

## MINI REVIEW

# THE STRUCTURE OF BACTERIAL QUINOPROTEIN DEHYDROGENASES

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### GENERAL INTRODUCTION

Quinoproteins are proteins having pyrrolo-quinoline quinone (PQQ) as their prosthetic group (Fig. 1). PQQ has now been found in all the main types of living organism and, as with other prosthetic groups and coenzymes, it may be a vitamin in higher organisms. Although quinoproteins occur in many different classes of enzymes, the most thoroughly characterized so far have been the bacterial dehydrogenases and it is the structure of three of these that is the subject of this review. These are the dehydrogenases for methanol, isolated from methylotrophs; for alcohol, isolated from acetic acid bacteria; and for glucose, isolated from a range of bacteria including enteric bacteria such as *Escherichia coli*. Comprehensive reviews of quinoproteins and their functions have been published previously (Anthony, 1982, 1986, 1988; Duine and Jongejan, 1989). Since these reviews were published, major advances have been made in gene cloning and sequencing which has provided information on the primary structures of these dehydrogenases. The aim of the present review is to collate this information in such a way that it may have the greatest impact on future studies relating structure and function of this important class of enzymes.

### THE QUINOPROTEIN DEHYDROGENASES

The first quinoproteins to be described and shown to have unusual prosthetic groups were those for methanol and for glucose. The prosthetic groups of most dehydrogenases that are not NAD(P)-linked are riboflavin derivatives, and that of glucose dehydrogenase (GDH) was first thought to be a novel flavin (Hauge, 1964). Methanol dehydrogenase (MDH), by contrast, appeared to have as prosthetic group either a novel pteridine or a completely novel compound (Anthony and Zatman, 1964, 1967a,b). It has subsequently been shown that both these dehydrogenases and other dehydrogenases have PQQ as prosthetic group (Salisbury *et al.*, 1979; Duine *et al.*, 1980), and Duine *et al.* have coined the name 'quinoprotein' to include them all. Methylamine dehydrogenase was previously thought to have PQQ as its prosthetic group but it is now known that this is not the case

(Vellieux and Hol, 1989; Vellieux *et al.*, 1989; Chistoserdov *et al.*, 1990). Its prosthetic group, derived from tryptophan residues, also contains a quinone and it has been suggested that it could also be classified as a quinoprotein. The electron acceptors used *in vivo* are not the same for all the quinoproteins and include ubiquinone, cytochromes or blue copper proteins. By contrast, the quinoproteins are similar in that they are usually assayed *in vitro* with phenazine ethosulphate or Wursters Blue as artificial electron acceptors.

For the purpose of this review only those dehydrogenases that have PQQ as prosthetic group are considered.

### Glucose dehydrogenases

Aerobic bacteria such as pseudomonads, acetic acid bacteria and *Acinetobacter*, which do not have the glycolytic pathway or the phosphotransferase system for glucose uptake, oxidise glucose by way of glucose dehydrogenase (GDH) [for review of this enzyme and other quinoproteins, see Anthony (1988)]. GDH is usually membrane-bound and is arranged so that its catalytic site is available for periplasmic oxidation of glucose to gluconic acid, which is further oxidised or released into the growth medium. Some strains of *Acinetobacter calcoaceticus* have an active GDH but are unable to further oxidise gluconic acid, whereas *Ac. lwoffii* has the apoenzyme but is unable to synthesise PQQ and so does not oxidise glucose. The same is true for two enteric bacteria: *Klebsiella aerogenes* oxidises glucose by GDH but *Escherichia coli* is only able to do so when PQQ is supplied in the growth medium (Hommes *et al.*, 1984; Neijssel *et al.*, 1983). GDH has been especially significant in the study of PQQ function because active holoenzyme can be reconstituted from apo-enzyme plus PQQ and related derivatives (Duine *et al.*, 1980). The role of GDH is not always immediately obvious because it occurs in some bacteria where the product is not further metabolised, and so glucose does not support growth of these bacteria. There is now ample evidence, however, that energy is obtained from glucose by way of GDH and that this leads to increased growth yields for some bacteria when glucose is a supplementary carbon source (van

