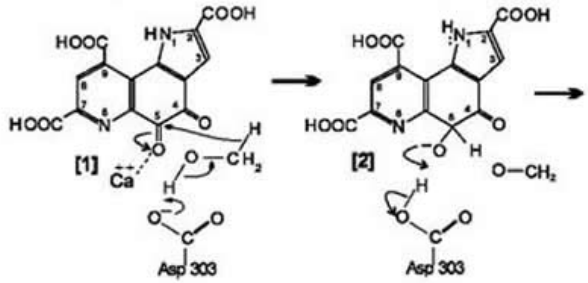


FIGURE 1: Disulfide bridge, PQQ, and Ca^{2+} in the active site of MDH.

The path of electrons and protons during the oxidative part of the reaction mechanism of MDH

The Direct Hydride transfer mechanism Anthony et al (1993--)



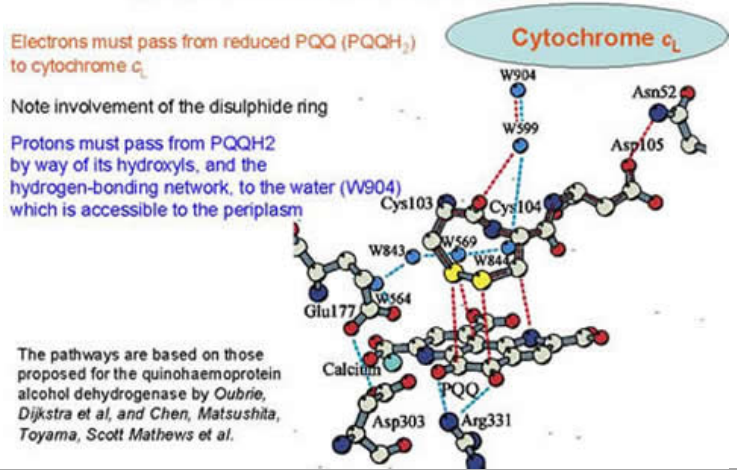
On balance the direct evidence favours the hydride transfer mechanism;

This is strongly supported by work on other quinoproteins
[Oubrie & Dijkstra, 2000; Dewanti & Duine; Chen, Matsushita, Yamashita, Fujuii, Toyama, Adachi, Bellamy & Mathews]

Electrons must pass from reduced PQQ ($PQQH_2$) to cytochrome c_L

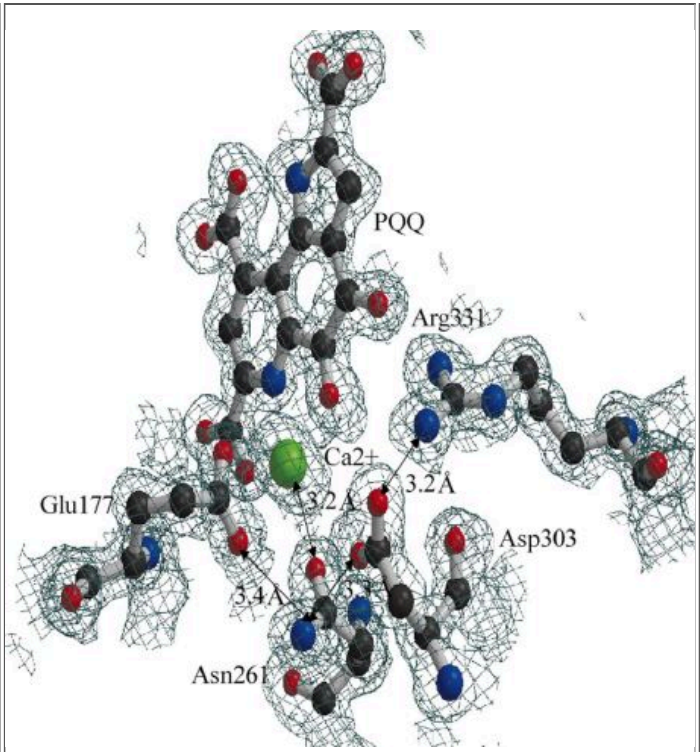
Note involvement of the disulphide ring

Protons must pass from PQQH2 by way of its hydroxyls, and the hydrogen-bonding network, to the water (W904) which is accessible to the periplasm

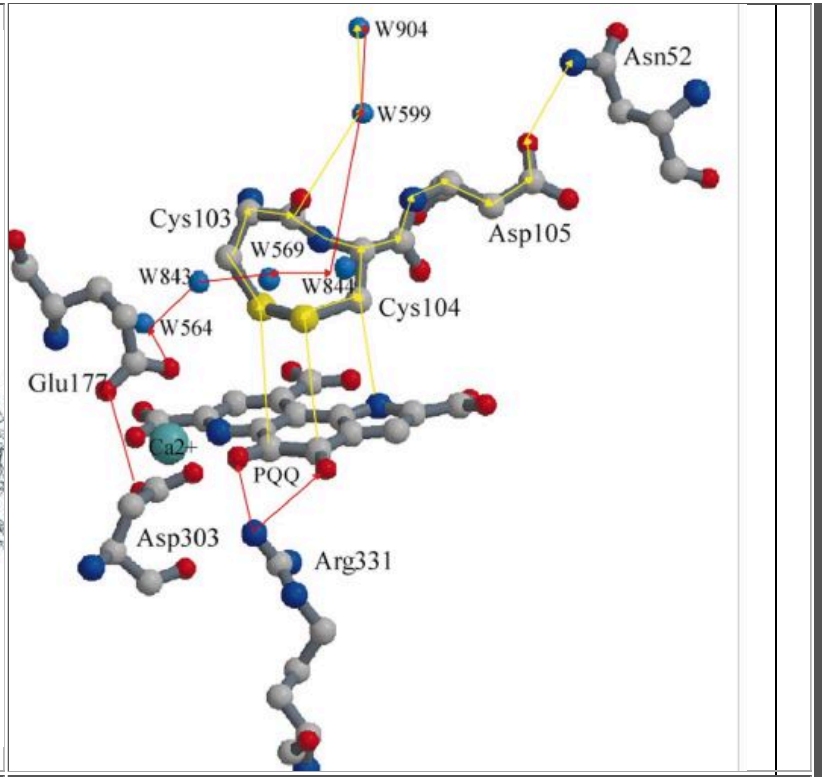


The pathways are based on those proposed for the quinohaemoprotein alcohol dehydrogenase by *Oubrie, Dijkstra et al, and Chen, Matsushita, Toyama, Scott Mathews et al.*

Update on structure Our last paper on MDH structure. The atomic resolution (1.2 Ang) structure of methanol dehydrogenase from Methylobacterium extorquens by Paul Williams in John cooper's Lab. PDF



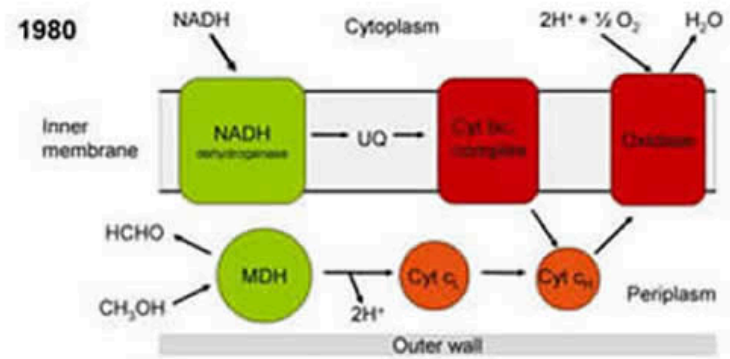
In the 1.2 A Ê resolution structure reported here, the bond length from the calcium ion to Asn261 is 3.18 A , suggesting that this side chain does not actually coordinate to the metal ion. However, Asn261 does hydrogen bond to the catalytic base Asp303 through its sidechain O atom. This hydrogen bond serves to orient the Asp303 carboxylate group for an efficient proton abstraction from the substrate hydroxyl group in the initial step of the reaction.



