Minireview

Structure and mechanism of proton-translocating transhydrogenase

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Abstract Recent developments have led to advances in our understanding of the structure and mechanism of action of proton-translocating (or *AB*) transhydrogenase. There is (a) a high-resolution crystal structure, and an NMR structure, of the NADP(H)-binding component (dIII), (b) a homology-based model of the NAD(H)-binding component (dI) and (c) an emerging consensus on the position of the transmembrane helices (in dII). The crystal structure of dIII, in particular, provides new insights into the mechanism by which the energy released in proton translocation across the membrane is coupled to changes in the binding affinities of $NADP^+$ and NADPH that drive the chemical reaction.

Key words: Transhydrogenase; Membrane protein; Proton translocation; X-ray structure; Nucleotide binding

1. Introduction

Transhydrogenase couples the transfer of reducing equivalents between NAD(H) and NADP(H) to the translocation of protons across a membrane.

$$NADH + NADP^+ + xH_p^+ \Leftrightarrow NAD^+ + NADPH + xH_n^+ \quad (1)$$

where H_p^+ and H_n^+ signify the involvement of protons in the *p* and *n* aqueous phases, respectively. The enzyme is found in the inner membranes of higher-animal mitochondria, and in the cytoplasmic membranes of many, though not all, bacteria. In some protozoan parasites lacking functional mitochondria, transhydrogenase is located in other membranes of the cell [1]. It is absent from *Saccharomyces cerevisiae* [2]. There was a suggestion from biochemical experiments that transhydrogenase is present in plant mitochondria [3], although it has not been identified in the available genome sequence of *Arabidopsis thaliana* [4]. It is present in the green alga *Acetabularia acetabulum* [5].

NAD(H) and NADP(H) are the major, soluble redox cofactors of living cells. Although there are exceptions to the rule, NAD(H) is generally involved in catabolism, and NADP(H) in anabolism. Thus, the redox potentials of NAD(H) and NADP(H) in the same aqueous compartment of the cell can be separately regulated to permit independent control of the energy-yielding and energy-consuming arms of metabolism. Transhydrogenase sits at the interface between these two important cofactor pools. Under most physiological conditions the enzyme probably operates from left to right (Eq. 1), consuming the proton electrochemical gradient (Δp) generated by respiratory (or, in some bacteria, photosynthetic) electron transport. Its function is organism- and tissue-dependent [6]. It can contribute to NADPH formation for biosynthesis and glutathione reduction [7], in the regulation of flux through the tricarboxylic acid cycle [8], and, operating in reverse in some invertebrates, it can transfer reducing power from NADPH to NAD^+ to fuel an anaerobic respiratory chain [9].

Extensive earlier reviews on transhydrogenase have been published [10–14]. The non-proton-pumping, or *BB* transhydrogenase [15] will not be discussed here.

2. An overview of the structure and elementary properties of transhydrogenase

Transhydrogenase has a tripartite arrangement of subunits (Fig. 1). Even though there is some domain rearrangement at the genetic level (see [16]), there appears to be little variation in the structural organisation of the enzyme from different species. The dI component (~400 amino acid residues), which binds NAD^+ and NADH, and the dIII component (~200 residues), which binds $NADP^+$ and NADPH, protrude from the membrane, on the matrix side in animal mitochondria and on the cytoplasmic side in bacteria [16–20]. The dII component (~400 residues) spans the membrane. The native enzyme is thought to be dimeric [21,22], though this might not be of functional significance.

As with all the 'soluble' dehydrogenases [23], it is not known whether the redox step in transhydrogenase involves the transfer of H^-, ($2e^- + H^+$) or ($e^- + H^\cdot$) between the nucleotides. In this review, the term 'hydride' is taken formally to represent 'hydride-ion equivalent'. Though it remained undetected for many years, there is a deuterium isotope effect ($k_H/k_D = 1.8$–4.5) in the transhydrogenation reaction, in both steady-state [24,25] and transient-state [26] conditions. The isotope effect has an interesting temperature dependence [26]. Hydride transfer is between the 4*A* (or *pro-R*) position on the nicotinamide ring of NAD(H) and the 4*B* (or *pro-S*) position on that of NADP(H) [27].

