The same domain motif for ubiquinone reduction in mitochondrial or chloroplast NADH dehydrogenase and bacterial glucose dehydrogenase

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The respiratory chain NADH:ubiquinone oxidoreductase (NADH dehydrogenase or Complex I) of mitochondria comprises some 30 different subunits, and one FMN and 4 or 5 iron-sulfur clusters as internal redox groups. The bacterial glucose dehydrogenase, which oxidizes glucose to gluconolactone in the periplasmatic space and transfers the electrons to ubiquinone, is a single polypeptide chain with pyrolloquinoline quinone as the only redox group. We report here that the two different enzymes have the same ubiquinone binding domain motif and we discuss the predicted membrane folding of this domain with regard to its role in the proton translocating function of the two enzymes.

NADH dehydrogenase; Glucose dehydrogenase; Ubiquinone; Piericidin; Proton translocation; DNA, mitochondrial

1. INTRODUCTION

The respiratory chain NADH dehydrogenase of mitochondria couples electron flow from NADH to ubiquinone with proton translocation according to the equation:

$$NADH_n + Q + 5\ H_n^+ = NAD_n^+ + QH_2 + 4\ H_p^+$$

where Q refers to ubiquinone-10, n to the negative matrix side and p to the positive intermembrane side of the mitochondrial inner membrane. The enzyme is extraordinarily complex. It consists of some 30 different subunits of which 7 are encoded by the mitochondrial ND genes and synthesized in the mitochondria. The remaining subunits are nuclear-encoded and imported from the cytoplasm. The electron passage through the enzyme is carried out by 1 FMN, 4 or 5 iron-sulfur clusters and probably an internal ubiquinone. The last electron transfer step from cluster N-2 to ubiquinone is selectively blocked by piericidin A which binds with high affinity to the ubiquinone catalytic site [1,2]. There is some evidence that a respiratory NADH dehydrogenase exists also in chloroplasts [3].

The glucose dehydrogenase found in many aerobic bacteria (e.g. *E. coli, G. suboxydans, A. calcoaceticus, Z. mobilis*) oxidizes glucose to glucono-1,4-lactone at the periplasmatic site of the plasma membrane and delivers the two electrons to ubiquinone. In doing so, the enzyme builds up a direct glucose oxidase system [4]. The enzyme consists of a single polypeptide which carries pyrrolloquinoline quinone as a redox group [5].

The observation that the glucose dehydrogenase is also inhibited by piericidin A prompted us to consider the possibility that NADH dehydrogenase and glucose dehydrogenase have the same domain motif for reducing ubiquinone.

The amino acid sequence is known for the glucose dehydrogenase of *A. calcoaceticus* [6]. In the major part of the sequence (residues 141–801), no hydrophobic stretch long enough to span the membrane is found. This part must therefore lie peripherally on the periplasmatic side of the membrane and contain the glucose catalytic centre. The smaller N-terminal part of the sequence (residues 1–140) is more hydrophobic and could span the membrane in the form of 4 helices (see below). Only this part is capable of binding ubiquinone.

In the case of NADH dehydrogenase, three observations pointed to the product of the mitochondrial ND1 gene as ubiquinone binding subunit. Firstly, this subunit is photolabeled by a rotenone analogue which, like piericidin A, is believed to bind at the ubiquinone site [7]. Secondly, the ND1 gene is the most conserved among the rapidly diverging mitochondrial genes of NADH dehydrogenase [8]. Thirdly, when mitochondrial protein synthesis in the fungus *N. crassa* is inhibited by chloramphenicol, a related but smaller form of NADH dehydrogenase is made which is devoid of the mitochondrially encoded subunits. The electron pathway in this small NADH dehydrogenase is shortened by the iron-sulfur cluster N-2 and the piericidin-sensitive site for ubiquinone [9–11].

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Our approach in this work was to compare the sequence of the hydrophobic part of the glucose dehydrogenase with sequences of ND1 gene product which are known for many different species.

2. MATERIALS AND METHODS

Bacteria were cultivated and membranes were prepared as described in [12]. The activity of glucose dehydrogenase was measured in an assay containing 50 mM sodium phosphate, pH 6.5, 5 mM $MgCl_2$, 5 mM KCN, 60 μg/ml protein, 3–15 μM ubiquinone-2 and 0–40 μM piericidin A. The reaction was started by adding 15 mM glucose and the reduction of ubiquinone-2 was followed with a dual wavelength spectrophotometer using the wavelengths 275 nm and 302 nm and the extinction coefficient of 14 $mM^{-1} \cdot cm^{-1}$. Calculations were performed with the program PC/GENE from Amos Bairoch. Sequence alignment was done with the subprogram PALIGN using the amino acid sequences deduced from the mitochondrial ND1 gene of mammals [2,8], insects [13,14], echinodermata [15,16], one amphibium [17], fungi [18–20] and plants [21–23], from the product of the homologous open reading frame found in the genome of chloroplasts