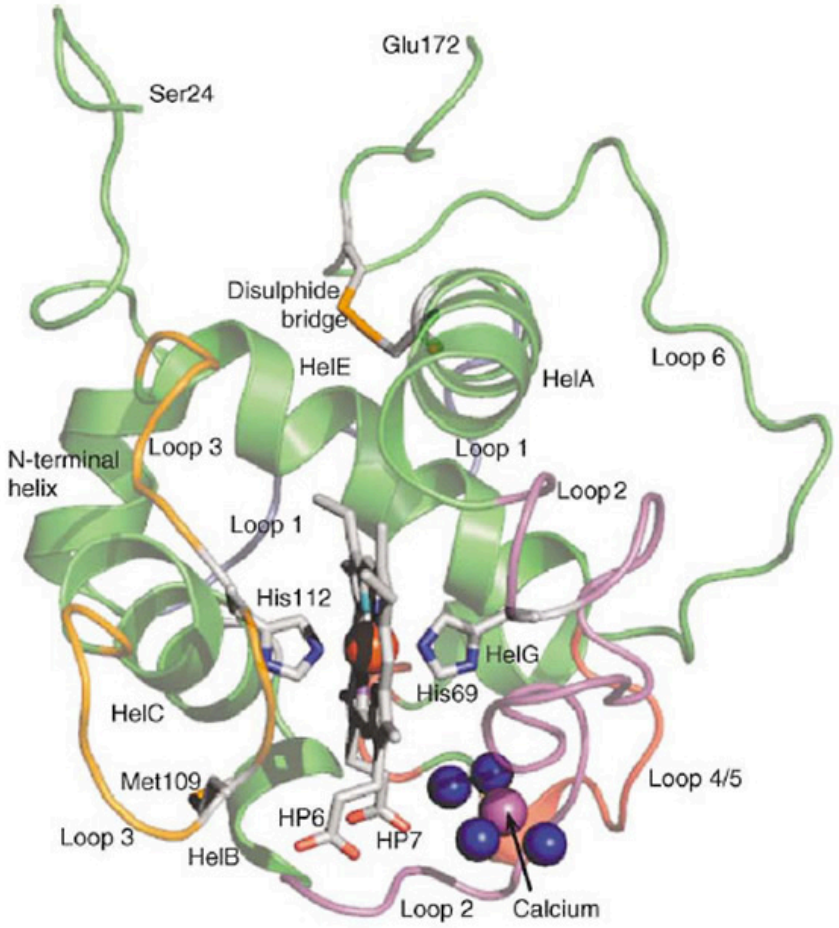
Proposed pathways for electron transfer from PQQ to cytochrome cL (shown in yellow) and proton translocation from PQQ to the periplasm (shown in red). This isBoth pathways are predicted on the basis of the structures of the quinohaemoprotein alcohol dehydrogenase from the bacterium C. testosteroni (Oubrie et al., 2002).

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The electron transport chain from MDH to oxygen: This is very unusual as it consists of 3 periplasmic proteins and a typical cytochrome oxidase. The primary electron acceptor from MDH is a completely novel type of cytochrome called cytochrome cL; this passes electrons to a more typical small cytochrome cH which is the electron donor to the oxidase.



Cytochrome c_L: The structure of cytochrome cL from Methylobacterium extorquens has been determined by X-ray crystallography to a resolution of 1.6 A ° . This unusually large, acidic cytochrome is the physiological electron acceptor for the quinoprotein methanol dehydrogenase in the periplasm of methylotrophic bacteria. Its amino acid sequence is completely different from that of other cytochromes but its X-ray structure reveals a core that is typical of class I cytochromes c, having a-helices folded into a compact structure enclosing the single haem c prosthetic group and leaving one edge of the haem exposed. The haem is bound through thioether bonds to Cys65 and Cys68, and the fifth ligand to the haem iron is provided by His69. Remarkably, the sixth ligand is provided by His112, and not by Met109, which had been shown to be the sixth ligand in solution.
Cytochrome c_L is unusual in having a disulphide bridge that tethers the long C-terminal extension to the body of the structure. The crystal structure reveals that, close to the inner haem propionate, there is tightly bound calcium ion that is likely to be involved in stabilization of the redox potential, and that may be important in the flow of electrons from reduced pyrroloquinoline quinone in methanol dehydrogenase to the haem of cytochrome cL. As predicted, both haem propionates are exposed to solvent, accounting for the unusual influence of pH on the redox potential of this cytochrome.
For Protein Data Bank file [pdb]: cytochrome c_L


Glu172
Ser24
Disulphide bridge
HelE
HelA
Loop 6
N-terminal helix
Loop 3
Loop 1
Loop 2
Loop 1
His112
HelG
His69
HelC
Met109
Loop 4/5
HP6
HP7
Loop 3
HelB
Loop 2
Calcium

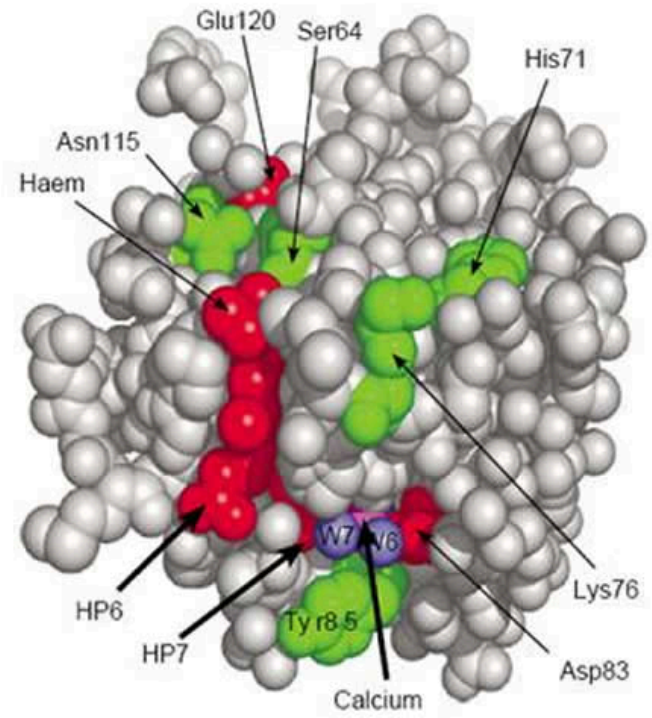


Figure 7. Surface view (space-filling) of cytochrome c_L. The outer haem propionate (HP6) is clearly exposed to solvent, as are the two water molecules (Wat6 and Wat7). Wat7 is bonded to the inner haem propionate (HP7). Asp83, one of the ligands to the calcium ion, has been proposed to be involved in interaction with methanol dehydrogenase together with Glu120, with the outer haem propionate (HP6) and with those residues coloured green.[24]

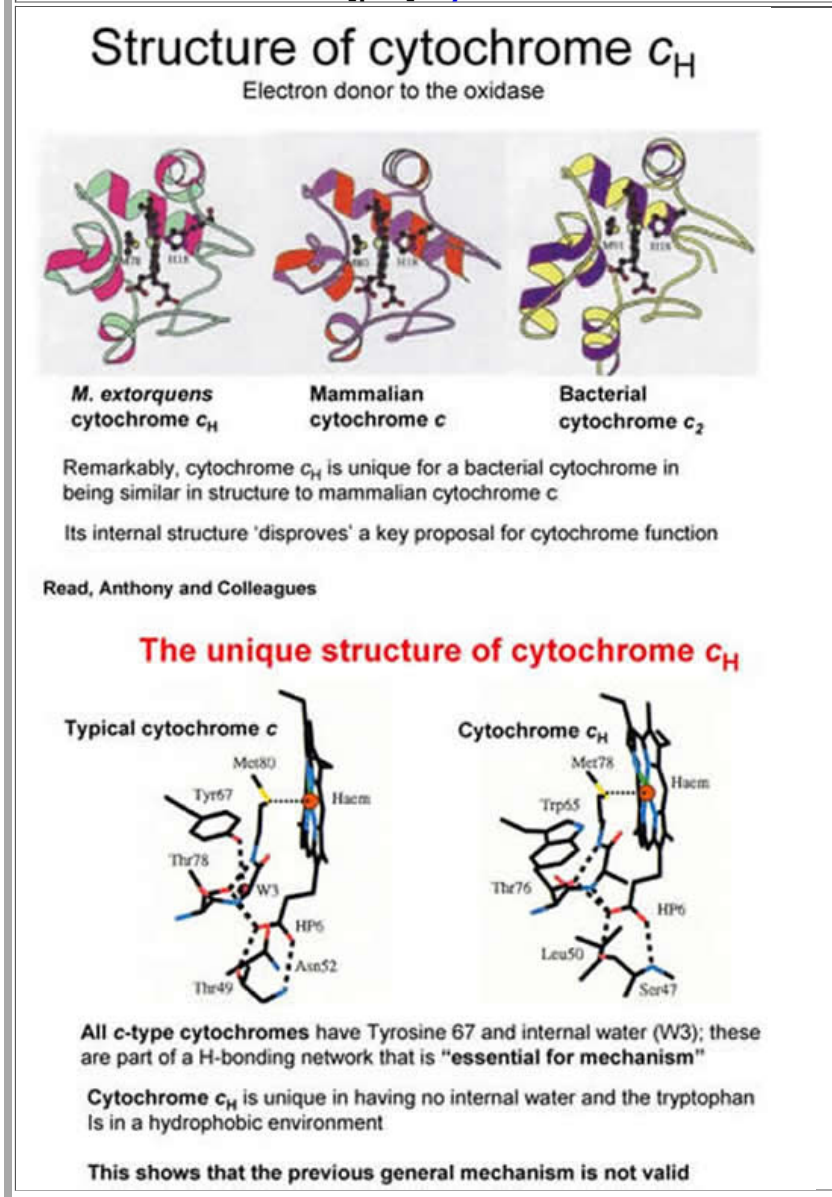
The main structural features of cytochrome cL. Although there is no sequence identity with other cytochromes, the helices A, C and E constitute the typical haem-enclosing fold seen in all cytochromes c. Helices are labelled HelA, HelB, etc. Loop 1 (grey) joins the N-terminal helix and helix A; loop 2 (purple) between helix A and helix B, carries the haem-binding sequence and the amino acid residues that coordinate to the calcium ion; loop 3 (orange) is the exceptionally flexible loop that joins helix C to helix E, and carries the sixth ligand to the haem (His112) and the methionine (Met109) that is the sixth ligand in solution. The red sphere is the iron atom at the centre of the haem prosthetic group. HP6 is the outer haem propionate group and HP7 is the inner haem propionate group. The blue spheres are the water molecules (Wat6-Wat9) that coordinate to the calcium ion (magenta sphere). Met109 is the residue that forms the sixth ligand to the haem in solutions of the cytochrome.