Recombinant forms of isolated dI and dIII have now been isolated from a number of species [28–33]. Simple mixtures of dI and dIII proteins, even from different species, catalyse a rapid, single turnover of hydride transfer between bound nu-

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Abbreviations: $AcPdAD^+$ and AcPdADH, oxidised and reduced forms, respectively, of acetylpyridine adenine dinucleotide; NAD(H), NADP(H) and AcPdAD(H), etc., indicate *both* the oxidised and reduced forms of a dinucleotide

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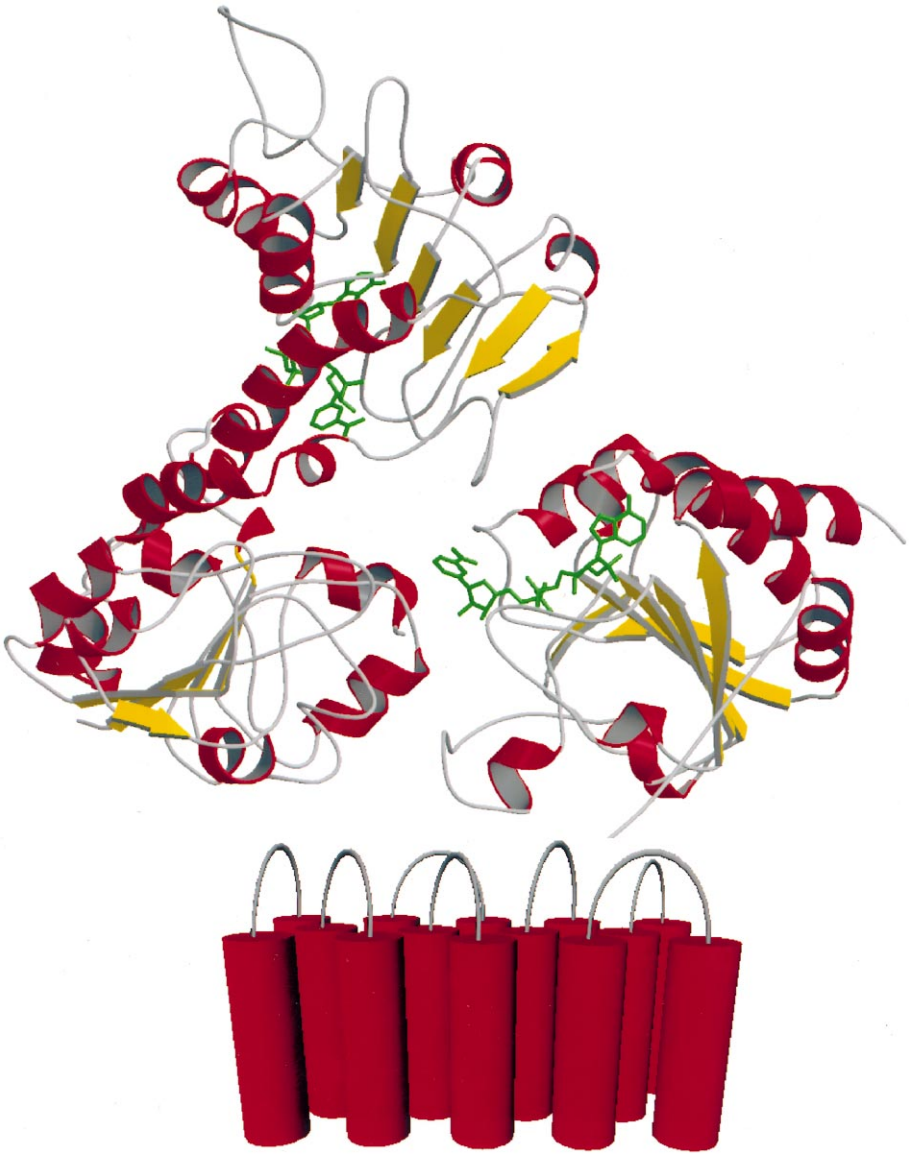

Fig. 1. The structure of transhydrogenase, facts and fantasies. The ribbon diagram of dIII (left) is a representation of the crystal structure of human dIII.NADP$^+$ [69]. That of dI (right) is a homology-based model [59] using the crystal structure of alanine dehydrogenase [62] as a template. Fourteen TM helices in dII (below) are indicated, the consensus number for mitochondrial transhydrogenase (see text). The figure (in an 'exploded' view) indicates possible interactions between dI, dII and dIII. The ridge on dIII is proposed to insert into the putative cleft of dI to bring the nicotinamide rings of NADP$^+$ and NADH into apposition, and helix-D/loop-D of dIII is proposed to interact with the interstitial loops between TM helices on dII.

