

Dehydrogenase and transhydrogenase properties of the soluble NADH dehydrogenase of bovine heart mitochondria

(nicotinamide nucleotides/dehydrogenation/nonenergy-linked and energy-linked transhydrogenation)

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ABSTRACT The soluble NADH dehydrogenase of low molecular weight, isolated from complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) of the respiratory chain, has been shown to have NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase activities. Both activities are greatly increased in the presence of added guanidine-HCl and at pH values <6.5 . The chromophores of the soluble enzyme (flavin and iron-sulfur centers) are reduced by NADH and NADPH to the same extent. The latter reduction is extremely slow, and is considerably stimulated in the presence of guanidine-HCl.

The soluble dehydrogenase has little or no NADH \rightarrow NADP and NADPH \rightarrow NADP transhydrogenase activity. The former reaction is known to be energy-linked in submitochondrial particles; the latter was shown in the present studies also to be energy-linked.

In view of the above and earlier results, possible mechanisms for dehydrogenation and transhydrogenation (nonenergy-linked and energy-linked) involving reduced and oxidized NAD and NADP are proposed.

The mitochondrial electron transport system can oxidize NADPH directly, i.e., without the intervention of NAD and the transhydrogenase reaction (1, 2). Under optimal conditions, the rate of NADPH oxidation by submitochondrial particles is ≥ 250 nmol \cdot min $^{-1}\cdot$ mg $^{-1}$ protein at 30°, and under phosphorylating conditions NADPH oxidation is coupled to ATP synthesis with P/O of 2.4-2.9 (2, 3). Both NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase activities fractionate mainly into complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3), the iron-sulfur centers of which have been shown in recent electron paramagnetic resonance studies to be reduced to about the same extent by NADPH and the slowly oxidized NADH analog, reduced acetylpyridine adenine dinucleotide (AcPyrADH) (4).

Resolution of complex I with chaotropic agents yields a soluble NADH dehydrogenase with a molecular weight of 70,000-80,000 (5, 6). The enzyme contains 1 mol of FMN, 4 g-atoms of nonheme iron, and 4 mol of labile sulfide per mol, and catalyzes the oxidation of NADH by quinoid structures (menadione, ubiquinones, 2,6-dichloroindophenol, methylene blue), ferric compounds (ferricyanide, cytochromes *c*), and NAD (for a recent review of complex I and the soluble NADH dehydrogenase, see ref. 7).

The present studies show that the soluble NADH dehydrogenase* also catalyzes NADPH dehydrogenation and NADPH \rightarrow NAD transhydrogenation. Little or no transhydrogenase activity from NADH \rightarrow NADP and NADPH \rightarrow NADP could be demonstrated with the soluble dehydrogenase. The former reaction is known to be energy-linked in submitochondrial particles (8). The latter reaction, i.e., transhydrogenation from

NADPH \rightarrow NADP, was shown in the present studies also to be energy-linked.

METHODS AND MATERIALS

Phosphorylating submitochondrial particles (9), complex I (10), and NADH dehydrogenase using either urea or 0.5 M NaClO₄ for complex I resolution (5) were prepared according to published methods. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (11), protein determination (biuret), and dehydrogenase and transhydrogenase assays were carried out as published (1). Other details are given with the results. Spectrophotometric studies were carried out with Cary 118 and Aminco-Chance dual wavelength/split beam spectrophotometers. All nucleotides were obtained from P. L. Biochemicals. The sources of other chemicals were the same as detailed elsewhere (1).