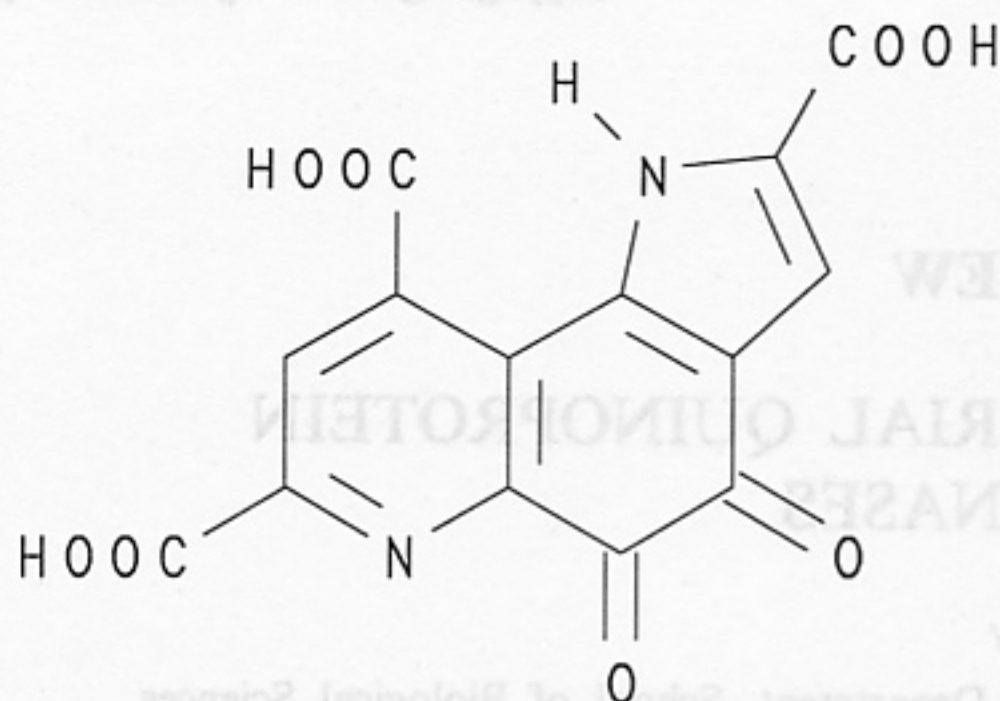


Fig. 1. Pyrrolo-quinoline quinone (PQQ); the prosthetic group of quinoproteins.

Schie *et al.*, 1985, 1987a,b,c; Neijssel *et al.*, 1989; Mueller and Babel, 1986; Matsushita *et al.*, 1987).

GDH was first isolated from membranes and purified from *Acinetobacter calcoaceticus* by Hauge (1964), and subsequently shown to be a quinoprotein by Duine *et al.* (1979). This was confirmed for membrane GDHs from other bacteria by Ameyama *et al.* (1981) and the enzymes were shown to be closely related serologically by Matsushita *et al.* (1986). Studies with the isolated GDH from *Pseudomonas* and *Ac. calcoaceticus* (Matsushita *et al.*, 1982, 1987, 1989a) indicated that the electron acceptor is ubiquinone and this was confirmed *in vivo* for *Acinetobacter* by Beardmore-Gray and Anthony (1986). Membrane GDH is a monomeric integral membrane quinoprotein of about 87 kDa, as recently confirmed for both *Ac. calcoaceticus* and *E. coli* by Cleton-Jansen *et al.* (1988, 1990). It is assumed that there is one PQQ per molecule and there is some evidence that  $Mg^{2+}$  or  $Ca^{2+}$  may be involved in binding it to the apoenzyme (Ameyama *et al.*, 1985; van Schie *et al.*, 1987a; Gorisch *et al.*, 1989; Imanaga, 1989).

In the past, there has been some confusion about the location of GDH in *Ac. calcoaceticus*; this has now been resolved. In addition to the integral membrane protein described above (GDH-A) there is also a soluble GDH that occurs in the periplasm (GDH-B). There is no evidence that GDH-B occurs in other bacteria. This GDH is a dimer of identical subunits of 50 kDa (Dokter *et al.*, 1986; Geiger and Gorisch, 1986; Matsushita *et al.*, 1989b,c; Cleton-Jansen *et al.*, 1988, 1989). The periplasmic GDH-B is associated with a specific cytochrome b-562 but it has not been established that it is involved in electron transport from glucose in whole bacteria (Beardmore-Gray and Anthony, 1986; Dokter *et al.*, 1988; Geerlof *et al.*, 1989).