cleotides [26,33–35]. This shows that the apparatus for hydride transfer is located entirely within dI and dIII, i.e. the membrane-spanning dII component is not involved in the redox step. Analysis of steady-state kinetics of complete transhydrogenase established that the reaction proceeds through a ternary complex (e.g. enzyme.NADH.NADP$^+$), as originally shown [36]. There is now little doubt that transfer of hydride proceeds *directly* between the two C4 atoms on the nicotinamide rings of the bound nucleotides: (a) isolated dI and dIII are not associated with detectable redox cofactors or prosthetic groups [34], (b) in stopped-flow experiments with mixtures of dI and dIII, the single turnover reduction of AcPdAD$^+$, bound to dI, has similar kinetics to the oxidation of NADPH, bound to dIII [34]; equivalently, the single turnover oxidation of AcPdADH, on dI, matches the reduction of NADP$^+$, on dIII [35]. Thus, there are no reduced enzyme intermediates in the reaction catalysed by transhydrogenase.

In early experiments to determine the value of x in Eq. 1, rates of transhydrogenation, and of proton leak, were both underestimated (sometimes producing fortuitously accurate ratios, as discussed in [37]). Recent measurements, performed on a much faster time scale to minimise errors, indicate that, for both the bacterial and mitochondrial proteins, one proton is translocated across the membrane per hydride transferred [38]. Complexes of dI and dIII catalyse a rapid single turnover of hydride transfer (see above) but, in the absence of dII, the steady-state reaction is very heavily limited by the extremely slow rate of release of either NADPH (during forward transhydrogenation), or of NADP$^+$ (during reverse) [30]. It would appear that, in the intact enzyme, interactions between dII

and dIII, probably coupled to proton translocation (see below), promote release of NADP(H).

3. A binding-change mechanism for proton translocation by transhydrogenase

The facts that hydride transfer between nucleotides is direct (see above), and that there is no isotopic exchange of the hydride transferred and the solvent water [27], together strongly suggest that the redox reaction is not itself coupled to proton translocation. In fact, the notion that conformational rearrangements in the protein are responsible for proton pumping was put forward by Rydström more than 20 years ago [39]. Following Boyer's proposals for energy coupling in ATP synthase (see [40]), it was suggested, if only in broad terms, that proton translocation by transhydrogenase might also be associated with changes in the binding of NAD(H) and NADP(H) [41,42]. The observation that susceptibility of transhydrogenase to inhibitory modification by

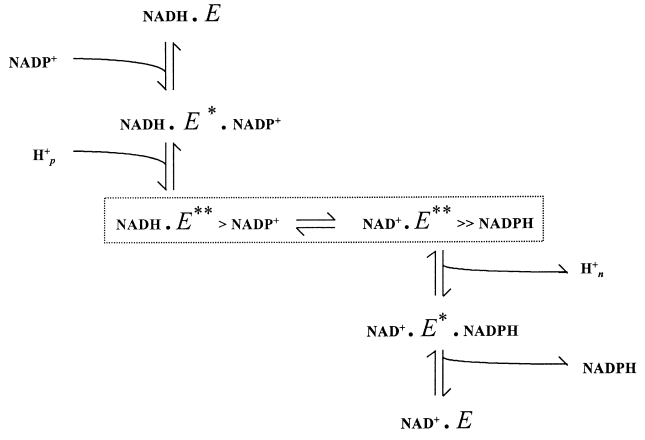

Fig. 2. A binding-change mechanism for transhydrogenase. *E*, *E** and *E*** are different conformations of the complete, membrane-bound transhydrogenase. The catalytic site is linked to the solvent by two proton channels, on the outside of the membrane, by the *p* channel, and on the inside, by the *n* channel. The reaction is gated to prevent hydride transfer without proton translocation, and vice versa. a: Hydride transfer can take place in *E*** but not in *E* or *E**. b: The *p* channel is open, and the *n* channel is closed, when $NADP^+$ is bound to the enzyme, whereas the *p* channel is closed, and the *n* channel is open, when NADPH is bound. c: In both the *E* and *E** states, NADP(H) on the enzyme can exchange rapidly with nucleotide in the solvent (binding is signified by '.'), but the exchange is blocked in *E*** (binding signified by '>' and '≫'). d: In contrast, NAD(H) can freely and rapidly exchange with the solvent from all states. The model shown here (and that discussed in the text) is based on the notion that protonation from the *p* phase follows $NADP^+$ binding, and that deprotonation to the *n* phase precedes NADPH release [45], but equivalent considerations apply to an alternative model (in which deprotonation to the *n* phase follows $NADP^+$ binding, and protonation from the *p* phase precedes NADPH release). Note that the equilibrium constant for hydride transfer on the enzyme with physiological nucleotides is approximately 10 (compare with the value of ~1.0 in free solution), and this biases the reaction in the forward direction at this step [35,77]. Since the binding affinity of dI for NADH is ~10-fold greater than that for NAD^+ [46], the affinity of dIII for NADPH in the *E*** state (signified '≫') is 100-fold greater than that for $NADP^+$ (signified '>'). Isolated complexes of recombinant dI and dIII can only perform the reactions within the dashed box; in the absence of interactions with dII, inter-conversion between *E** and *E*** is prevented (see text).

