Internal sequence repeats and the path of polypeptide in mitochondrial ADP/ATP translocase

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Received 18 June 1982

1. INTRODUCTION

Adenine nucleotide translocase (ATP carrier) is an integral protein of the inner mitochondrial membrane. It exchanges cytoplasmic ADP for ATP synthesized inside the mitochondrion. It appears that the active form of the protein is a dimer [1,2]. Klingenberg [2] has proposed that substrates bind at the subunit interface and that this 'translocational centre' is exposed alternately to the 2 sides of the membrane. Two translocational states can be differentiated by sensitivity to inhibitors: in the 'm' (matrix) state bongkrekic acid inhibits the enzyme; in the 'c' (cytoplasmic) state it is sensitive to atractyloside [2,3]. In both states translocase shows unchanged affinities for ADP and ATP. In the absence of membrane potential it translocates these nucleotides symmetrically. Asymmetric translocation (ATP out, ADP in) is achieved only in respiring mitochondria where a membrane potential (positive on the outside) tends to drive out ATP^{4-} ions and select for ADP^{3-} as the ingoing ion [4].

Recently, the sequence of 297 amino acids of bovine ADP/ATP translocase was reported [5]. A feature of this sequence of an integral membrane transport protein is the large number of polar residues. Only 3 segments of >20 amino acids with apolar character were noted [5]. We have now analysed this sequence and found a striking pattern of homologous repeats. The protein appears to have evolved by a process involving 2 gene duplications. The conserved regions are centred on 3 cysteine residues found in 3 hydrophilic segments. At least 2 of these segments appear to be linked by a long hydrophobic segment probably folded into 2 transmembrane α-helices that makes a loop back and forth (IV and V, in fig.3). This arrangement would orient 2 similar hydrophilic segments on the same side of the membrane. It is probable that the arrangement of these repeated sequences is important for the function of the protein.

2. METHODS

Two computer programs were employed in this analysis: DIAGON and HYDROPLOT. DIAGON [6] is an interactive graphics program derived from MDM78 [7]. The scoring matrix is based upon accepted point mutations in 71 families of homologous proteins and found to be the most powerful matrix for detection of distant relationships [8]. HYDROPLOT is a direct application of SOAP [9], a program to look for hydrophobic and hydrophilic regions in proteins. It uses a hydropathy index based on the relative amphiphilicities of amino acid side chains [9].