#### Methanol dehydrogenase

Methanol dehydrogenase is a soluble protein that occurs in the periplasm of most bacteria growing on methane or methanol (for comprehensive reviews of this enzyme and its cytochrome electron acceptor see Anthony (1982, 1986, 1988)). It is probable that any enzyme found to be associated with membrane fractions is a result of preparation procedures because when sphaeroplasts are prepared all the MDH is liberated into the periplasmic fraction. MDH reacts with a specific electron acceptor which is a novel c-type cytochrome, cytochrome  $c_L$ . This passes its electrons by way of a typical small Class I cyto-

chrome c (originally called cytochrome  $c_H$ ) to the membrane-bound oxidase. Although thought for many years to be a dimer of identical subunits ( $M_r$  about 60 kDa), the MDH of *Methylobacterium extorquens* has recently been shown to be a tetramer arranged in an  $\alpha_2\beta_2$  configuration, having  $\alpha$  and  $\beta$  subunits of about 66 and 8.5 kDa respectively (Nunn *et al.*, 1989). This configuration has now been confirmed for MDH of the obligate methylotroph *Methylobacillus glycogenes* (Adachi *et al.*, 1990a) and a gene coding for the 'new' small subunit has been identified in *Paracoccus denitrificans* (de Vries *et al.*, 1990). Each tetramer has two molecules of PQQ. It has proved impossible to separate the subunits in any way that does not also lead to denaturation and loss of PQQ; similarly, all methods for removal of PQQ lead to denaturation of the enzyme. Earlier work had indicated that MDH does not contain any metal atoms (Anthony and Zatman, 1967a) but recent work suggests that calcium or strontium are important in the structure of the MDH of some bacteria (Adachi *et al.*, 1990b).

During the reaction cycle two hydrogen atoms are passed from methanol to PQQ to give the quinol ( $PQQH_2$ ) which is then oxidised (presumably sequentially by way of the semiquinone) by two molecules of cytochrome  $c_L$  in single electron transfer reactions (Dijkstra *et al.*, 1989). It is not known whether or not the complete tetramer can oxidise two molecules of methanol at the same time or if there is any co-operativity in the reaction mechanism between two  $\alpha/\beta$  dimers, each containing one PQQ molecule. One possibility is that one molecule of methanol reduces one PQQ to  $PQQH_2$  which then partially reduces the second PQQ of the tetramer, giving rise to two semiquinones, each of which reduces a single cytochrome  $c_L$ . Evidence suggesting that this is the case is that a semiquinone is detectable on the enzyme. Furthermore, when the enzyme is dissociated, half of the PQQ is in the reduced (quinol) form and half in the completely oxidised form; this could occur by disproportionation of two semiquinone species (Dijkstra *et al.*, 1989; Frank *et al.*, 1989).

The brief outline given above makes it obvious that when consideration is given to the structure of MDH, the following regions and interactions are of particular interest: the methanol-MDH binding site, the site of PQQ binding, the region involved in 'docking' with cytochrome  $c_L$  and electron transfer, and binding of  $\alpha$  and  $\beta$  subunits to each other.

#### Alcohol dehydrogenases

There are a number of different types of alcohol dehydrogenase in bacteria. Some are soluble and similar in many ways to the methanol dehydrogenases of methylotrophic bacteria. These include ADHs from *Pseudomonas aeruginosa* (Groen *et al.*, 1984; Gorisch and Rupp, 1989); from *Pseudomonas putida* (Gorisch and Rupp, 1989); and from *Rhodopseudomonas acidophila* (Bamforth and Quayle, 1978).

A second type of ADH is found, however, in *Pseudomonas testosteronii*. This is a soluble monomeric ADH of about 67 kDa which contains only one PQQ per molecule and a substrate-reducible haem c; it was suggested that this ADH, which has a structural requirement for  $Ca^{2+}$  or  $Mg^{2+}$  ions,