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          63                                                          116
          |                                                           |
H.s.   PAT|STITLYITAPTLALTIALLL|WTPLPMPNPLV--NLNL|GLLFILATSSLAVYSI
N.c.   LTQ|ANMTLFFLGPVITLIFSLLG|YAVIPYGPSLVIQDVNL|GILYMLAVSSLATYGI
C.r.   PDS|SSAGAFAASPMISFVLSQVA|WVGI------CISDASF|QGLVIMAISSLAVYGV
M.p.   PAQ|GDVWLFNIGPILVLIPVFLS|YLVIPFFEYNVILANFSI|GVFFWIAVSSVVPLGL
Cons.  ···|·····*···P·········|*··#P·····J·····|=#··##··=S#····#
A.c.   ATL|GGSIYYIIAGVLLLIVAWQL|YKRAS----------|TA--LWFYAALMLGTI
          |                                                           |
          33                                                          74

          117                                                         170
          |                                                           |
H.s.    LWSG|WASNSNYALIGALRAVAQTI|SYEVTLA-IILLSTLLMS|GSFNLSTLITTQE
N.c.    LLAG|WSANSKYAFLGSLRSAAQLI|SYELVLSSAILLVIML-T|GSFNLGVNTESQR
C.r.    MLAG|WASNSKYAFLGCLRSVALMV|SYELSLGAALLSIGLFVT|DGTGMKCLNFAEM
M.p.    LMAG|YGSNNKYSFLGGLRAAAQSI|SYEIPLALSVLSIA/SLS|TVDIVEAQSKYGF
Cons.   #··G|*···==·Y··JG·JR·#A··#|=YE··#···#L·····|·············
A.c.    IWSV|WEVGTDFWAL-APRL-----|--DILGILGLWLLVPAVT|RGINNLG--SSKV
          |                                                           |
          75                                                          119