3. RESULTS AND DISCUSSION

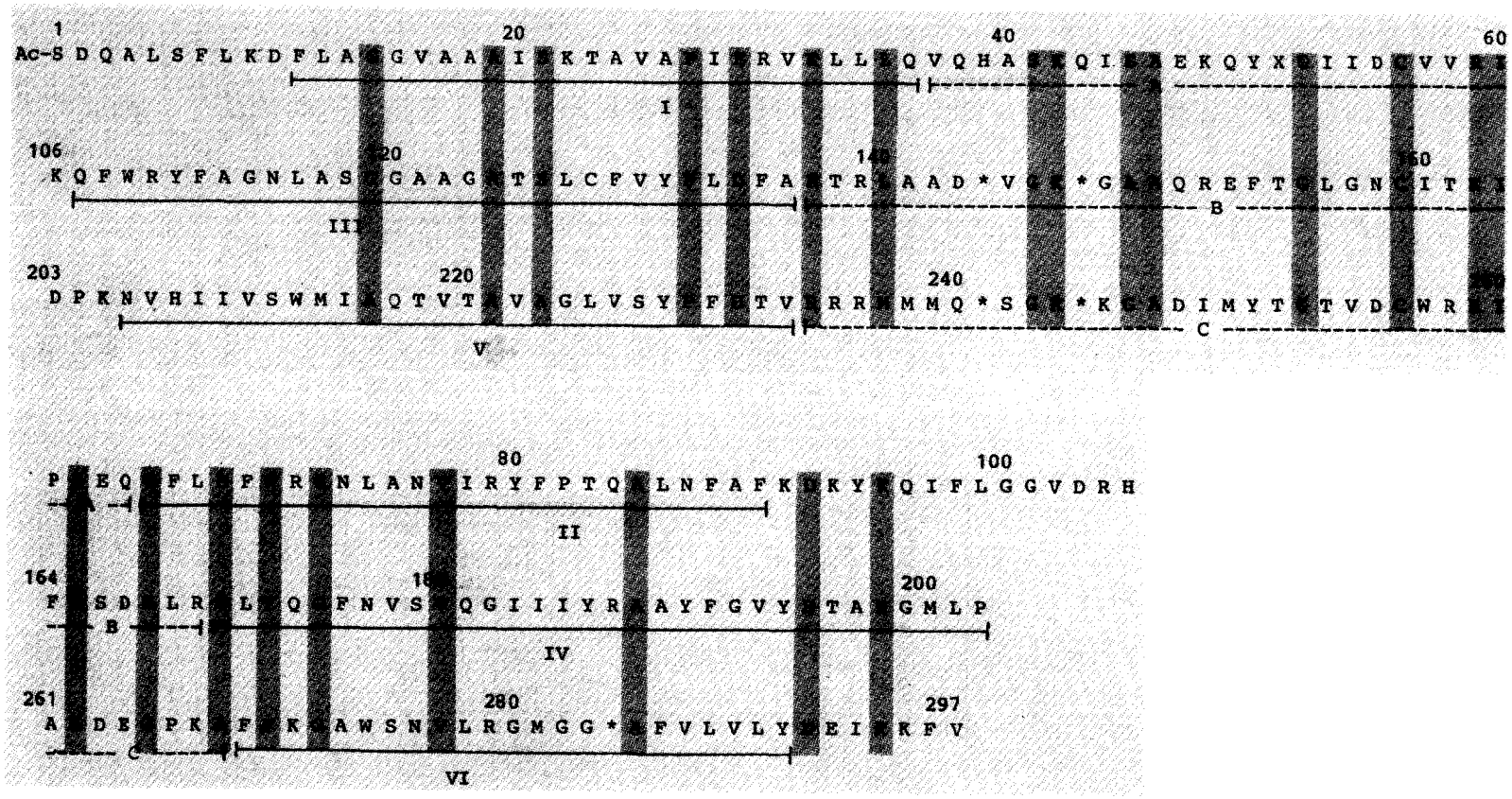
3.1. *Sequence repeats*

The comparison matrix of the ATP/ADP translocase sequence with itself is very distinctive. It contains 3 related sequences (fig.1) that are aligned in table 1. In these 3 sequences of ~ 100 amino acids 24 residues are identical or conservatively substituted. We note that the third repeat includes a sequence near the C-terminus (residues 279–291) that is weakly homologous to related sequences in a number of adenine nucleotide binding proteins [10] and has been proposed to contribute to a nucleotide binding site. However, this does not exclude the possibility that the other regions in translocase may also be involved in binding the nucleotides.

Published by Elsevier Biomedical Press

Table 1

Alignment of repeated sequences in ADP/ATP translocase according to fig.1



* is a deletion

Each repeat contains two hydrophobic segments linked by an extensive hydrophilic region. The first hydrophobic segment is preceded by an additional short stretch (residues 1–8) at the N-terminus of the protein and segments II and III are linked by a segment of 8 amino acids (98–105). Identical residues and conserved substitutions are boxed

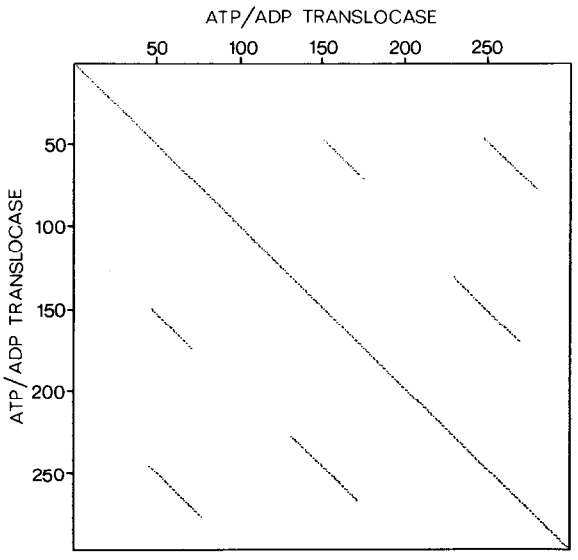


Fig.1. Comparison matrix of the sequence of ADP/ATP translocase with itself. A span of 31 was used in the calculation. Points of the figure represent homology with a double matching probability [8] $>5 \times 10^{-4}$.