various agents was differentially affected by $NADP^+$ and NADPH led to the suggestion that conformational changes at the NADP(H)-binding site are of particular importance [43]. However, the view persisted that changes in binding of both NAD(H) and NADP(H) are coupled to proton translocation [44]. In 1994, on the basis of observations on the 'cyclic reaction' in *Escherichia coli* transhydrogenase [45], we proposed that proton translocation is coupled specifically to changes in the binding of $NADP^+$ and NADPH, and the gating requirements of such a mechanism were defined. Present evidence strongly supports the notion that NADH serves only as a passive donor of reducing equivalents in the reaction [46–48]. A working hypothesis is shown in Fig. 2. Binding of $NADP^+$ to dIII leads to a state of the enzyme (*E**.$NADP^+$) in which protonation from the *p* side of the membrane is permitted. Protonation drives the enzyme into a state (*E***) capable of hydride transfer – the two C4 atoms on the nicotinamide rings of $NADP^+$ and NADH are brought into apposition. Following hydride transfer, deprotonation of the enzyme to the *n* side then regenerates *E** which allows rapid release of NADPH.

4. Transmembrane helices in the dII component of transhydrogenase

A range of predictive algorithms and experimental approaches has been employed to determine the number and position of transmembrane (TM) helices in dII [16–18,49–52]. The emerging consensus (extensively reviewed [52]) is that there are 13 TM helices in transhydrogenases from prokaryotes (including *E. coli*, *Rhodospirillum rubrum*, and others), and certain protozoan parasites [53], and 14 in the enzyme from mitochondria of higher animals; the extra helix in the last group is located in a short sequence which is missing in bacteria. A comparison of the organisation of genes coding for transhydrogenase indicates that there are two clusters of TM helices (numbers 1–4 and 6–14 in the adopted nomenclature [18,52]), which in many species are separately transcribed. Whether this is of structural or functional significance remains to be seen. All the conserved, protonatable residues in the putative TM helices of *E. coli* transhydrogenase, and their interstitial loops, have been replaced by site-directed mutagenesis [50,54–58], but most substitutions have, at most, only a partial effect on activity. Replacement of βH91 by other residues gave rise to decreased transhydrogenation rates, and it was suggested that this residue is involved in proton pumping [50,55,58]. However, there are five recently determined genome sequences from which it appears that the equivalent histidine residue is replaced by an asparagine. It is unlikely, therefore, that the residue in this position plays other than a passive role in proton translocation in transhydrogenase. The probability is that the proton channel through dII comprises mainly bound water molecules and the side chains of serine and threonine residues.

5. A model of the 3-D structure of the dI component of transhydrogenase based on the high-resolution structure of alanine dehydrogenase

The existence of a fingerprint region [59] in the amino acid sequences provided evidence that a part of dI has the common, dinucleotide-binding fold [16–18,49,60]. It was noted

[61] that the amino acid sequence of dI is similar to that of the soluble enzyme, alanine dehydrogenase, whose crystal structure was solved to 2.0 Å resolution in 1998 [62], and on this basis, a model of the 3-D structure of dI has been proposed [63] (see Fig. 1). The model structure has two domains. The NAD(H)-binding domain, dI.2, is linked by two long helices to domain dI.1, of unknown function. As in alanine dehydrogenase, the nicotinamide ring of the bound nucleotide is thought to be located deep within a cleft between domains I.1 and I.2.

In the dI protein, but not in alanine dehydrogenase, there is a segment of 20 amino acid residues, which is sufficiently mobile to be observable by NMR spectroscopy [47,63,64]. Upon NAD(H) binding, this 'mobile loop' closes down on the surface of the protein and makes contact with the nucleotide. Mutational analysis [65–68] shows that the loop is important in catalysis; it was suggested to have a role in the relative positioning of the nicotinamide rings of NADH and $NADP^+$ prior to hydride transfer.