RESULTS

Effect of Guanidine on NADH Dehydrogenase. Table 1 summarizes the molecular and NADH dehydrogenase properties of complex I and the soluble NADH dehydrogenase isolated from complex I. As compared to complex I, the soluble dehydrogenase has a low dehydrogenase activity per mole of flavin and a higher K_m for NADH. As seen in Fig. 1, addition of guanidine hydrochloride (up to about 150 mM) to the assay mixture increases the V_{max} and lowers the K_m^{NADH} of the enzyme, thus bringing these values closer to those of the complex I-bound dehydrogenase. Alkyl guanidines, including arginine and arginyl methyl ester, also activate the soluble dehydrogenase, but on a molar basis are less effective than guanidine-HCl (12). Guanido groups of enzyme arginyl residues have been demonstrated to be involved, apparently as substrate binding sites, in nicotinamide nucleotide and adenine nucleotide linked enzymes (13-16), including the NADPH \rightarrow NAD transhydrogenase activity of the respiratory chain (2). The ability of guanidine-HCl and alkyl guanidines to restore the kinetic characteristics of respiratory chain-linked dehydrogenase to the soluble enzyme suggests that the soluble dehydrogenase contains fewer (e.g., by loss of a polypeptide) or less favorably positioned arginyl residues for substrate binding as compared to its membrane-bound counterpart.

NADPH Dehydrogenase Activity of the Soluble NADH Dehydrogenase. As seen in Fig. 2, the soluble dehydrogenase has undetectable NADPH dehydrogenase activity at pH ≥ 6.5 . Indeed, under the assay conditions applied to complex I, little NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase activity was found in any of the chaotrope-resolved fractions of complex I. However, as seen in Fig. 2, addition of 75 mM guanidine-HCl allowed measurement of substantial NADPH dehydrogenase activity, which greatly increased as the assay pH was lowered. At pH 5.0, this activity was 13.1 μ mol of NADPH oxidized \cdot min $^{-1}\cdot$ mg $^{-1}$ protein. (For comparison the NADH dehydrogenase activities of the enzyme in the absence

Abbreviations: AcPyrAD, acetylpyridine adenine dinucleotide; ETP_H, phosphorylating submitochondrial particles.

* The designation "NADH dehydrogenase" has been applied by others to preparations comparable to complex I (for review, see ref. 7). In the present studies, this term is applied only to the soluble, low-molecular-weight enzyme specified in the preceding paragraph.

Table 1. Molecular and enzymatic properties of complex I and the soluble, low-molecular-weight NADH dehydrogenase

Parameter	Complex I	Dehydrogenase
g of protein/mol of flavin	7×10^5	$7-8 \times 10^4$
Flavin: Fe:S*	1:16-18:16-18	1:4:4
Turnover number†	5×10^5	2.9×10^4
K_m^{NADH} (μM)	~ 45	~ 80

* S, acid-labile sulfide.

† Mol of NADH oxidized by potassium ferricyanide/mol of flavin per min.

and presence of 75 mM guanidine are also shown.) Fig. 3 shows a similar effect of guanidine on the reduction of the enzyme prosthetic groups [FMN and iron-sulfur center(s)] by NADPH. At pH 6.0, the reduction of oxidized enzyme by NADPH from trace 1 to trace 6 of Fig. 3 took 25 min at 22°, allowing multiple tracings of the intermediate stages of reduction to be made (Fig. 3 and inset, dashed traces). In the presence of 50 mM guanidine, however, NADPH reduced the soluble dehydrogenase from trace 1 to trace 6 in less than 1 min. As seen in Fig. 2, guanidine stimulation of NADPH dehydrogenase activity, especially at pH ≥ 6.0 , is much greater than stimulation of NADH dehydrogenase activity, and at pH ≤ 5.5 the enzyme has considerable NADPH dehydrogenase activity in the absence of added guanidine.

Transhydrogenase Activities of the Soluble NADH Dehydrogenase. Table 2 summarizes the dehydrogenase and transhydrogenase activities of a typical preparation of the enzyme at two pH values in the absence and presence of 75 mM guanidine-HCl. Dehydrogenase and transhydrogenase activities were measured using, respectively, ferricyanide and the 3-acetylpyridine analogs of the oxidized nucleotides as acceptors. It is seen that at the appropriate pH values (8 for NADH, 5.5 for NADPH), the dehydrogenase exhibits both NADH \rightarrow AcPyrAD and NADPH \rightarrow AcPyrAD transhydrogenase activities, which are again stimulated in the presence of 75 mM guanidine-HCl. It may also be noted that when the NADP analog is the acceptor, there is little or no transhydrogenase activity in the absence or presence of guanidine, regardless of whether the