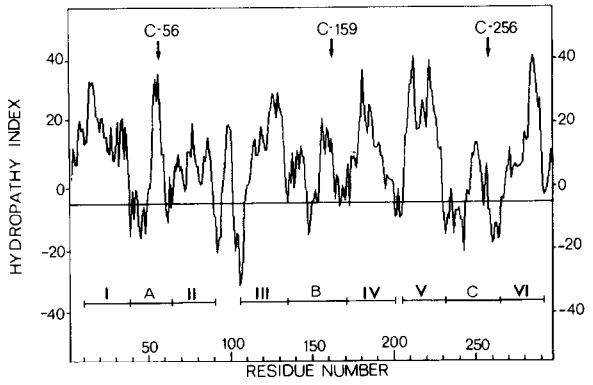


Fig.2. Detection of hydrophobic stretches in ADP/ATP translocase with HYDROPLOT. The program calculates a hydropathy index (ordinate) for a span of seven amino acids along the sequence (abcissa). Solid line indicates the mean hydropathy index for amino acid residues [9]. The maxima correspond to centres of hydrophobic regions, the minima to hydrophilic ones. I–VI are hydrophobic and A, B and C hydrophilic segments. The positions of conserved cysteine residues are indicated by arrows.

3.2. *Hydrophobic segments*

Buried α-helical segments of membrane proteins appear to be characterised by a number of features [11]. They are composed predominantly of non-polar stretches of 25–28 amino acids, which is sufficient to cross a membrane 40 Å thick. (In bacteriorhodopsin [11] a small number of charged residues appear to be buried in the membrane.) These segments are linked by more polar regions presumed to be in a more hydrophilic environment at or near the membrane surface. These linkers often contain proline residues that help to make a bend in the polypeptide chain.

A hydroplot of translocase (fig.2) indicates that there could be six segments (I–VI) with overall hydrophobic character and sufficient length to traverse the membrane in α-helices. Three of them (also noted in [5]), III (residues 105–137), IV (170–202) and V (205–234) have many of the features outlined above to qualify as transmembrane α-helices: charged residues (with the exception of Arg-187) are near the periphery, and IV and V are linked by the sequence Pro–Asp–Pro which could make a tight bend.

Three other long hydrophobic segments are detected by the HYDROPLOT, I (9–39), II (64–90) and VI (265–294). Although sufficient in length, these contain several acidic and basic residues and would require special accommodation (e.g., charge neutralisation by ion-pairing) to be buried in the membrane. However, segments I, III and V are homologous to each other as are II, IV and VI (table 1) and so are likely to have related secondary structures.

Between the hydrophobic segments pairs I and II, III and IV, and V and VI are hydrophilic segments A, B and C, also related to each other in sequence (fig.1). A conserved cysteine in A, B and C is very striking given that reaction of the protein with *N*-ethylmaleimide inhibits translocase activity [12,13]

3.3. *Topography of translocase*

How are these various hydrophobic and hydrophilic segments arranged in the membrane? It seems likely that B and C will be oriented to the same side of the membrane being linked by helices IV and V as depicted in fig.3. The disposition of A is rather more uncertain. Should segment II also cross the membrane, A would then be placed on the same side as B and C. However, this would require that 2