Table 1. Quinoproteins whose primary sequences are available

|                                           | EMBL<br>no. | Swiss-Prot<br>no. | No. of<br>amino<br>acids | Mol.<br>wt | Location | Signal<br>peptide | References                                            |
|-------------------------------------------|-------------|-------------------|--------------------------|------------|----------|-------------------|-------------------------------------------------------|
| <b>Methanol dehydrogenase</b>             |             |                   |                          |            |          |                   |                                                       |
| <b><math>\alpha</math>-Subunit (MDHA)</b> |             |                   |                          |            |          |                   |                                                       |
| <i>M. extorquens</i>                      | M31108      | DHM1\$MET.EX      | 599                      | 65,795     | P        | 27                | Anderson <i>et al.</i> (1990)                         |
| <i>M. organophilum</i>                    | M22629      | DHM1\$MET.OR      | 599                      | 65,996     | P        | 27                | Machlin and Hanson (1988)                             |
| <i>P. denitrificans</i>                   | M17339      | DHME\$PAR.DE      | 599                      | 66,845     | P        | 32                | Harms <i>et al.</i> (1987)                            |
| <b><math>\beta</math>-Subunit (MDHB)</b>  |             |                   |                          |            |          |                   |                                                       |
| <i>M. extorquens</i>                      | X15792      | DHM2\$MET.EX      | 74                       | 8463       | P        | 22                | Nunn and Anthony (1988);<br>Nunn <i>et al.</i> (1989) |
| <i>P. denitrificans</i>                   | —           | —                 | 84                       | 9500       | P        | 20                | van Spanning<br>(personal communication)              |
| <b>Alcohol dehydrogenase (ADH)</b>        |             |                   |                          |            |          |                   |                                                       |
| <i>A. aceti</i>                           | M26951      | DHETSACE.AC       | 707                      | 78,028     | P        | 35                | Inoue <i>et al.</i> (1989, 1990)                      |
| <b>Glucose dehydrogenase (GDH)</b>        |             |                   |                          |            |          |                   |                                                       |
| <b>A-Type (GDHA)</b>                      |             |                   |                          |            |          |                   |                                                       |
| <i>A. calcoaceticus</i>                   | X07235      | DHGASACI.CA       | 801                      | 86,956     | M        | 0                 | Cleton-Jansen <i>et al.</i> (1988)                    |
| <i>E. coli</i>                            | X51323      | DHG\$ECO.LI       | 796                      | 86,993     | M        | 0                 | Cleton-Jansen <i>et al.</i> (1990)                    |
| <b>B-Type (GDHB)</b>                      |             |                   |                          |            |          |                   |                                                       |
| <i>A. calcoaceticus</i>                   | X15871      | DHGB\$ACI.CA      | 454                      | 50,237     | P        | 24                | Cleton-Jansen <i>et al.</i> (1989)                    |

Only those quinoproteins whose prosthetic group is undoubtedly PQQ are included. The cellular location given in the table is based on the primary sequence data: P, periplasmic protein; M, integral membrane protein.

should be called a quinohaemoprotein (Groen *et al.*, 1986).

No information is available on the location of the soluble alcohol dehydrogenases. However, if their electron acceptors are cytochrome c then they are likely to be periplasmic because all c-type cytochromes in Gram-negative bacteria are either membrane-bound or periplasmic.

By contrast with these soluble enzymes, the ADHs from acetic acid bacteria are membrane-bound quinohaemoprotein complexes. They are enzymes having considerable importance in the production of acetic acid from ethanol. The enzymes from *Gluconobacter suboxydans* and *Acetobacter aceti* (IFO 3284) have been completely purified, crystallized and their subunit structure determined by electrophoresis (Ameyama and Adachi, 1982; Adachi *et al.*, 1982; Ameyama *et al.*, 1991). ADH from *G. suboxydans* dissociates into three components, of 85 kDa (the dehydrogenase, which contains 1 mol of haem c), 49 kDa (a di-haem cytochrome c) and 14.4 kDa. The ADH from *A. aceti* dissociates into three subunits of

63 kDa (dehydrogenase), 44 kDa (cytochrome c), 29 kDa and 13.5 kDa. The only ADH whose primary structure has been determined (from gene sequencing) is from another strain of *A. aceti* (strain K6033); it was purified from membranes and shown to consist of at least two subunits of 72 and 45 kDa (Inoue *et al.*, 1989, 1990).

Relatively little information is available on the coupling of alcohol oxidation by ADHs to the electron transport chain. In the case of the soluble ADHs from *Pseudomonas* species this is most likely (by analogy with MDH) to be by way of periplasmic c-type cytochromes and an oxidase that oxidises cytochrome c. In the case of the acetic acid bacteria this is not likely to be the case because the only oxidases present are cytochrome bd and cytochrome ba (previously cytochrome a<sub>1</sub>) and the substrate for these oxidases is ubiquinol whose redox potential is lower than that of the c-type cytochromes (Matsushita *et al.*, 1990). It has recently been proposed that ubiquinol is an essential component of the respiratory chain involving ADH in the acetic acid