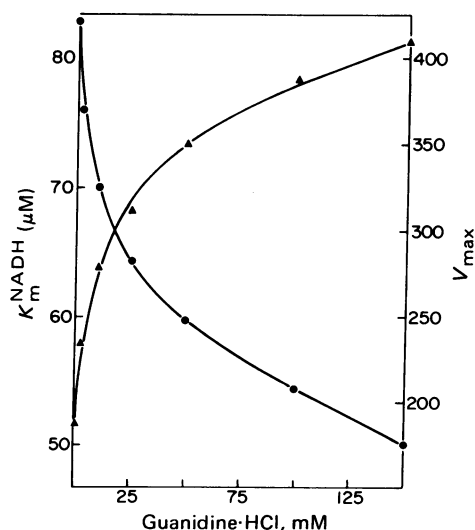


FIG. 1. Effect of guanidine-HCl on the K_m (●) and V_{max} (▲) of the reaction NADH \rightarrow $\text{K}_3\text{Fe}(\text{CN})_6$, catalyzed by NADH dehydrogenase. V_{max} is expressed as μmol of NADH oxidized $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein at 38°.

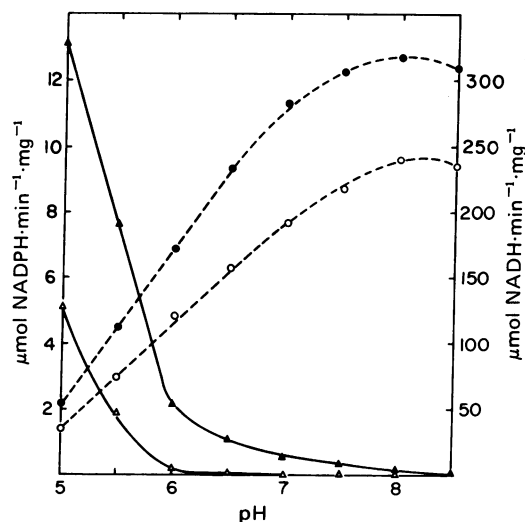


FIG. 2. NADH and NADPH dehydrogenase activities of NADH dehydrogenase as a function of pH. Conditions: 100 mM K acetate for pH 5 and 5.5, 100 mM K phosphate for pH 6-8.5, 150 μM NADH, 300 μM NADPH, and 0.2 mM 2-methylnaphthoquinone (K_3) as electron acceptor at 38°. The NADH dehydrogenase used was a 42-51% ammonium sulfate fraction of a complex I supernatant resolved with 0.5 M NaClO_4 . (○ - - - ○, ● - - - ●) NADH \rightarrow K_3 ; (△ - △, ▲ - ▲) NADPH \rightarrow K_3 ; (●, ▲) 75 mM Gdn-HCl.

hydride ion donor is NADH or NADPH. Fig. 4 shows the effect of pH on the NADPH \rightarrow AcPyrAD transhydrogenase activity of the soluble enzyme in the absence and presence of 75 mM guanidine. As in the case of NADPH dehydrogenase activity shown in Fig. 2, the enzyme has negligible NADPH \rightarrow NAD transhydrogenase activity at pH ≥ 6.5 , but it is substantially stimulated by lowering of pH and/or addition of guanidine-HCl.

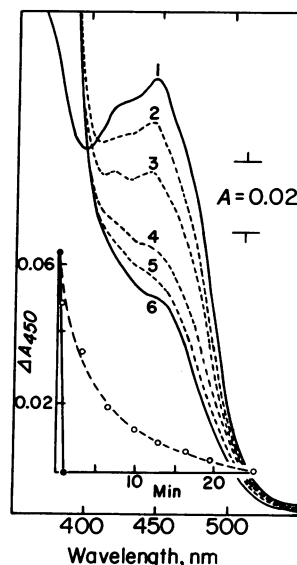


FIG. 3. Reduction of NADH dehydrogenase chromophores by NADPH in the absence and presence of 50 mM guanidine-HCl. The enzyme at a concentration of 0.5 mg of protein per ml was dissolved in 0.1 M potassium phosphate at pH 6.0, and placed in a stoppered cuvette. The cuvette was evacuated and filled with argon five times. Then 50 μl of 15 mM NADPH, or 30 μl of 5 M guanidine-HCl followed by 50 μl of 15 mM NADPH, was added and spectra recorded as shown. When NADH was used as substrate, the degree of reduction of the enzyme was the same as shown by trace 6. Inset: decrease of 450 nm absorbance (ΔA_{450}) as a function of time in the absence (- - - -) and presence (—) of 50 mM guanidine-HCl. For other details, see text.