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Cytochrome cH: This small cytochrome mediates electron flow from cytochrome cL to the oxidase. Cytochrome cH is the electron donor to the oxidase in methylotrophic bacteria. Its amino acid sequence suggests that it is a typical Class I cytochrome c, but some features of the sequence indicated that its structure might be of special interest. The structure of

oxidized cytochrome cH has been solved to 2.0 Å resolution by X-ray diffraction. It has the classical tertiary structure of the Class 1 cytochromes c but bears a closer gross resemblance to mitochondrial cytochrome c than to the bacterial cytochrome c2. The left-hand side of the haem cleft is unique; in particular, it is highly hydrophobic, the usual water is absent, and the "conserved" Tyr67 is replaced by tryptophan. A number of features of the structure demonstrate that the usual hydrogen bonding network involving water in the haem channel is not essential and that other mechanisms may exist for modulation of redox potentials in this cytochrome.

For Protein Data Bank file [pdb]: Cytochrome cH



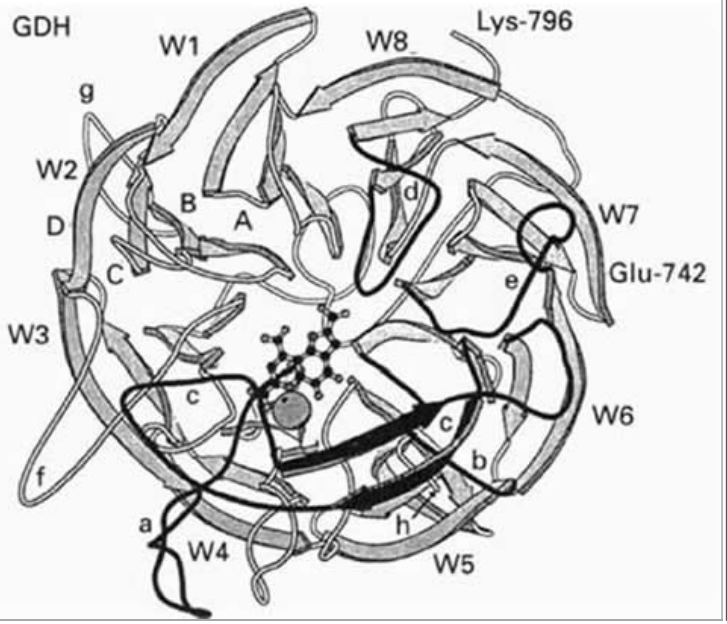
TOP

The structure and mechanism of the quinoprotein Glucose Dehydrogenase (GDH) of *Escherichia coli*

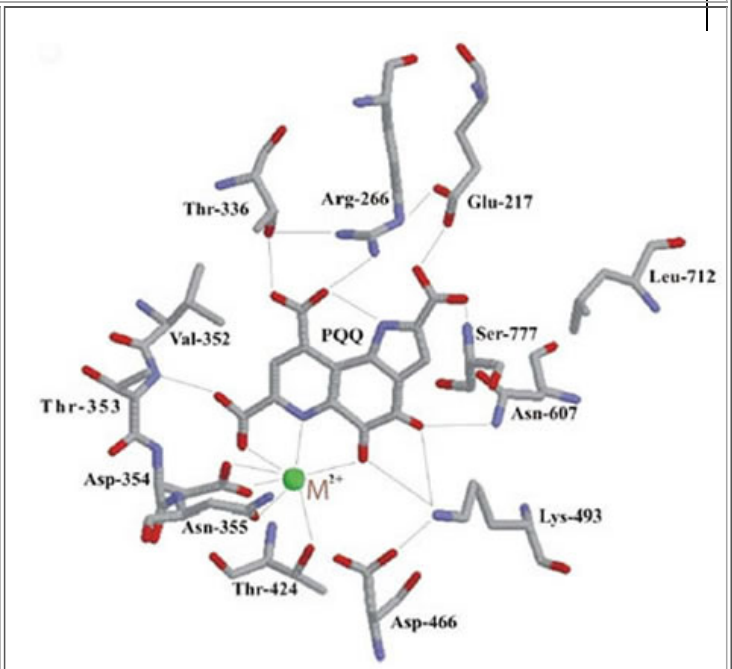
This quinoprotein is responsible for oxidation of glucose to gluconic acid in enteric bacteria, acetic acid bacteria and pseudomonads. It is anchored in the periplasmic membrane by the C-terminal domain made up of 5 membrane-spanning helices. the reaction is catalysed in the periplasm by the N-terminal domain; the amino acid sequence of this domain is sufficiently similar to that of methanol dehydrogenase (MDH) for its structure to be modelled using the MDH X-ray coordinates. Particularly important in achieving this were the conserved beta sheet structures that form the 'propeller blades', and the 'tryptophan-docking motifs' that hold these together [5]. Some sequence that was absent from MDH could not be modelled; this probably forms a loop [80 amino acids] that forms a domain close to the membrane which may contain the site for binding the membrane electron acceptor ubiquinone [UQ]. The active site of the GDH is very similar to that of MDH with the important difference that the novel disulphide ring in MDH is absent from GDH. This is because this ring is involved in the transfer of electrons [one at a time] from reduced PQQ in MDH to through the protein to cytochrome *c*L. In GDH the electrons are passed by way of a bound ubiquinone to membrane ubiquinone, which is then oxidised by a quinol oxidase.

The model GDH has been used very successfully by ourselves and by other groups as the basis for site-directed mutagenesis of GDH [see References].

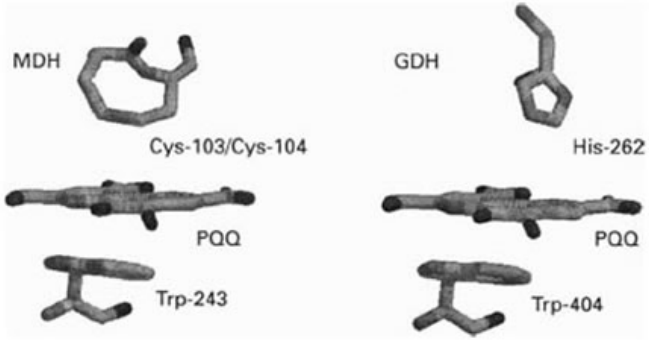
For Protein Data Base [pdb]file: **GDH H262Y mutant GDH**



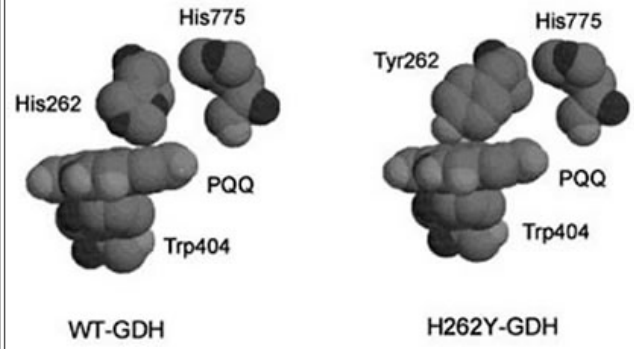
The 'propeller' structure of the model GDH [1]



The active site of GDH [1]. The Metal may be Mg or Ca [3]



A



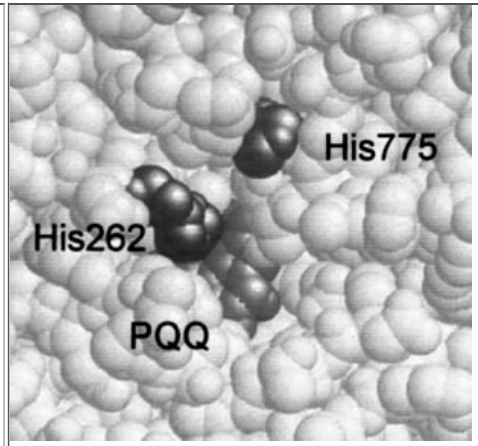
B

The active site of the model GDH.