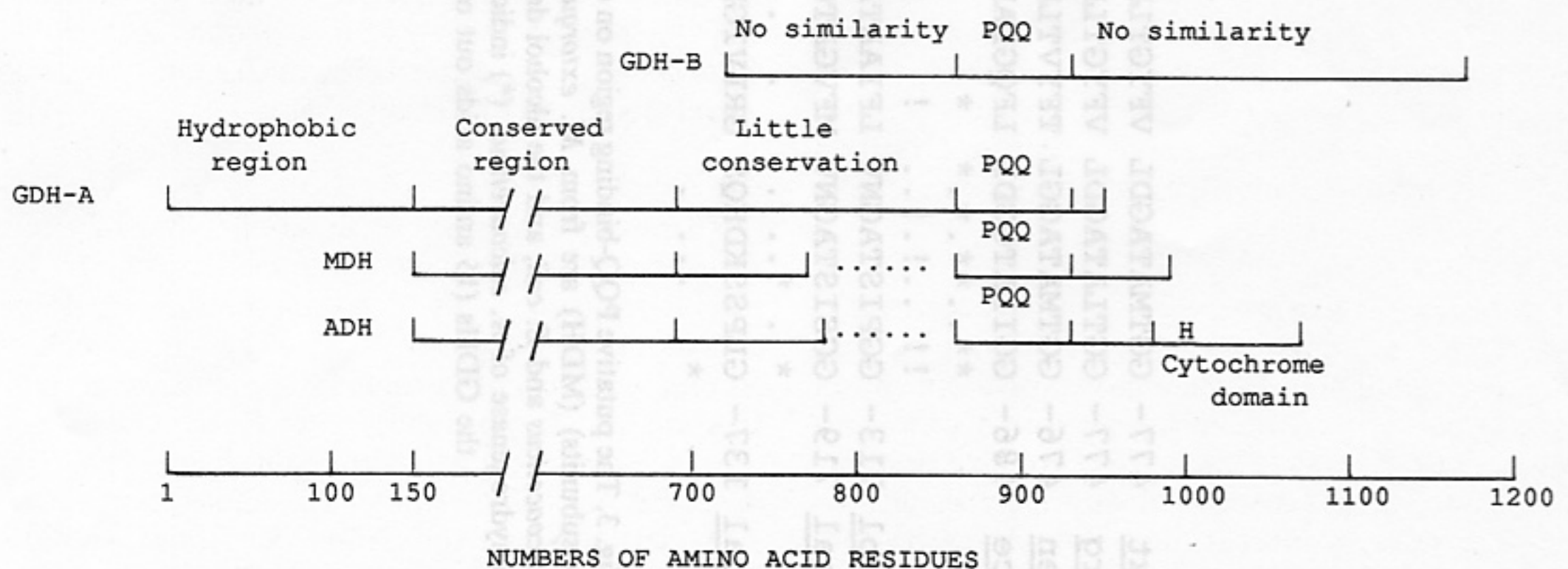


Fig. 2. Alignment of the amino acid sequences of the quinoproteins. The lengths of sequence are approximately to scale (but note the break in the scale). The sequences of MDH ( $\alpha$ -subunit) and ADH are very similar throughout the proteins; the comments on degree of conservation in the diagram refer to similarities between these two proteins and the GDH-A. The only region of the GDHs showing similarity is the postulated PQQ-binding region. H marks the position of a possible haem-binding site.

|            |      |            |           |            |            |            |            |            |                               |
|------------|------|------------|-----------|------------|------------|------------|------------|------------|-------------------------------|
| MDH M.ext  | 477- | GGTMATAGDL | VFYGTLDGY | L-KARDSDTG | DL-LWKFKIP | SGAIGYPMT  | YTHKGTQYVA | IYYGVGG    | -Identity in MDHs and ADH     |
| MDH M.org  | 477- | GGTLATAGDL | VFYGTLDGY | L-KARDSDTG | DL-LWKFKIP | SGAIGYPMT  | YTHKGTQYVA | IYYGVGG    | -Identity in MDHs, ADH & GDHA |
| MDH P.den  | 476- | GGTMATAGGL | TFYVTLDF  | I-KARDSDTG | EL-LWKFKLP | SGVIGHPMT  | YKHDGRQYVA | IMYGVG     |                               |
| ADH A.ace  | 486- | GGILATGDL  | LFQGLANGE | F-HAYDATNG | SD-LYKFDAQ | SGIIAPPMT  | YSVNGKQYVA | VEVGWGG    |                               |
|            |      | **..**.*.* | *.*       | *.*        | *.*        | *.*        | *.*        | *.*        |                               |
|            |      | !!..!!..!! | !         | !!..!!..!! | !!..!!..!! | !!..!!..!! | !!..!!..!! | !!..!!..!! |                               |
| GDHA E.col | 713- | GGPISTAGNV | LFIAATADN | YLRAYNMSNG | EK-LWQGRLP | AGGQATPMT  | YEYVNGKQYV | ISAGGHG    |                               |
| GDHA A.cal | 719- | GGSISTAGNV | MFVGATQDN | YLRAFNVTNG | KK-LWEARLP | AGGQATPMT  | YEINGKQYV  | IMAGGHG    |                               |
|            |      | *.*        | *.*       | *.*        | *.*        | *.*        | *.*        | *.*        |                               |
| GDHB A.cal | 137- | GLPSSKDHQS | GRLVIGPDQ | KIYYTIGDQG | RNQLAYLFLP | NQAQHTPTQ  | QELNGKDYHT | YMGKVLR    |                               |
|            |      | *          | *         | *          | *          | *          | *          | *          |                               |