6. The high-resolution structure of the dIII component of human transhydrogenase

The crystal structure of recombinant dIII from human-heart transhydrogenase was recently solved by X-ray diffraction at 2.0 Å resolution [69]. The structure comprises an open, twisted β sheet of six parallel strands, flanked by helices. As in the 'classical' lactate-dehydrogenase fold, a paradigm for the structure of many other dinucleotide-binding proteins [70] (and see review [71]), there are two βαβαβ motifs linked by a crossover helix. $NADP^+$ is bound to dIII by loops at the C-terminal edge of the β sheet but its orientation is reversed relative to nucleotide binding in classical structures. Thus, the adenosine moiety in dIII is bound predominantly within the first βαβαβ motif, and the NMN moiety within the second. Remarkably, a fingerprint sequence (G-X-G-X-X-A/V) is found in its 'usual' position in the first βαβαβ motif but, in dIII, the sequence is not associated with pyrophosphate binding.

As in other dinucleotide-binding sites, the adenine ring is buried in a hydrophobic crevice (formed predominantly by amino acid side chains in the C-terminal loops of strands β1, β5, β6) (see Figs. 3 and 4). The specificity of dIII for NADP(H) relative to NAD(H) is determined by numerous H-bond and electrostatic interactions between K999, R1000 and S1001 and the 2′-phosphate group of the adenosine ribose. The nicotinamide ring of $NADP^+$ is bound only by a single loop at the C-terminus of strand β2. It is positioned at the end of a ridge, defined mainly by the well-conserved and hydrophobic helix B, which separates two, relatively flat protein surfaces. In the complete enzyme, the ridge on dIII is presumably inserted into the putative cleft on dI (see above, and Fig. 1) to permit mutual approach of the two nicotinamide rings. In the crystal structure of isolated dIII the *pro-R* (or *A*) face of the bound $NADP^+$ is shielded from the solvent, but its *pro-S* (or *B*) face is largely exposed, notably around the C4 atom. Thus the *pro-S* face will be involved in the apposition with NADH, explaining the 4*B* stereochemistry of direct hydride transfer to $NADP^+$ [27].

The 'back' of dIII is covered by weakly conserved amphipathic α helices (0, A and F), which are probably exposed to the solvent also in the complete enzyme. Taking due account of the likely position of the dI component, this strongly suggests that the membrane-spanning dII must interact with the 'front' of dIII (Fig. 1), where we find the distinctive structural feature, helix-D/loop-D. Helix-D projects almost perpendicularly from the β sheet. It emanates from the C-terminus of strand β4, where the chain turns sharply under the pyrophosphate group of the nucleotide. At the C-terminus of helix-D, the polypeptide makes a series of turns and, as the relatively extended loop-D, runs back towards the sheet, approximately antiparallel with helix-D. If the expected arrangement of the three subunits in the complete enzyme is correct, then helix-D/loop-D of dIII makes contacts with the loops between the TM helices of dII. The N-terminus of the recombinant dIII protein (M837) is close to the C-terminus of the last predicted TM helix of dII. The first 21 residues are disordered in the crystal structure of dIII, and the region is protease-sensitive in the complete enzyme [19,20], indicating that it might be flexible linker with the membrane component.

Adjacent to helix-D is loop-E, which arises from the C-terminus of strand β5, and which arches over the nucleotide like a lid. At the apex of loop-E is Y1006. This residue, and Y890, which protrudes from the C-terminus of strand β1, 'sandwich' the guanidinium group of R925 (from the loop which binds the nicotinamide ring, see above). Y890 (actually, the second residue in the fingerprint sequence), is positioned by a kink introduced into strand β1 by P888.

There is an extremely interesting set of H-bond interactions between helix-D, loop-E, including Y1006 and its associated Y890 and R925, and the ribose and pyrophosphate groups of the bound $NADP^+$, that we believe are involved in energy transmission in the complete enzyme. Thus, there are extensive H-bond contacts between residues in helix-D (N966, D967 and T968) and the pyrophosphate, and both ribose groups, of the nucleotide. Each of the residues in the sandwich triad, also makes an H-bond with the pyrophosphate (and R925 also with the NMN ribose). Residues in helix-D (again including N966 and D967) are in H-bond contact with residues in loop-E (including G1003, Y1006, A1007 and N1011). The fact that N966 and D967 have H-bonds with both the pyrophosphate group and loop-E might be particularly significant (see below). The carboxylate of D967 and the NMN-phosphate group are both buried in the protein. Their charges are partly compensated by H-bond interactions; that between the carboxylate of D967 and the amide of A1007 is a short-range interaction (2.62 Å).