Table 2. Dehydrogenase and transhydrogenase activities of the soluble, low-molecular-weight NADH dehydrogenase isolated from complex I

Reaction	pH	Activity*	
		-Gdn·HCl	+Gdn·HCl†
NADH → ferricyanide	8	242	485
NADH → AcPyrAD	8	12	30
NADH → AcPyrAD	5.5	6	8
NADH → AcPyrADP	5.5	<0.04	<0.04
NADPH → ferricyanide	8	Nil	0.3
NADPH → ferricyanide	5.5	4.2	20.8
NADPH → AcPyrAD	8	<0.05	0.3
NADPH → AcPyrAD	5.5	0.7	1.54
NADPH → AcPyrADP	5.5	Nil	Nil

Conditions are described in ref. 1. Gdn·HCl, guanidine·HCl.

* Data are $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein at 38° .

† Concentration was 75 mM.

Energy Requirement of the NADPH → NADP Transhydrogenase Reaction. As seen in Table 2, the soluble dehydrogenase has little or no NADH → NADP and NADPH → NADP transhydrogenase activity. The former reaction is known to be slow in submitochondrial particles in the absence of an energy supply, and stimulated severalfold when energy is supplied to the system by ATP hydrolysis or substrate oxidation. The latter reaction, i.e., transhydrogenation from NADPH to NADP (or AcPyrADP), is shown in Fig. 5 also to be energy-linked. The reaction mixture contained oligomycin-treated phosphorylating submitochondrial particles (ETP_H), NADPH, rotenone, and succinate whose oxidation by ETP_H would supply energy. The acceptor was AcPyrADP whose reduction was monitored in the dual wavelength spectrophotometer at 400 minus 450 nm (1). It is seen in Fig. 5 that AcPyrADP was reduced in this system and that its reduction was inhibited by uncouplers or by antimycin A, which interrupted succinate oxidation. Because preparations of ETP_H contain ≤ 0.2 nmol of bound NAD per mg of protein (1), the possibility existed that in the experiment of Fig. 5 AcPyrADP reduction might have occurred in the following manner. First, nonenergy-linked transhydrogenation would result in reduction of the bound NAD by the added NADPH; then, the NADH so formed would reduce AcPyrADP *via* the known energy-linked transhydrogenation reaction. To

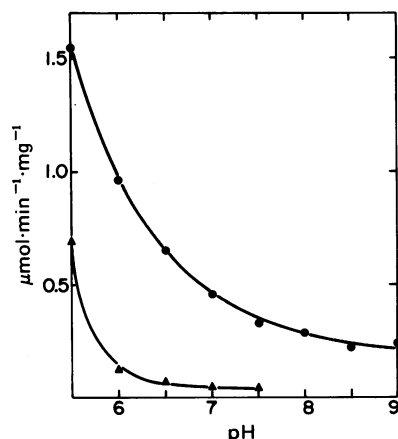


FIG. 4. Effects of pH and 75 mM guanidine·HCl on the NADPH → 3-acetylpyridine adenine dinucleotide (AcPyrAD) transhydrogenase activity of NADH dehydrogenase. Conditions: 1 mM AcPyrAD, 0.9 mM NADPH, and the same buffers at 38° as in Fig. 2. (●) +75 mM guanidine·HCl; (▲) no guanidine·HCl added.