Fig. 3. The putative PQQ-binding region on quinoproteins. The residue numbers refer to the mature proteins with signal peptides excluded. The methanol dehydrogenases ( $\alpha$ -subunits) (MDH) are from *M. extorquens*, *M. organophilum* and *P. denitrificans*. The glucose dehydrogenases (GDHA and GDHB) are from *Acinetobacter calcoaceticus* and *E. coli*, and the alcohol dehydrogenase is from *Acetobacter aceti*. (!) Indicates identity in all quinoproteins except for the periplasmic B-type glucose dehydrogenase of *A. calcoaceticus*. (\*) indicates a conserved residue. It should be noted that although there is considerable identity between all the GDHs (15 amino acids out of 65), only six of these are the same as the 19 amino acids that are identical in all the other six quinoproteins.

numbers and references to the original work. The sequences considered here do not include the signal peptides which are cleaved from the precursor protein during or after passage through the periplasmic membrane. They have been omitted because inclusion of the signal peptides in sequence comparisons can lead to misleading conclusions, and they have no relevance to the structure and function of the mature protein except to indicate its location in the cell.

Table 1 summarises the proteins discussed in this section and provides EMBL database accession

Fig. 4. Comparison of primary sequences of methanol dehydrogenase, alcohol dehydrogenase and glucose dehydrogenase. The first residue is of the *N*-terminal amino acid of the mature proteins without signal peptides. MDH is the  $\alpha$ -subunit of methanol dehydrogenase of *M. extorquens*; ADH is the alcohol dehydrogenase of *Acetobacter aceti* and GDHA is the glucose dehydrogenase (A-type) of *Acinetobacter calcoaceticus*. (\*) Indicates where the GDHA of *E. coli* (sequence not shown) is identical to that of *A. calcoaceticus* recorded here. (.) Indicates a conserved residue. (!) Indicates where all three MDHs, both GDHAs and ADH are identical. (NB: The alignments of the proteins are usually the optimal alignments. However, in the boxed region MDH aligns with ADH as shown here, but there is so little similarity between these proteins and GDHA that no optimal alignment of all three proteins is presented in this region.) The underlined regions in the *N*-terminal sequence of GDHA are probable membrane-spanning regions; there is considerable flexibility in interpretation of the exact location of these. The other regions in bold print are the putative PQQ-binding region (477–539 for MDH) and the possible haem-binding site on ADH (614–618) (see also Fig. 6).

Searches of the EMBL database show similarities between all these quinoproteins but no similarities to any other proteins, except for a small region of the alcohol dehydrogenase which showed some similarity to cytochrome c (see below). The  $\beta$ -subunit of MDH is included here as part of the structure of this protein; it has no similarities to the quinoproteins listed here nor to any other quinoproteins. Methylamine dehydrogenase was previously thought to have PQQ as its prosthetic group. This is now known not to be the case, and its light subunit, which bears the prosthetic group has no structural or sequence similarity to any quinoprotein (Vellieux *et al.*, 1989; Chistoserdov *et al.*, 1990).

The regions of similarity between the different types of quinoprotein are summarised in Fig. 2, and for convenience are summarised here before the more detailed analyses. The only region of similarity between all four types of quinoprotein has been suggested to be the PQQ-binding site. In addition to this, the MDHs and ADH show considerable similarity, with the ADH having an extra 'cytochrome domain'. The GDH-As have an extra *N*-terminal membrane-anchoring region followed by a region with some similarity to MDH and ADH. The only region of similarity to GDH-B is the putative PQQ-binding region.