A: The disulphide ring of MDH is replaced by His262 [1, 2]

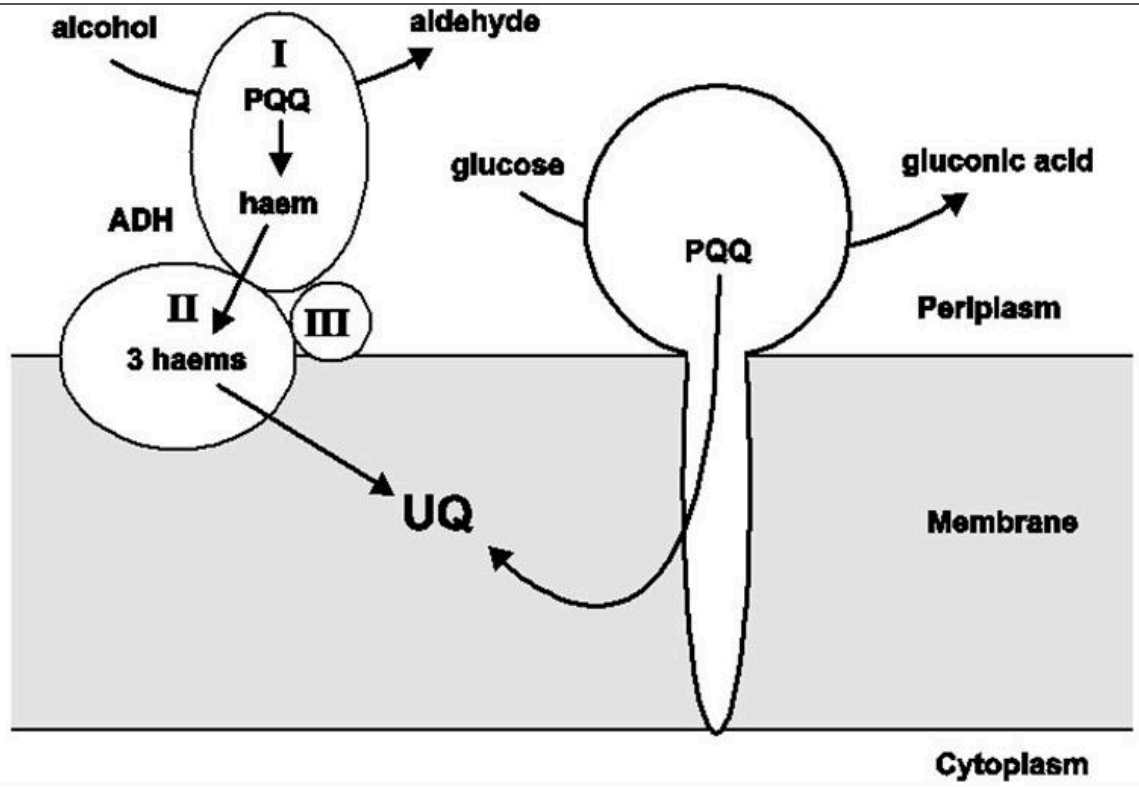
B: His262 was replaced by Tyrosine [2]

C: View of the 'open' active site of GDH [2]



C

Electron transport from membrane GDH and ADH



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References to Glucose Dehydrogenase

1. Cozier, G.E. & Anthony, C. (1995). The structure of the quinoprotein glucose dehydrogenase of Escherichia coli modelled on that of methanol dehydrogenase from Methylobacterium extorquens. Biochemical Journal 312, 679-685.
2. Cozier, G.E., Salleh, R.A. & Anthony, C. (1999). Characterisation of the membrane glucose dehydrogenase from *Escherichia coli* and characterisation of a site directed mutant in which His262 has been changed to tyrosine. *Biochem. J.* 340, 639-647.
3. James, P.L. and Anthony, C. (2003). The metal ion in the active site of the membrane glucose dehydrogenase of *Escherichia coli*. *Biochim. Biophys. Acta* 1467, 200-205.

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4. Anthony, C. & Ghosh, M. (1998). The structure and function of the PQQ-containing quinoprotein dehydrogenases. *Progress in Biophysics and Molecular Biology* 69, 1-21.
5. Goodwin P M, Anthony C (1998) The biochemistry, physiology and genetics of PQQ and PQQ-containing enzymes. In: *Advances in Microbial Physiology.* (Poole R K eds), 40, 1-80.Academic Press, London.
6. Anthony, C. (2004) The Pyrroloquinoline Quinone (PQQ)-Containing Dehydrogenases. In Zannoni D. (ed): Respiration in Archaea and Bacteria. Vol. 1. Diversity of Prokaryotic Electron Transport Carriers, pp. 203-225. Kluwer Academic Publishers. Printed in The Netherlands.

7. Anthony, C. (2004). The quinoprotein dehydrogenases for methanol and glucose. *Archives of Biochemistry and Biophysics* 428, 2–9.

Publications on GDH by other important research groups
(Note: much of this work was based on our model GDH described above).

M. Yamada, K. Sumi, O. Adachi, Y. Yamada (1993), Topological analysis of quinoprotein glucose dehydrogenase in Escherichia coli and its ubiquinone binding site, *J. Biol. Chem.* 268, 12812– 12817.

M.D. Elias, M. Tanaka, H. Izu, K. Matsushita, O. Adachi, M. Yamada (2000) Functions of amino acid residues in the active site of Escherichia coli pyrroloquinoline quinone-containing quinoprotein glucose dehydrogenase. *J. Biol. Chem*. 275, 7321– 7326.

MD. Elias, Satsuki Nakamura, Catharina T. Migita, Hideto Miyoshi, Hirohide Toyama, Kazunobu Matsushita, Osao Adachi and Mamoru Yamada (2004). Occurrence of a Bound Ubiquinone and Its Function in *Escherichia coli* Membrane-bound Quinoprotein Glucose Dehydrogenase. *Journal of Biological Chemistry* 279, 3078 – 3083.

Mamoru Yamada, M D Elias, Kazunobu Matsushita, Catharina T Migita, Osao Adachi (2003). Escherichia coli PQQ-containing quinoprotein glucose dehydrogenase: its structure comparison with other quinoproteins. B*iochimica et biophysica acta* 1647, 185-92.

Kazuo Kobayashi, Golam Mustafa, Seiichi Tagawa, Mamoru Yamada (2005). Transient formation of a neutral ubisemiquinone radical and subsequent intramolecular electron transfer to pyrroloquinoline quinone in the Escherichia coli membrane-integrated glucose dehydrogenase . *Biochemistry.* 44, 13567-72.

Golam Mustafa, Catharina T. Migita, Yoshinori Ishikawa, Kazuo Kobayashi, Seiichi Tagawa and Mamoru Yamada (2008). Menaquinone as Well as Ubiquinone as a Bound Quinone Crucial for Catalytic Activity and Intramolecular Electron Transfer in *Escherichia coli* Membrane-bound Glucose Dehydrogenase. *Journal of Biological Chemistry* 283, 28169–28175.

Sode K, Yoshida H, Matsumura K, Kikuchi T, Watanabe M, Yasutake N, Ito S, Sano H (1995) Elucidation of the region responsible for EDTA tolerance in PQQ glucose dehydrogenase by constructing Escherichia coli and *Acinetobacter calcoaceticus* chimeric enzymes. *Biochem. Biophys. Res. Commun.* 211, 268-273.

H. Yoshida, K. Sode (1996). Thr424 to Asn substitution alters bivalent metal specificity of pyrroloquinoline quinone glucose dehydrogenase, *J. Biochem. Mol. Biol. Biophys.* 1 89– 93.