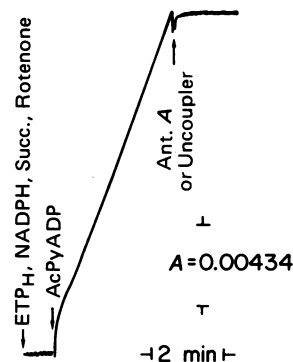


FIG. 5. Energy-linked transhydrogenation from NADPH to (AcPyrADP) as catalyzed by ETP_H and assayed at 400–450 nm. The reaction mixture contained in 0.25 M sucrose, 50 mM Tris-sulfate at pH 6.8, 0.23 mg ETP_H protein per ml, 8 mM MgSO_4 , 0.9 mM NADPH, 6.6 mM sodium succinate (Succ.), 5 μM rotenone, and 1.3 μg of rutamycin per ml. Where indicated 0.33 mM AcPyrADP and 50 μM antimycin A (Ant. A) or 1 μM carbonyl cyanide *m*-chlorophenylhydrazone (uncoupler) were added. Assay temperature was 20° .

test for this possibility, we replaced the source of reducing power in the experiment of Fig. 5 (i.e., NADPH) with 3-hydroxybutyrate. It was found that no AcPyrADP was produced without the addition of NAD to the reaction mixture. To obtain a rate of AcPyrADP reduction comparable to that shown in Fig. 5, it was necessary to add 3.2 μM NAD, i.e., 50 times as much as might have been bound to the added ETP_H .

DISCUSSION

It has been shown in the preceding section that the soluble NADH dehydrogenase of the respiratory chain isolated from complex I catalyzes the following reactions: NADH and NADPH dehydrogenation, and NADH and NADPH transhydrogenation to NAD. The enzyme does not catalyze appreciable transhydrogenation from either reduced nucleotide to NADP, both of which reactions ($\text{NADPH} \rightarrow \text{NADP}$ shown in the present studies) are energy-linked in submitochondrial particles. All the reactions catalyzed by the soluble dehydrogenase are stimulated in the presence of guanidine·HCl, especially those involving NADPH. The rate of NADH oxidation by the soluble enzyme is considerably greater than that of NADPH oxidation. In the absence of guanidine, the respective rates given in Table 2 at pH 8.0 for NADH and pH 5.5 for NADPH show that the NADH reaction is about 60-fold faster than the NADPH one. The same ratio of ferricyanide reductase activities is obtained in submitochondrial particles for the oxidation rates of NADH and NADPH at the pH values indicated. These results and the fact that the prosthetic groups of the soluble NADH dehydrogenase are reduced by NADPH as shown in Fig. 3 indicate, therefore, that the NADH dehydrogenase component of the respiratory chain is the site of direct NADPH oxidation by the electron transport system.

Whether the soluble dehydrogenase is also related to the membrane-bound transhydrogenase enzyme has yet to be established. Several preparations of the mitochondrial transhydrogenase enzyme have been obtained by others, by using digitonin (17), lysolecithin (18), and cholate (19) as solubilizing agents. Our survey has indicated that these preparations are all particulate membrane fragments containing, among other things, complex I components.

Recent studies on the inhibition of a number of enzymes by the arginine-binding reagents butanedione, cyclohexanedione, and phenylglyoxal have suggested that the guanido moieties of protein arginyl residues serve as positively charged sites for

recognition of anionic substrates, especially the phosphate groups of nicotinamide nucleotides and adenine nucleotides (13–16). The respiratory chain-linked transhydrogenase enzyme was also shown recently to be inhibited by treatment of the particles with butanedione or trypsin (2). In view of these findings, the possibility was considered that the change in the kinetic characteristics of NADH dehydrogenase upon removal of the enzyme from the membranes might be due to loss (in a separate polypeptide) or unfavorable positioning of positively charged groups at the enzyme active site involved in substrate binding. This consideration prompted the use of guanidine-HCl, which as shown above changed the kinetic constants (K_m and V_{max}) of the soluble NADH dehydrogenase in the direction of the values obtained for the membrane-bound enzyme. Addition of guanidine also solved another dilemma, namely the observation that after resolution the NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase activities of complex I could no longer be detected under the same assay conditions in any of the resolved fractions of complex I. As seen in Figs. 2 and 4, and in Table 2, both activities are present in the soluble dehydrogenase when the assay is conducted in the presence of guanidine-HCl and/or at low pH.