#### *The putative PQQ-binding region*

This sequence of about 65 amino acids was independently proposed as the PQQ-binding site by Cleton-Jansen *et al.* (1990) and Inoue *et al.* (1990), and my proposal is derived from these (Fig. 3). At the outset it is important to consider what is meant by 'binding site'. Binding sites in proteins usually involve relatively few amino acids, and these are not necessarily adjacent or even on the same part of the primary sequence. In the present case, this length of 65 amino acids probably includes amino acids that are involved in specific interactions with PQQ, and it will also contribute essential secondary structural features and perhaps substrate-binding regions. Of the 65 amino acids included in this possible site, 19 are identical in all the quinoproteins except for the B-type GDH which has only six amino acids that are identical to all the others. This might suggest at first sight that GDH-B does not have a similar PQQ-binding site. However, the only region of GDH-B that has any similarity to other quinoproteins (and therefore is a candidate for a conserved PQQ-binding site) is within this same region. If the sequences for the two types of GDH are compared, they are found to have 15 out of 65 identical amino acids. There are thus two obvious possibilities. The first is that the GDH-B is

exceptional in its PQQ-binding site. The second possibility is that the MDHs and ADH have one site (of 29 identical amino acids) whereas the GDHs have a second site (of 15 identical amino acids); and this has only six amino acids in common with the MDH/ADH PQQ-binding site. If the region described here is also involved in binding substrates and electron acceptors then the second suggestion is perhaps more likely because these ligands are completely different for the two types of enzyme; for glucose dehydrogenase they are glucose and ubiquinone, and for the other enzymes they are alcohols and (probably always) a cytochrome c.

#### *Glucose dehydrogenase (A-type)*

The gene sequences and predicted protein sequences of this type of membrane dehydrogenase have been determined for the enzymes from *Acinetobacter calcoaceticus* and *E. coli* (Cleton-Jansen *et al.*, 1988, 1990). The primary structure of the enzyme from *A. calcoaceticus* is shown in Fig. 4. The close similarity of the GDH from the two bacteria is indicated in the figure. The expected membrane-spanning regions are found entirely in the first 140 amino acids. After this region, which functions to anchor the protein in the membrane, there is a sequence of about 400 amino acids which shows considerable identity to the *N*-terminal regions of the other quinoproteins, MDH and ADH (Figs 2 and 4). There is then a region of about 170 amino acids with little similarity, and this is followed by the putative PQQ-binding region which reaches almost to the *C*-terminus (Fig. 3).

#### *Glucose dehydrogenase (B-type)*

This type of periplasmic glucose dehydrogenase has only been described in *A. calcoaceticus* and its sequence is shown in Fig. 5. Its sequence shows no similarity to that of any other protein except for a small region, which has similarity to the putative PQQ-binding sites in other quinoproteins (Fig. 3). As has been pointed out by Cleton-Jansen *et al.* (1990), it is striking that two quinoproteins having the same substrate (glucose) should bear less resemblance than quinoproteins having different substrates (methanol, alcohol or glucose). This perhaps emphasises the possibility that the proposed PQQ-binding site is also responsible for binding substrates and/or electron acceptors.

#### *Methanol dehydrogenase*

*Large ( $\alpha$ ) subunit.* The three  $\alpha$ -subunits of MDH shown in Fig. 6 are identical in the number of amino acids present and they show an extremely close similarity to each other. The proteins from the two

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1- DVPLTPSQFA KAKSENFDDK VILSNLNKPH ALLWGPNDQI WLTERATGKI LRVNPESGSV
61- KTVFQVPEIV NDADGQNGLL GFAFHPDFKN NPYYIISGTF KNPKSTDKEL PNQTIIRRYT
121- YNKSTDTLEK PVDLLAGLPS SKDHQSGRLV IGPDQKIYYT IGDQGRNQLA YLFLPNQAQH
181- TPTQQELNGK DYHTYMGKVL RLNLDGSIPK DNPSFNGVVS HIYTLGHRNP QGLAFTPNGK
241- LLQSEQGPNS DDEINLIVKG GNYGWPNAVAG YKDDSGYAYA NYSAAANKSI KDLAQNGVKV
301- AAGVPVTKEE EWTGKNFVPP LKTLTYVQDT YNYNDPTCGE MTYICWPTVA PSSAYVYKGG
361- KKAITGWENT LLVPSLKRGV IFRIKLDPTY STTYDDAVPM FKSNNRYRDV IASPDGNVLY
421- VLTDTAGNVQ KDDGSVTNTL ENPGSLIKFT YKAK

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Fig. 5. Amino acid sequence of the periplasmic glucose dehydrogenase (GDH-B) from *Acinetobacter calcoaceticus*. The underlined part corresponds to a possible PQQ-binding region (see Fig. 3). The signal peptide of 24 amino acids has been omitted.