          171                                                   202
          |                                                     |
          ┌ - - - - - - - - ┐
H.s.      |HLWLLLPSWPLAMMWFIST|-LAETN---RAPFDLAE
N.c.       AVLFVLPLLPIFIIFFIGS -IAETN---RAPFDLAE(Intron)
C.r.      |PTTPQYAMLPLCLIFLVCI|-LAETK---RDPFDLPA
M.p.       LSWNLWRQPIGFIVFFIAS -LAECE---RLPFDLPE
Cons.     |··············*····|-#AE=·---R·PFD#··
A.c.       ALSSTLAIAIVLMVYSIFN DPQEINGEIKTPQPETA
          └ - - - - - - - - - ┘
          |                                                     |
          120                                                   155
```

Fig. 1. Alignment of the amino-acid sequences of the NADH dehydrogenase subunit encoded by the mitochondrial ND1 gene of *H. sapiens* (H.s.), *N. crassa* (N.c.) and *C. reinhardtii* (C.r.), the chloroplast ND1 gene of *M. polymorpha* (M.p.), and the glucose dehydrogenase of *A. calcoaceticus* (A.c.). Numbers indicate the positions of the *H. sapiens* and *A. calcoaceticus* sequence, respectively. Cons. means the consensus sequence deduced from the sequences of the NADH dehydrogenase subunit of 17 different species. The dark, hatched areas show identities, whereas the light, hatched areas show similarities among the species. The blocked areas indicate the calculated transmembranous helices, and the dashed block indicates the surface helix. The begin of the *N. crassa* intron is shown. Note the missing amino acids Leu-168 to Gln-172 in the *M. polymorpha* sequence. Symbols for side chains are: (*) aromatic; (#) neutral hydrophobic; (=) neutral hydrophilic. Other similar pairs are (I, L), (D, E) and (K, R, H).

[24] and the glucose dehydrogenase from *A. calcoaceticus* [6]. Hydrophobicity was plotted according to [25]. The prediction of the protein folding was calculated according to [26,27].

3. RESULTS AND DISCUSSION

The type of inhibition of the glucose dehydrogenase by piericidin A was assayed using membrane preparations of *G. oxydans, Z. mobilis,* and *A. calcoaceticus* and following the reduction of added ubiquinone-2 by glucose photometrically. Plots of $1/v$ against 1/[ubiquinone-2] at different concentrations of piericidin A clearly showed competitive inhibition with regard to the ubiquinone-2. Analysis of the kinetic data gave K_i values of approximately 1 μM piericidin A.

The alignment of the amino-acid sequences deduced from the mitochondrial ND1 gene of 3 mammals, 2 insects, 2 echinodermata, 1 amphibium, 3 fungi and 4 plants, and from a homologous reading frame found in the chloroplast genome of 2 plants, reveal 70–90% similarity within each kingdom and 35–40% among the kingdoms. A consensus sequence was derived from this alignment and was compared with the N-terminal sequence region of the glucose dehydrogenase. A stretch of ~80 residues was found with 65% similarity followed by ~35 residues without similarity and then ~15 residues with 40% similarity (Fig. 1).

The comparison of sequences alone is too restrictive from a functional standpoint. We therefore tried to predict the membrane folding of this sequence region on the basis of its hydrophobicity profile (Fig. 2). In both NADH dehydrogenase and glucose dehydrogenase, 3 helices are expected to span the membrane and 1 helix to be located at the outer membrane surface (Fig. 3). The related helices of the two enzymes show very good conformities concerning their positions and lengths. This predicted folding would locate the invariant amino acids at the corresponding membrane sides.

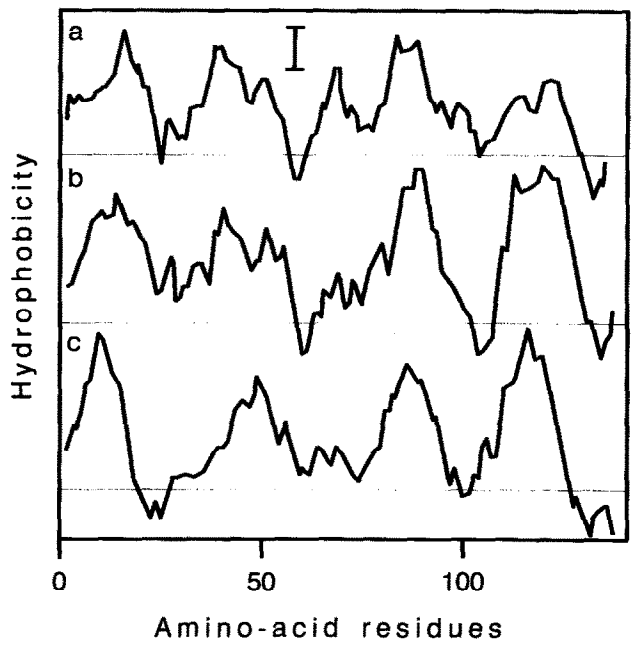

Fig. 2. Hydrophobicity profiles of the sequence region shown in Fig. 1 for *H. sapiens* (a), *N. crassa* (b), and *A. calcoaceticus* (c). The bar indicates 10 hydropathy index units.

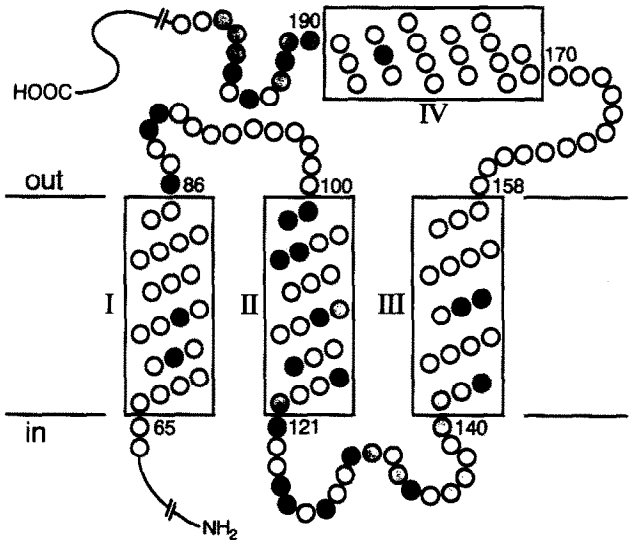

Fig. 3. Model of the ubiquinone binding domain by example of the *H. sapiens* sequence. The grey circles indicate the positions of amino acid similarity among all investigated ND1 encoded NADH dehydrogenase subunits, and the black circles the positions of similarities among the NADH dehydrogenase subunits and the glucose dehydrogenase.

Contemporary theories state that new genes may be assembled from fragments of preexisting genes, rather than created de novo. Such fragments are often organized as exons and may encode discrete domains, i.e. structural and functional units formed from continuous amino acid sequences. An exon-intron junction in a gene therefore often marks the edge of a coenzyme binding domain. Interestingly, in the ND1 gene of *N. crassa* an intron begins precisely where the similarity among the NADH dehydrogenases and between themselves and the glucose dehydrogenase ends.

The invariant Arg_{134} and Glu_{143} in human NADH dehydrogenase (corresponding to Arg_{91} and Asp_{93} in *A. calcoaceticus* glucose dehydrogenase) could be used to stabilize the semiubiquinone anion. Such residues were predicted from molecular modeling with piericidin derivatives [28]. In the glucose dehydrogenase, these residues lie at the opposite membrane side in comparison to the glucose binding moiety of the enzyme, in a region of highest similarity within the NADH dehydrogenase. Ubiquinone reduction concomitant with the uptake of two protons could then occur at the negative side of the membrane as is postulated for the NADH dehydrogenase [1]. For the glucose dehydrogenase, this would imply also a proton translocating reaction according to the equation:

$$Glucose_p + Q + 2H_n^+ = Gluconolactone_p + QH_2 + 2H_p^+$$

where n and p refer to the negative inner and positive outer side of the plasma membrane.

Taken together, the properties which we found to be common to the mitochondrial NADH dehydrogenase and the bacterial glucose dehydrogenase, namely electron delivery to ubiquinone, sensitivity to piericidin A, sequence similarity in a membranous protein moiety, and conformity in the folding of this protein moiety, indicate that the two enzymes have the same ubiquinone binding domain motif. This is an example where an exon of a mitochondrial gene can be traced to a domain of a totally different bacterial enzyme. The observation further supports our view that the mitochondrial NADH dehydrogenase emerged from (at least) two preexisting electron transfer enzymes which joined to create a new enzyme [10].

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