However, because added guanidine cannot be considered to act as a counterpart of enzyme arginyl (or guanido) residues for *binding* of substrates to the protein by electrostatic attraction, it is possible that substrate charge neutralization by guanidinium ions might be the main effect in the case studied above. This interpretation agrees with the observation that, in the absence of added guanidine, a lowering of the assay pH (presumably protonation of substrate phosphate groups) resulted in considerable stimulation of NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase activities of the soluble enzyme. It further agrees with the fact that (i) as compared to NAD and NADH, the dehydrogenase and transhydrogenase reactions involving NADPH (i.e., the nucleotide with an extra 2'-phosphate group) have their pH optima below neutrality in both the membranous and the soluble enzyme systems, and (ii) in the latter system added guanidine activates NADPH dehydrogenase and NADPH \rightarrow NAD transhydrogenase much more than NADH dehydrogenase and NADH \rightarrow NAD transhydrogenase, especially at pH \geq 6.0. In addition, if we conceive of an enzyme active site accommodating a reduced and an oxidized nucleotide for direct hydride ion transfer (as is the case with the mitochondrial transhydrogenase reactions), then coulombic repulsion of the phosphate anions of the closely located nucleotides might require charge neutralization by appropriate groups on the protein active site. One of these groups might well be an arginyl residue crucial for binding of the 2'-phosphate of NADP or NADPH during transhydrogenation, because in submitochondrial particles the transhydrogenase reactions NADPH \rightarrow NAD and NADH \rightarrow NADP are considerably more sensitive to trypsin and butanedione than NADH and NADPH oxidation and NADH \rightarrow NAD transhydrogenation (2, 20).

As a working hypothesis, we are considering that the dehydrogenase contains two closely related "active" sites: site 1 for dehydrogenation of NADH and NADPH (or reduction of NAD and NADP by reverse electron transfer in submitochondrial particles), and site 2 for binding of a second nucleotide for transhydrogenation (Fig. 6). Because in the dehydrogenation reaction of the particle-bound enzyme the K_m for NADPH is very high ($K_m \approx 550 \mu\text{M}$ versus $45 \mu\text{M}$ for NADH), it is possible that in transhydrogenation site 1 binds NADH or NAD in preference to NADPH or NADP, while site 2 binds the latter nucleotides. In transhydrogenation from NADH to NAD, however, site 1 would bind one nucleotide (possibly NADH) and site 2 the other. Such a scheme would require the trypsin-

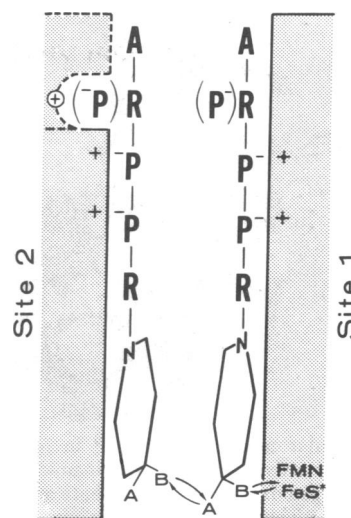


FIG. 6. Proposed arrangement of nicotinamide-adenine dinucleotides at the active sites of NADH dehydrogenase for dehydrogenation (site 1) and transhydrogenation (sites 1 and 2). The essential arginyl residue is shown by a plus sign inside a circle. The dashed lines indicate that the segment of the enzyme containing the arginyl residue mentioned above may be a polypeptide which may or may not be present in the soluble, low-molecular-weight enzyme. For simplicity, the carbamyl groups have been deleted from the nicotinamide rings, and both nucleotides have been shown in reduced form to indicate the stereospecificities of hydrogen abstraction (curved arrows) in dehydrogenation and transhydrogenation. A, R, and P⁻ stand for adenine, ribose, and phosphate, respectively. The P⁻ in parentheses is 2'-phosphate when the nucleotide is NADP or NADPH. The shaded areas represent portions of the enzyme around the active sites. For details see text. S* refers to acid-labile sulfide.