Determinations of secondary-structure elements by NMR, firstly of *R. rubrum* dIII [72,73], and later of *E. coli* dIII [74,75], have been published. The secondary-structure assignments are generally consistent with the crystal structure of human dIII (see above) though, in both cases, there are minor differences. From both sets of NMR data, it was suggested that dIII adopts the Rossmann fold. However, the 'global fold' model predicted for the *E. coli* protein [75] has major differences in the relative disposition of the helices, strands and loops, and in the identification of some of the short helices, with the tertiary structure of human dIII determined by X-ray crystallography. These differences might simply arise from the rather limited number of measurements (31 NOEs between the backbone amide groups of β strands, and 16 NOEs between side-chains). The NMR structure of *R. rubrum* dIII (with bound $NADP^+$), which is based on >1800 unambiguous NOEs, of which >100 are between β strands, >100

are between strands and helices and >560 are between protons more than 6 residues apart, and on angle constraints (M. Jeeves, K.J. Smith, P.G. Quirk, N.P.J. Cotton and J.B. Jackson, manuscript in preparation), is considerably closer to the crystal structure of human dIII.

7. Conclusions and comments on the mechanism of energy transduction in transhydrogenase, from observations on the crystal structure of dIII

7.1. The $E^{**} > NADP^+$ intermediate in catalysis

It was suggested [11,30,35,48] that isolated, recombinant dIII.$NADP^+$ in solution (and in its complex with isolated dI in solution) is locked into a conformation which is similar to that of dIII in the reaction intermediate, $E^{**} > NADP^+$, of the complete enzyme (Fig. 2). This putative intermediate directly precedes the hydride transfer step, and, to minimise slip in the mechanism, exchange of nucleotide between the protein and the solvent must be prevented in this state (see legend to Fig. 2). These are, indeed, well-characterised properties of isolated dIII; the protein can carry out rapid hydride transfer when in complex with recombinant dI [26,34,35], and it releases bound $NADP^+$ and NADPH at only extremely low rates [30,32,33]). Thus, we suggest that the crystal structure of isolated dIII.$NADP^+$ is representative of that of dIII in $E^{**} > NADP^+$. In this conformation, the nicotinamide ring, exposed on the helix-B 'ridge', is poised ready to accept a hydride equivalent (see above). The restricted rate of release of nucleotide is probably a consequence of the arrangement of loop-E and the Y890/R925/Y1006 triad, which form a lid, or cage, over the pyrophosphate and NMN ribose moieties (Figs. 3 and 4).

7.2. Generation of the $E^{**} > NADP^+$ intermediate

In the scheme of Fig. 2, $E^{**} > NADP^+$ is generated from E^{*}.$NADP^+$ by protonation of the enzyme from the *p* side of