H. Yoshida, K. Kojima, A.B. Witarto, K. Sode,(1999). Engineering a chimeric pyrroloquinoline quinone glucose dehydrogenase: improvement of EDTA tolerance, thermal stability and substrate specificity, *Protein Eng.* 12 63– 70.

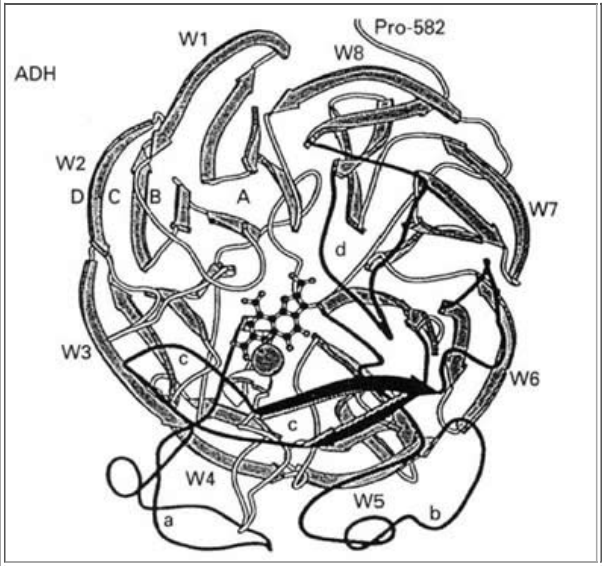
Goldstein A H, Lester T, Brown J (2003) Research on the metabolic engineering of the direct oxidation pathway for extraction of phosphate from ore has generated preliminary evidence for PQQ biosynthesis in *Escherichia coli* as well as a possible role for the highly conserved region of quinoprotein dehydrogenases. *Biochem. Biophys. Acta* 1647, 266-271.

Tripura C B, Podile A R, (2007) Properties of chimeric glucose dehydrogenase improved by site directed mutagenesis. *J. Biotechnol*. 131, 197-204.

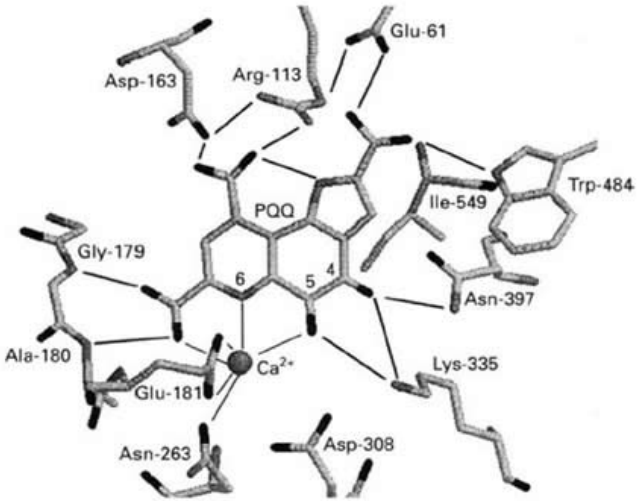
TOP OF QUINOPROTEINS
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A model for Alcohol Dehdrogenase (ADH) of acetic acid bacteria

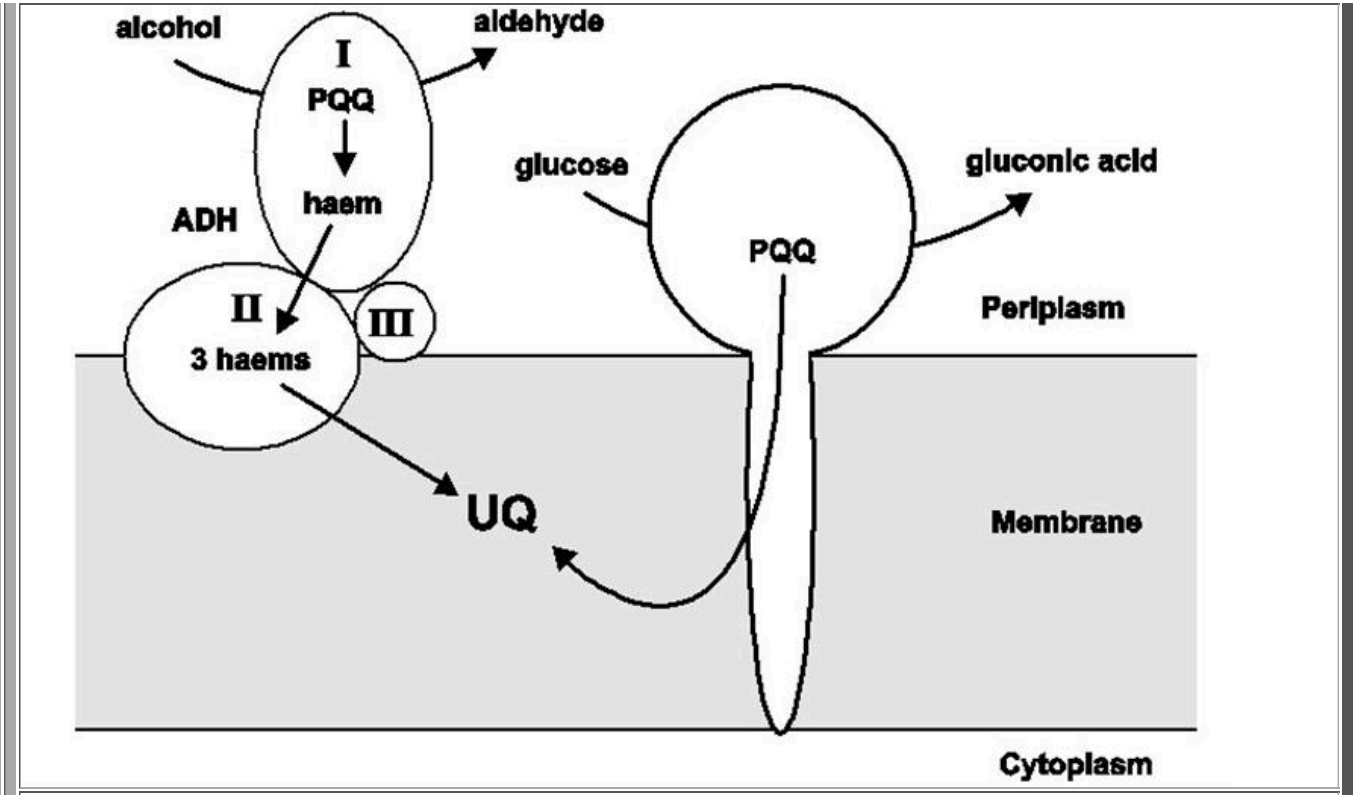
This model was achieved using the same methods as that used for GDH [above].
The ADH of *Acetobacter aceti* is a membrane quinohaemoprotein. We modelled its structure on the X-ray coordinates of methanol dehydrogenase. The basic superbarrel structure and the active-site region are retained, indicating essentially similar mechanisms of action, but there are considerable differences in the external loops, particularly those involved in formation of the shallow funnel leading to the active site.
Cozier, G.E. & Anthony, C. (1995). The structure of the quinoprotein glucose dehydrogenase of *Escherichia coli* modelled on that of methanol dehydrogenase from *Methylobacterium extorquens*. *Biochemical Journal* 312, 679-685.
Protein Data Base [pdb] coordinates for **ADH**



'Propeller fold' structure of ADH



Active site of ADH



Electron transport from memrane GDH and ADH

TOP OF QUINOPROTEINS
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The proposed role of PQQ as a vitamin

CLICK HERE For extensive discussion of PQQ and its role in quinoproteins and especially its role in humans go to my new page on PQQ

In 2003 a paper in Nature claimed to demonstrate that PQQ is a vitamin. To me thee were so many misunderstandings in this paper that it was clearly completel wrong so i ignored it. I then read that, on the basis of this paper, Mitsubishi were planning to invest a huge amount of money to produce PQQ as a vitamin. I persuaded Nature that they should publish a refutation which they did (Felton and Anthony, 2005) together with a second refutation of some of the ntritional statements. I contacted Mitsubishi scientists and the following week two of them came from Japan to Southampton to discuss it. They understood perfectly

and said that Mitsubishi would continue but sell PQQ as a nutritional supplement. They arranged for me to attend a conference on vitamins in Japan and to visit them at Nigata (Click here for pictures).