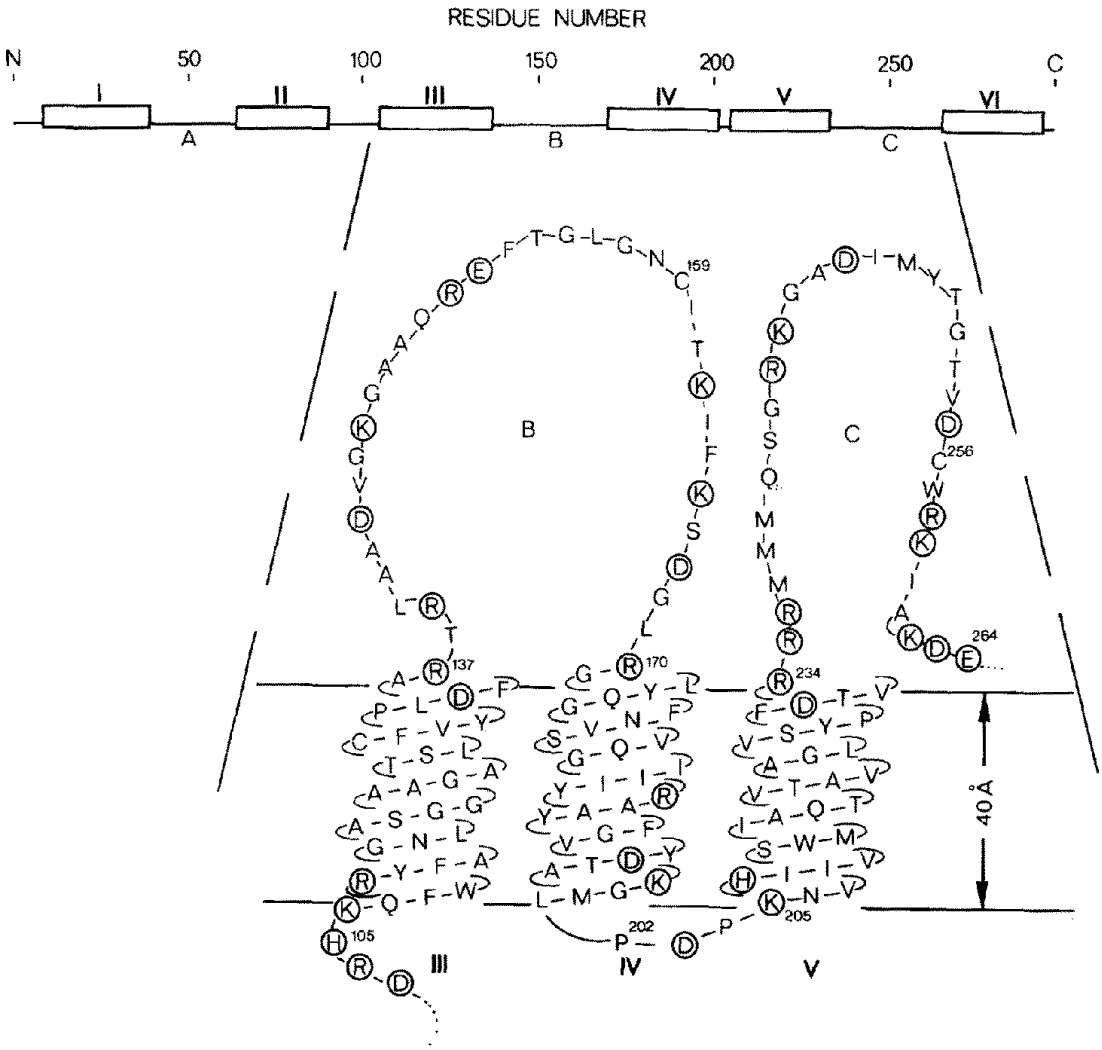


Fig.3. Hydrophilic and hydrophobic domains in translocase. The upper diagram illustrates the disposition of hydrophobic (I–VI) and hydrophilic regions A, B and C within the consecutive sequence repeats (table 1). Below sequence 103–263 is drawn to the proposed topography. Segments III–V are plotted on helical surfaces as transmembrane elements. Charged amino acids are encircled. Although segments B and C are depicted as being outside the lipid bilayer it is equally possible that they may at least in part be in the membrane.

arginines in II be within the lipid bilayer. Similar difficulties arise with I (1 acid and 2 basics to be buried) and VI (2 basics to be buried).

Whether or not A is on the same or opposite side of the membrane as B and C, it is difficult to reconcile either of these arrangements with symmetrical translocation of adenine nucleotides in the absence of membrane potential in the dimeric translocase. A structure symmetrical with respect to the membrane surfaces can be achieved if the dimer has a 2-fold symmetry axis in the plane of the membrane and not perpendicular to it [2]. This in turn poses difficulties for biosynthetic assembly. However, difference in inhibition of 'm' and 'c' states indicates that in at least this one respect the protein is asymmetric in the membrane.

ACKNOWLEDGEMENTS

We thank R. Staden for making an interactive graphics program HYDROPLOT. M.S. is a long-term EMBO Fellow.

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