Fig. 6. Sequences of three methanol dehydrogenases, alcohol dehydrogenase and cytochrome c-555, and cytochrome c<sub>L</sub>. Methanol dehydrogenases ( $\alpha$ -subunits) (MDHs) are from *Methylobacterium extorquens* (*M. ext*), *Methylobacterium organophilum* (*M. org*) and *Paracoccus denitrificans* (*P. den*). The alcohol dehydrogenase (ADH) is from *Acetobacter aceti*. The cytochrome that shows most similarity to the C-terminal region of ADH is a small c-type cytochrome from *Methylococcus capsulatus* (Ambler *et al.*, 1986). The sequence of cytochrome c<sub>L</sub> is that of the electron acceptor for MDH in *M. extorquens* (Nunn and Anthony, 1988). A possible PQQ-binding region is placed in a box and so is a potential haem-binding site. The cytochrome c<sub>L</sub> has a methionine (in bold print) which may form the sixth ligand with the haem iron which corresponds to a methionine residue in ADH. Numbers refer to number of residues in the mature protein excluding signal peptides. (!) Indicates identical amino acids in all MDHs and ADH. (\*) Indicates identical amino acids in the three MDHs, and, in the C-terminal region, similarity of the ADH to the two c-type cytochromes.



(non-covalent) of its PQQ; it is necessary to dissociate the MDH into its subunits to release its prosthetic group.

#### *Alcohol dehydrogenase of acetic acid bacteria*

The sequence of this dehydrogenase is shown in Fig. 6 together with those of the  $\alpha$ -subunits of methanol dehydrogenase. There is considerable similarity between the first 596 amino acids of the ADH and the 599 amino acids of the three MDHs shown here, with the putative PQQ-binding region near the C-terminus.

The remaining 110 residues constitute a separate domain that was suggested to be a cytochrome domain (Inoue *et al.*, 1990). The isolated ADH contains haem c, as indicated by its spectrum, and there is a typical haem-binding site (CXXCH; residues 614–618). The cytochrome c that shows most identity with this region of the ADH is a small Class I cytochrome c (cytochrome c-555) of no known function from the methanotroph *Methylococcus capsulatus* (Ambler *et al.*, 1986). It should be noted that the similarity to this particular cytochrome c is not necessarily especially significant because other small cytochromes are also similar at this relatively low level. Furthermore, the sixth ligand to the iron (the fifth being the His residue) in c-type cytochromes is a methionine, and there is no methionine residue on the ADH corresponding to the single methionine residue (59) in the cytochrome c-555. An alternative possibility is suggested in Fig. 6, in which the electron acceptor for MDH, cytochrome  $c_L$ , is also aligned with the C-terminal region of ADH. This does not show as many identical residues as does cytochrome c-555 (20 by contrast with 25) but the methionine residue that is conserved in the two examples of cytochrome  $c_L$  that have been sequenced is also present on ADH very near a region with four other identical amino acids. Because there is so much similarity between the sequences of MDH and ADH, it is perhaps to be expected that the electron acceptor for ADH will be similar to that for MDH which is the cytochrome  $c_L$  drawn here. There is no region on ADH that corresponds to the  $\beta$ -subunit of MDH.

There is some difficulty in reconciling the structure of ADH as shown by the work of Inoue *et al.* (1989, 1990) with what is known of its properties when isolated. The quinohaemoprotein ADH is isolated from membranes using the detergent Triton X-100 and this is included in all subsequent purification steps (Ameyama and Adachi, 1982). It appears, therefore, to be a typical membrane protein. Inoue *et al.* (1989) concluded that their ADH structure (Fig. 6) is consistent with this conclusion. However, there are no strongly hydrophobic regions in the structure, and certainly no membrane-spanning helices. The only hydrophobic region predicted from the DNA sequence is the span of 35 amino acids at the N-terminus (not shown in Fig. 6). This region has the typical characteristics of a signal sequence with positively charged residues at the N-terminus followed by a hydrophobic core and an Ala-Xaa-Ala sequence at the C-terminus. Such signal sequences are more typical of periplasmic proteins than of membrane proteins, the signal peptide being hydrolysed to release the mature protein into solution in the

periplasm. That the published gene sequence corresponds to that for the quinoprotein ADH seems to be confirmed by the demonstration that the N-terminal region of the predicted mature protein (shown in Fig. 6) corresponds to the N-terminal sequence of amino acids determined for the ADH isolated and purified from the membrane. Because the ADH occurs as a complex with other proteins it is possible that one of these is a typical integral membrane protein and the periplasmic ADH is firmly bound to it. This remains a mystery which should be borne in mind in any discussion of the location and function of ADH in the acetic acid bacteria.

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