At this time a lot was written on Wikipedia that was wrong so I argued there at length. Most of this has now been removed but you can look at the discussion on a separate page (Wikipedia and PQQ)

It is generaly accepted that no mammalian PQQ-containing enzyme (quinoprotein) has been described. If such an enzyme does exist then PQQ would almost certainly be a vitamin [analogous to riboflavin, needed in the diet for production of essential flavoproteins].

On a separate page there is discussion of PQQ as a nutritional supplement.

My argument against PQQ as a vitamin can be downloaded as a Word document.

The following section covers all this material in greater detail.

For a summary go my powerpoint presentation

PQQ is now to be produced as a food supplement by Mitsubishi and sold as a vitamin by Maypro

Mitsubishi Announcement: "On August 15, 2008, Mitsubishi Gas Chemical (MGC) announced that it had received official acceptance from the U.S. Food and Drug Administration (FDA) for its notification of coenzyme pyrroloquinoline quinone (PQQ) as a new dietary ingredient. Having received this acceptance, MGC will begin developing the U.S. market for PQQ as an initial step toward the commercialization of PQQ". Mitsubishi statement.

NPI Center announcement: Highly-Anticipated PQQ (Pyrroloquinoline Quinone) Finally Commercialized As VitaPQQ(TM) From Maypro Industries:
"Hailed as a new vitamin by Nature1, VitaPQQ™ brand PQQ is now available from Maypro for use by vitamin and dietary supplement manufacturers. The launch of VitaPQQ™ is a critical milestone in a decades-long effort to commercialize Pyrroloquinoline Quinone (PQQ), a newly discovered vitamin that offers significant health benefits due to its vitamin-like activity, cognitive function and antioxidant capacity. VitaPQQ™ is manufactured utilizing a patented natural fermentation process. "
Announcement by NPI-Center.

A brief history of PQQ

PQQ as coenzyme. PQQ (Pyrroloquinoline quinone) was first discovered in the early 1960s as the novel prosthetic group (or coenzyme) of bacterial enzymes (now known as quinoproteins). Methanol dehydrogenase was one of the first of these, and was the enzyme subsequently most investigated [With Len Zatman, I discovered the enzyme in 1964 and published the purification and description of its coenzyme in 1967]. It is responsible for oxidizing methanol to formaldehyde in bacteria that grow on methane or methanol. The structure of this novel co-enzyme was determined by Olga Kennard's group by X-ray

crystallography in Cambridge University in 1979. Other quinoproteins containing PQQ include the enzyme responsible for production of vinegar from ethanol by acetic acid bacteria, and glucose dehydrogenase in (amongst others) enteric bacteria. We published the first high resolution structure of methanol dehydrogenase (using X-ray crystallography) in 1995. The unusual structure has radial symmetry and has been called a 'propeller structure' and this structure is shared by all the quinoproteins.
No plants or animals have PQQ-containing enzymes. If they did so then it is likely that PQQ would be a vitamin; quinoproteins having PQQ would be analogous to flavoproteins having riboflavin (vitamin B2).

PQQ as dietary supplement. PQQ is present in tiny amounts in animals and has been shown (mainly by the group of Robert Rucker) to improve growth and reproductive performance in mice fed on chemically-defined diets, but he does not claim that this shows it to be a vitamin. However, this sort of study has led to speculation that PQQ may eventually be found to be a vitamin for humans.

False claim that PQQ is a vitamin. Although nutritional experiments have indicated some (unknown) metabolic or nutritional role for PQQ in mammals, it cannot seriously be accepted as a vitamin until an enzyme can be shown to require it as its coenzyme. In 2003 Kasahara and Kato claim to have provided this evidence and announced 'A new redox-cofactor vitamin for mammals' in the top science journal Nature. This was greeted with enthusiasm - "The first new vitamin for 55 years". However, their claim was based on sequence analysis of an enzyme, predicted to be involved in mouse lysine metabolism, which showed that part of the protein has a 'propeller fold', which is a feature of all PQQ-dependent dehydrogenases. What the evidence actually suggests is that their (predicted) protein is an interesting novel protein part of which has a propeller structure; but there is no evidence that it is a PQQ-dependent dehydrogenase. In essence their argument is this: Our mouse protein has a 'propeller' structure [true]; All PQQ-containing proteins have a propeller structure [true]; Therefore our protein contains PQQ [false]. This is the equivalent of the well known false argument: All trees are green; My coat is green; My coat is a tree.

When I pointed out to the journal Nature that their high reputation was being used to justify investments of millions of dollars in the development of PQQ as a vitamin, they investigated the original paper, agreed with our objections and published our argument against it (Felton & Anthony, Nature Vol. 433, 2005). They also published (alongside ours) a paper by Rucker disagreeing with the conclusions of Kasahara and Kato on nutritional grounds, concluding "that insufficient information is available so far to state that PQQ uniquely performs an essential vitamin function in animals".

It should also be noted that the enzyme which they claim to be involved in lysine breakdown does not occur in mice; it is an irreversible enzyme involved in the opposite process of lysine biosynthesis in yeast. The whole of this part of their paper is biochemical nonsense.

CONCLUSION
Mitsubishi is producing PQQ to market as a *nutritional supplement*, because they know that the evidence for it being a *vitamin* is not valid. Maypro, and journalists who like a good story, are selling it as a *New Vitamin VitaPQQ*. They quote the original Nature paper but ignore the demonstration of its faultyconclusions that was subsequently published in Nature.

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For more detailed information see my lecture slides below

TOP OF QUINOPROTEINS
TOP OF MY RESEARCH

My presentation to a conference on vitamins in Osaka and to Mitsubishi scientists in Niigata

Click here for my photos of this visit

Introduction: PQQ cannot seriously be accepted as a vitamin until an enzyme can be shown to require it as its cofactor. About one year ago Kasahara and Kato claim to have provided this evidence and announced 'A new redox-cofactor vitamin for mammals' in Nature. This was greeted with enthusiasm by Reuters news agency "The first new vitamin for 55 years", and its exploitation by Mitsubishi seems to be underway (put vitamin and PQQ into Google for a good survey of this). However, the claim of Kasahara and Kato was based on sequence analysis of an enzyme, predicted to be involved in mouse lysine metabolism, using databases and search engines which inappropriately label beta propeller sequences as PQQ-binding sites. The 'sites' wrongly identified by the databases do not represent PQQ-binding sites but represent the Beta -sheets that form the 'blades' of the 'propeller fold' which happens to be a feature of all PQQ-dependent dehydrogenases, whose main structure is a superbarrel made up of either six or eight 'propeller blades'. What the evidence actually suggests is that their (predicted) enzyme is an interesting novel protein having an eight-bladed beta propeller structure; but there is no evidence that it is a PQQ-dependent dehydrogenase.

There is also no evidence that this protein has any relevance to lysine metabolism.

This material has been published the journal Nature (Felton and Anthony, 2005) (**Download PDF**).

Dr Leigh M. Felton



My friend and co-author of our Nature paper

Felton, L. M. & Anthony, C.(2005).

Role of PQQ as a mammalian enzyme cofactor? *Nature* doi:10.1038/nature03322

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There is *no evidence* from structural studies that PQQ is a mammalian enzyme cofactor

Chris Anthony and Leigh M. Felton

School of Biological Sciences
University of Southampton

Acknowledgements

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MGC

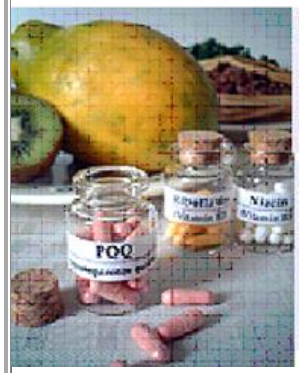
BACKGROUND

A new redox-cofactor vitamin for mammals

Takaoki Kasahara, Tadafumi Kato *Laboratory for Molecular Dynamics of Mental Disorders, Brain Science Institute, RIKEN, Japan*

NATURE | VOL 422 | 24 APRIL 2003 | www.nature.com/nature

"On the basis of our demonstration of its molecular function, we propose that PQQ should be classified as *a new B vitamin*"



IN THE NEWS

Japanese Discover First New Vitamin in 55 Years

April 24, 2003
By REUTERS

Scientists Add New Member to Vitamin B Family
—Sarah Graham, Scientific American, April 24, 2003.

A new page may need to be added to the vitamin B family album. According to a report published today in the journal Nature, researchers have determined that the compound known as pyrroloquinoline quinone (PQQ) should be classified as a vitamin in the same class as vitamins B2 and B3. Scientists first isolated PQQ from bacteria in 1979.