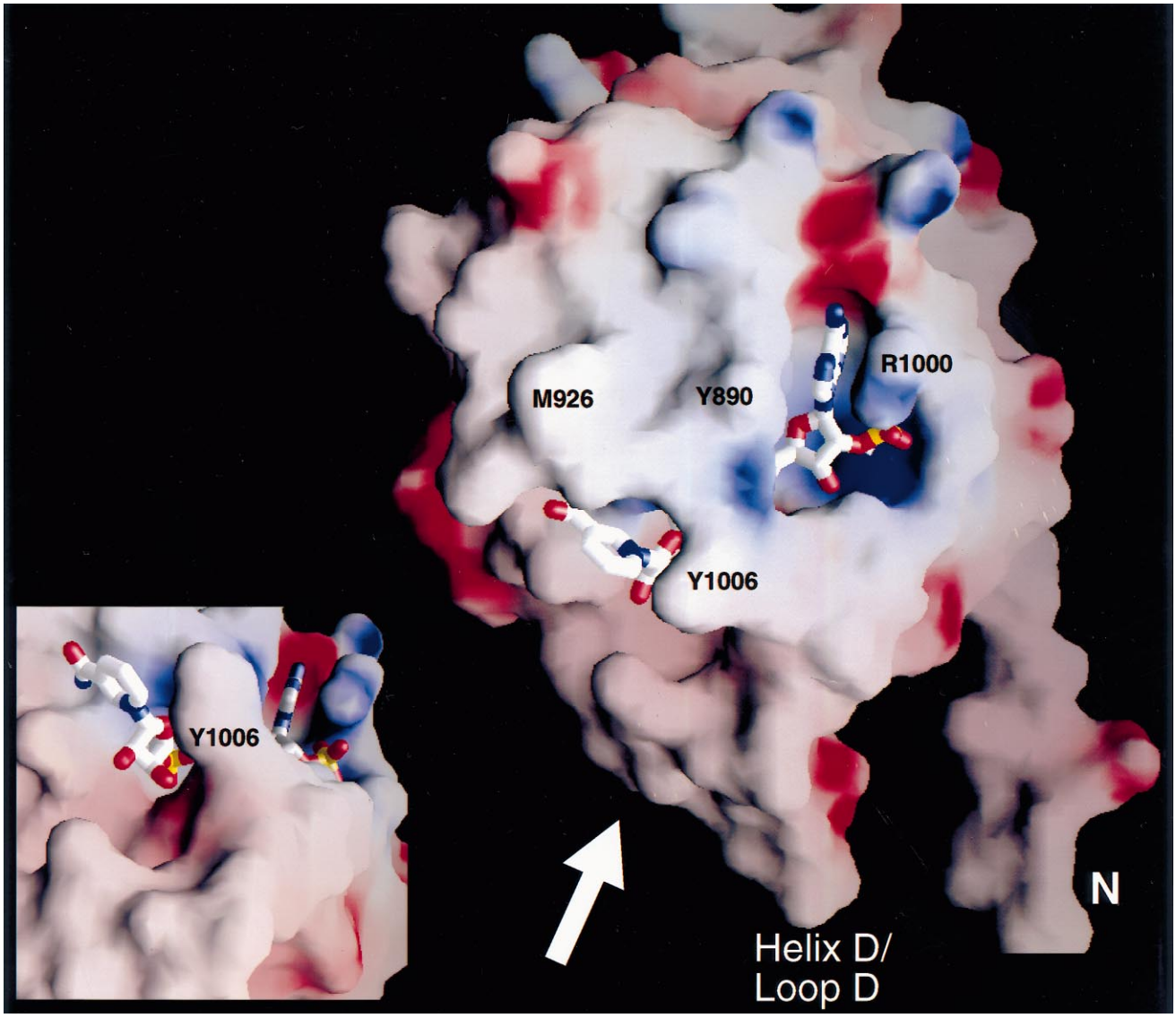

Fig. 3. High-resolution structure of dIII from human transhydrogenase. The program GRASP [78] was used to show the molecular surface of the protein. Positive and negative Coulombic fields are represented by blue and red, respectively. $NADP^+$ is shown as a stick model. The inset gives the view of the protein along the direction of the arrow. Note the 'lid' over the bound $NADP^+$ formed by R925 and Y1006 (see text).

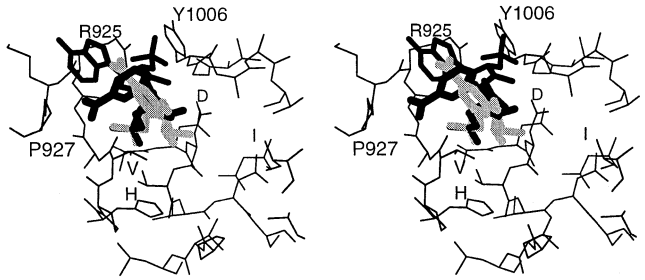

Fig. 4. The $NADP^+$-binding site and the 'glycerol pocket' of transhydrogenase dIII. The nucleotide in heavy shading indicates its position in the crystal structure; that in light shading indicates a possible position when the protein is in the *E** state (see text and Fig. 2). The numbers of some amino acid residues are shown; others are: H = H920, V = V922, D = D967, I = I981.

the membrane. This is the first power stroke in the reaction, the second being the conversion of *E***≫NADPH to *E**.NADPH with proton release to the *n* side. *E** is a state from which $NADP^+$ (or NADPH) can exchange rapidly with the solvent. It is suggested that, in this state, amino acid residues in the apex of loop-E (e.g. Y1006) are farther away from helix-D and from Y890/R925 than in the dIII.$NADP^+$ crystal form. With the lid in this open conformation, the pyrophosphate group of the nucleotide is less deeply buried, and this permits more rapid binding/release of nucleotide. To prevent redox slip on *E**, hydride transfer between nucleotides on dI and dIII, is blocked (Fig. 2). We propose that, in this conformation, the nicotinamide rings of NAD(H) and NADP(H) are farther apart than they are in *E***. A possible site for binding the nicotinamide ring of NADP(H) in the *E** state is an adjacent pocket, which in the crystal structure (*E***, see above) is occupied by glycerol. The necessary movement can be achieved by rotating the NMN-ribose moiety about the O–P–O bonds of the nicotinamide-pyrophosphate group (Fig. 4). The swivelling movement would be facilitated by moving back loop-E – as indicated above, loop-E is expected to be open in the *E** state.