The claim that PQQ is a vitamin

This was based on some false assumptions and misinterpretations. However, it appeared that the conclusions were generally accepted; as this could cause problems two refutations have been published: *NATURE: VOL 433 27 JANUARY 2005:-*

Role of PQQ as a mammalian enzyme cofactor?

Leigh M. Felton, Chris Anthony |www.nature.com/nature *doi:10.1038/nature03322*

"We contend that there is still no compelling evidence for a PQQ-dependent enzyme in the mouse and that the authors' announcement of a new vitamin was therefore premature".

Is pyrroloquinoline quinone a vitamin?

Robert Rucker, David Storms, Annemarie Sheets, Eskouhie Tchaparian, Andrea Fascetti www.nature.com/nature *doi:10.1038/nature03323*

"Our results call into question the identification of PQQ as a new vitamin"

Robert Rucker: Thursday, 16.50 session R13: Physiological and nutritional importance of PQQ

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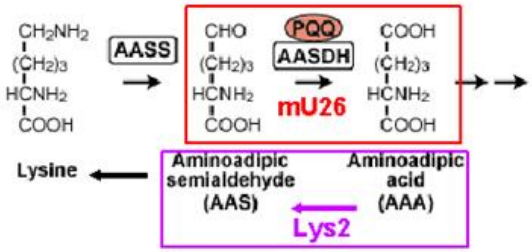
Robert Rucker: Thursday, 16.50 session R13: Physiological and nutritional importance of PQQ

Lysine metabolism

In yeast, aminoadipate reductase (Lys2) catalyses *reduction* of aminoadipic acid to the semialdehyde during lysine *biosynthesis*.

The predicted mouse protein mU26 is similar (<26% identity) to part of Lys2 in yeast.

It was proposed that, in the mouse, mU26 catalyses the *oxidation* of aminoadipic semialdehyde to aminoadipic acid.



However, the reaction catalysed by Lys2 is not reversible.

It is not needed in mammals because oxidation of the semialdehyde is catalysed by a specific NAD-linked aminoadipic semialdehyde dehydrogenase.

There is no evidence that the predicted enzyme (mU26) catalyses oxidation of aminoadipic semialdehyde in the mouse

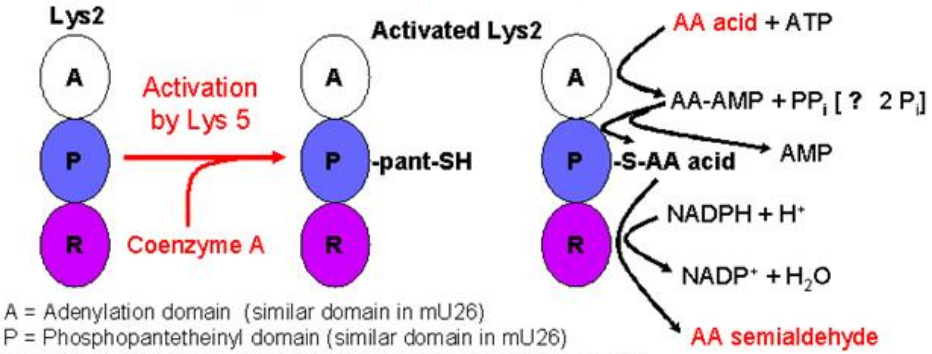
Reduction of aminoadipic acid to the semialdehyde by aminoadipate reductase (Lys2) in yeast

Overall reaction after activation of Lys2 by Lys5 + Coenzyme A :

Amino Adipic acid + ATP + NADPH + H^+

Lys2 ↓

Amino Adipic Semialdehyde + AMP + PP_i + $NADP^+$ + H_2O

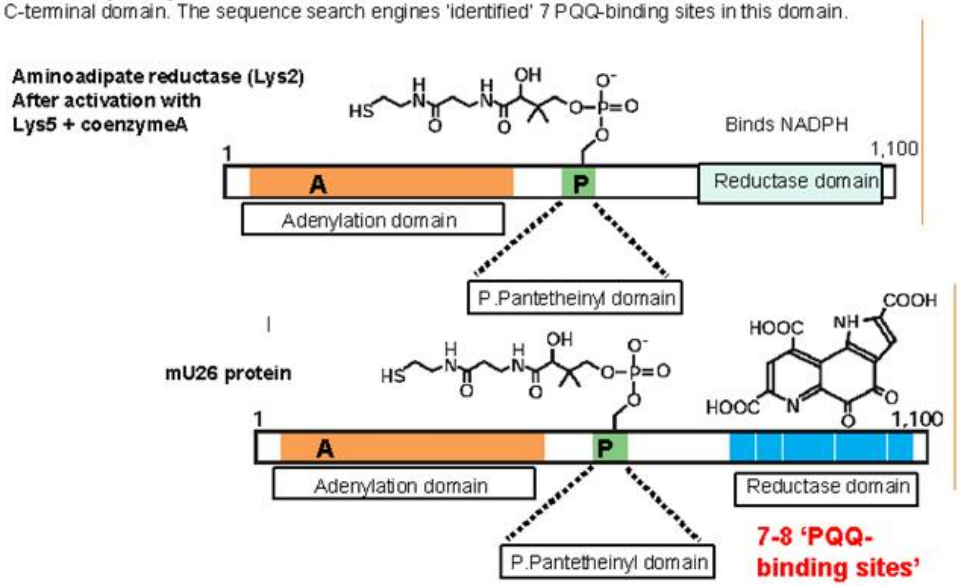


A = Adenylation domain (similar domain in mU26)
P = Phosphopantetheinyl domain (similar domain in mU26)
R = Reductase domain (replaced by 'propeller' structure in mU26)

This reaction is not reversible. In mammals the semialdehyde is oxidised to the acid by a specific NAD-linked dehydrogenase

Sequences of Aminoadipate Reductase (Lys2) and mU26

The cloned novel mouse gene *mU26* was cloned using the Adenylation (A) and Phosphoantetheinylation (P) domains of yeast *Lys2*. The reductase domain was not used. mU26 has the A and P domains and a different C-terminal domain. The sequence search engines 'identified' 7 PQQ-binding sites in this domain.



The search engines 'identified' PQQ-binding sites in the novel C-terminal 'Reductase' domain in mU26

There is no way to identify PQQ-binding sites

The search engine identified the presence of 7 *repeated sequences* that typically occur in PQQ-containing quinoproteins.

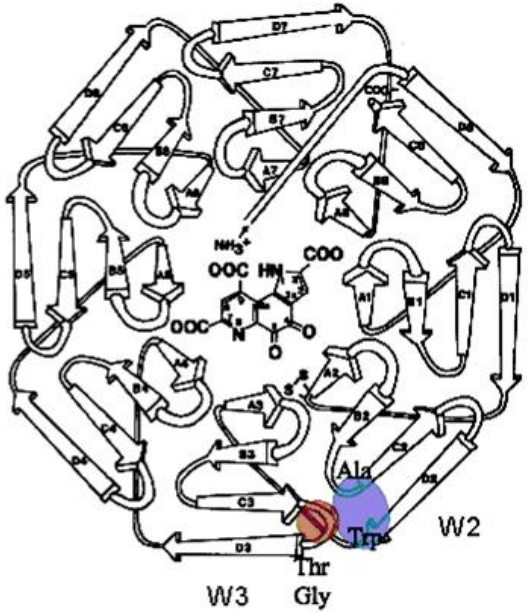
These *sequences* form into the 'propeller blades' of the well-known 'Propeller fold', in which 4-stranded β-sheets (the 'propeller blades') are radially arranged to form a superbarrel structure.

The propeller blades are held together by Tryptophan docking motifs.

The 'propeller blades' – wrongly identified by the search engine as 'PQQ-binding sites' are not directly involved in binding PQQ.

Methanol dehydrogenase is the best-characterised PQQ-containing quinoprotein

Propeller structure of MDH



The propeller blade W2 is bonded to W3 by Trp145 docking between Ala135 and the planar peptide bond between Thr191 and Gly192

The consensus sequence in the tryptophan-docking motifs

Propeller blades W1-W8

Position	1	2	3	4	5	-	6	7	8	9	10	11
Motif	C	C	C				D	D	D	D	D	D
W1	Ala 77	Leu	Gly	Leu	Asp	Asp	Pro	Gly	Thr	Ile	Leu	Trp 88
W2	Ala135	Leu	Asn	Ala	Glu	-	Thr	Gly	Gln	Thr	Val	Trp145
W3	Ala 186	Tyr	Asp	Val	Lys	-	Thr191-Gly192		Gln	Gln	Val	Trp 196
W4	Gly 282	Arg	Asp	Ala	Asp	-	Thr	Gly	Gln	Ala	Lys	Phe 292
W5	Thr 337	Leu	Asp	Arg	Thr	-	Asp	Gly	Ala	Glu	Val	Ser 347
W6	Ala 457	Tyr	Asn	Ala	Ile	-	Thr	Gly	Asp	Tyr	Lys	Trp 467
W7	Ala 489	Arg	Asp	Ser	Asp	-	Thr	Gly	Asp	Leu	Leu	Trp 508
W8	Val 577	Phe	Ser	Leu	Asp 581	-	Gln 39	Leu	Arg	Pro	Ala	Trp 44
MDH Consensus	Ala	X	Asp/ Asn	X	X	-	Thr	Gly	Asp/ Glu	X	X	Trp
GDH/ADH Consensus	Ala	X	Asp/ Asn	X	X	-	Thr	Gly	Lys	X	X	Trp

For example: **Trp145** of strand D in W2 is stacked between **Ala135** in strand C in W2 and **Thr-Gly** at position **6-7** in W3

The propeller blades of mU26

The protein mU26 has 8 tryptophan docking motifs.

Sequence analysis also suggests that mU26 has 8 propeller blades

The human homolog is similar

"Cloning and characterization of a novel human homolog of mouse U26, a putative PQQ-dependent AAS dehydrogenase"

Wang et al. 2005. *Mol Biol Reports*: DOI: 10.1007/s11033-003-2716-4

Taken from T. Kasahara and T. Kato
Nature: doi:10.1038/nature03324

Motif		Position										
		1	2	3	4	5	6	7	8	9	10	11
W1	MDH	A 77	L	G	L	D	D P	G	T	I	L	W 88
	mU26	A 795	V	D	L	S	- S	G	E	T	R	W 805
W2	MDH	A 135	L	N	A	E	- T	G	E	T	V	W 98
	mU26	V 847	L	K	S	N	- S	G	E	K	Y	W 805
W3	MDH	A 186	Y	D	V	K	- T	G	E	Q	V	W 196
	mU26	A 879	L	D	I	Y	- E	K	K	C	V	W 889
W4	MDH	G 242	R	D	A	D	- T	G	E	A	K	F 252
	mU26	A 921	L	N	P	A	- S	G	S	T	V	W 931
W5	MDH	T 347	L	D	R	T	- D	G	A	E	V	S 347
	mU26	L 960	C	F	T	H	- S	G	E	Q	V	W 970
W6	MDH	A 437	Y	N	A	I	- T	G	D	Y	K	W 497
	mU26	Y 1001	C	C	S	K	- E	G	H	L	R	W 1011
W7	MDH	A 489	R	D	S	D	- T	G	D	L	L	W 509
	mU26	V 1048	L	E	S	R	- S	G	E	L	R	S 1059
W8	MDH	V 577	F	S	L	D	- Q	L	R	P	A	W 44
	mU26	C 1086	L	D	L	L	- C	G	D 1095	E 1095	R	W 757

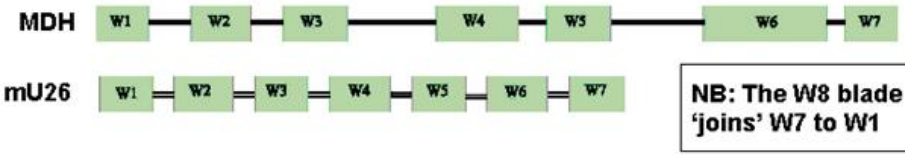
Consensus											
MDH	A	X	D/N	X	X	-	T	G	D/E	X	X	W
GDH/ADH	A	X	D/N	X	X	-	T	G	K	X	X	W
mU26	A	C/L	D	X	X	-	S	G	E	X	X	W

Docking motifs in human U26: hU26 A C/L D/N X X - S/T G E/K X X W

Comparison of mU26 propeller blades and the blades in the PQQ-containing methanol dehydrogenase

There is no sequence identity between mU26 and any PQQ enzyme except for the tryptophan docking motifs.

The C-terminal domain of mU26 is similar in *structure* in having 8 'propeller' blades. This structure is remarkable: it is the smallest possible 8-bladed 'propeller' structure, with only the smallest possible blades and the shortest possible loops between them.



NB: The W8 blade 'joins' W7 to W1

	MDH	mU26
Total residues in 'propeller'	543	341
Number of residues in each propeller 'blade'	30 – 69 With internal loops	30 (minimum possible)
Residues in loops between 'blades'	11 – 51 (av. 27)	9 – 18 (av. 13)

Conclusions

Mammals have a gene coding for a protein (mU26 in mouse, hU26 in humans) that codes for a 3-domain protein;

2 domains are similar (<26%) to a yeast gene for biosynthetic amino adipic acid reductase. This is an irreversible enzyme not known in mammals.

Domain A of mU26 is similar to domains in those enzymes that catalyse the ATP-dependent activation of carboxylic acids:-

a) Activation of fatty acids Fatty acid + ATP ? Fatty acyl-AMP + PP_i

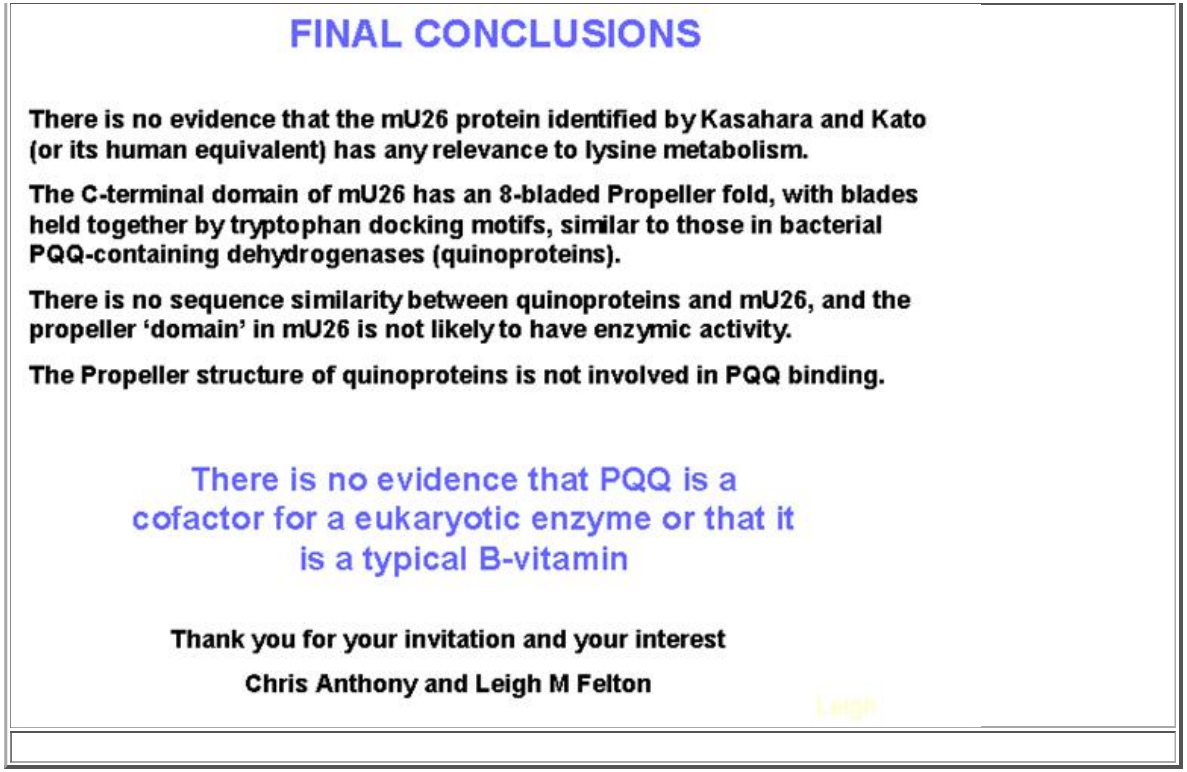
b) Activation of amino acids Amino acid + ATP ? Aminoacyl-AMP PP_i

Domain P is similar to domains that can be 'activated' by phosphopantetheinyl transferases which add the Phosphopantetheinyl part of Coenzyme A to a conserved serine residue:-

a) Acyl carrier protein (ACP) domain of fatty acid and polyketide synthases

b) Peptidyl carrier protein (PCP) domain of microbial non-ribosomal peptide synthases

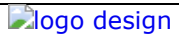
Domain R is a completely novel small superbarrel whose propeller fold has the minimum number of 'blades' held together by tryptophan docking motifs.



TOP of PQQ as a vitamin

TOP of page

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