It is probably of some significance that several distinctive structural features of the dIII protein focus on the pyrophosphate group of the bound nucleotide (see above). It is suggested that the seminal ionisation/conformational changes in dIII, which are primarily responsible for changing NADP(H) binding during the transition from *E** to *E*** to enable the hydride transfer step, take place at the N-terminus of helix-D, in the region of N966 and D967. Both residues are invariant, and mutation of the residue equivalent to D967 in *E. coli* transhydrogenase has pronounced effects on transhydrogenation [58,76]. We suggest this region is the catalytic centre for energy conversion. Concerted changes in the intricate set of interactions between N966, D967, residues in loop-E including Y1006, Y890/R925 and the nucleotide pyrophosphate group, all of which are in the same locality, could lead to the relative movements of the $NADP^+$ and protein which are responsible for this power stroke of the transhydrogenase reaction, (a) to bring the C4 atom of the nicotinamide ring, from a distal position (relative to that of NADH), e.g. in the 'glycerol pocket', into a proximal position, in the C-terminal loop of strand β2, and (b) to prevent release of the bound $NADP^+$, by closing the loop-E lid, over the pyrophosphate group, against loop-D and Y890/R925. These proposed conformational shifts would not involve significant changes in the interactions between the adenine, and the adenosine-ribose groups, and the protein. The extensive contacts between the 2′-phosphate of the adenosine ribose and the N-terminal region of loop-E, as well as determining the nucleotide specificity (see above), will also, therefore, provide a fulcrum for the opening of this loop.

The important question remains, as to how the energy released by proton conduction through dII is transmitted to the catalytic centre. Conceivably, dII and the protruding part of helix-D in dIII could serve as a conductance pathway to introduce protons into the centre; changes in the protonation state of, for example, D967 or the nucleotide pyrophosphate group can be envisaged to result in sufficiently large displacements of the nicotinamide ring and loop-E to account for the events depicted in Fig. 2. The proton efflux channel might involve the pyrophosphate group itself. Alternatively, conformational changes resulting from proton translocation could be propagated closer to, or below, the membrane surface, and transmitted to the catalytic centre through helix-D over a distance of several Ångstrom units. In this case the proton efflux channel need not proceed through dIII. There is very little biochemical evidence to discriminate between these two possibilities (see [48]).

*7.3. Conformational changes accompanying the conversion of E**.NADP⁺ to E**.NADPH*

The conversion of *E***.$NADP^+$ to *E***.NADPH by hydride transfer from NADH is accompanied by a change in access of the *p* and *n* proton channels of transhydrogenase (Fig. 2). Differences in the HSQC-NMR spectrum of isolated *R. rubrum* dIII.$NADP^+$ were observed upon substituting the bound nucleotide with NADPH [73]. The HSQC experiment provides information on changes in the magnetic environment of amide groups in the protein. Mapping the changes on to the crystal structure of human dIII indicates that reduction of $NADP^+$ causes conformational changes, not unexpectedly, in the backbone amides of residues in the nicotinamide-binding region, but also throughout helix-D/loop-D and in loop-E [69]. Evidently information on the redox state of the bound nucleotide is transmitted into the part of dIII that is thought to interact with dII (see above). It is tempting, therefore, to suggest that this information relay is associated with the gating of the proton channels.

8. Concluding remarks

There are strong indications that proton translocation by transhydrogenase is associated with changes in the mode of binding of $NADP^+$ and NADPH to the dIII component of the enzyme. Features in the high-resolution crystal structure of human dIII.$NADP^+$ provide a basis for understanding the mechanism of the reaction. (1) The stereochemistry of hydride transfer, and the specificity of NADP(H) over NAD(H), are clearly explained by structure in and around the nucleotide-binding pocket. (2) The movement of a 'lid' over the pyrophosphate group of the nucleotide is responsible for a necessary restriction in the rate of NADP(H) release during catalysis. (3) A cluster of amino acid residues at the N-terminus of helix-D is centrally involved in coupling the energy available from Δp to changes in NADP(H) binding.

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