susceptible arginyl residue to be located in site 2, which agrees with the observed results. Thus, in trypsin- or butanedione-treated particles NADH and NADPH dehydrogenation at site 1 would be unaffected. Nor would transhydrogenation from NADH to NAD be inhibited, because neither nucleotide contains a 2'-phosphate to bind to the trypsin/butanedione-susceptible arginyl residue. In membranes, the stereospecificity of hydride ion transfer is 4B for NADH and NADPH oxidation, 4A for NADH \rightarrow NADP transhydrogenation, and 4B for NADPH \rightarrow NAD transhydrogenation (7, 21–23). Assuming as discussed above that in NAD(H) \rightleftharpoons NADP(H) transhydrogenation site 2 would bind NADP(H), then these stereospecificities will be satisfied by arranging the nicotinamide rings as shown in Fig. 6.

Energy-Linked Transhydrogenation. As stated above, reduction of NADP by either NADH or NADPH is an energy-linked process in mitochondria. Studies on the kinetic and thermodynamic features of the reaction NADPH + NAD \rightleftharpoons NADP + NADH have shown that the forward reaction is unaffected (i.e., its initial rate) by the energized state of the membrane and proceeds to an equilibrium close to unity as expected from the redox potentials of the two nucleotides (23). The reverse reaction is slow in nonenergized membranes, but in the presence of an energy supply it is accelerated severalfold and results in NADP reduction far beyond the equilibrium point of the forward nonenergy-linked reaction. Various mechanisms have been proposed to explain the unusual thermodynamics of the energy-linked *versus* nonenergy-linked transhydrogenation (for a review, see 23).

However, an examination of the data of Table 2 suggests that the problem might rest with NADP, because the enzyme can catalyze dehydrogenation and transhydrogenation reactions with reduced and oxidized nucleotides, except when NADP is involved. This nucleotide differs structurally from NAD,

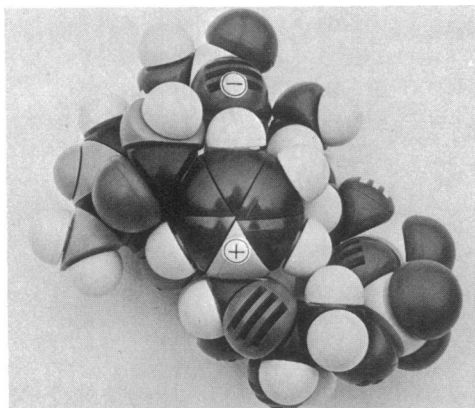


FIG. 7. Molecular model of β -NADP in folded (or stacked) conformation. The positively charged, nicotinamide ring nitrogen and the negatively charged oxygen of 2'-phosphate are marked with (+) and (-) signs, respectively. Note the close proximity of the negatively charged oxygen (or the hydroxyl group) of 2'-phosphate to the C-4 of the nicotinamide ring, which through the ring resonance carries a formal positive charge. The model shown is based on the structure of the stacked conformation of β -NADH proposed by Miles and Urry (25) and Kaplan and Sarma (26).

NADH, and NADPH by having both a negatively charged 2'-phosphate and a positively charged nitrogen in the nicotinamide ring. The folded structure of NADP in solution (Fig. 7) shows that the negatively charged oxygen (or the hydroxyl group) of the 2'-phosphate can be very close to the C-4 of the nicotinamide ring, which carries a formal positive charge. Thus, it is possible that this intramolecular electrostatic stabilization of the folded structure of NADP is chiefly responsible for the fact that NADP is a poor hydride ion acceptor in mitochondrial transhydrogenation. If this reasoning is correct and the extrapolation from the soluble enzyme model to the membrane-bound transhydrogenase is valid, then energy-linked transhydrogenation might mean a change in membrane structure (and/or surface charge) which permits of better interaction of the transhydrogenase active site with NADP. This interpretation agrees with the results of Ernster and coworkers (24) who found that the K_m values of all interacting nucleotides in energy-linked and nonenergy-linked transhydrogenation reactions changed very little except the K_m of NADP, which decreased from 40 μ M under nonenergy-linked conditions to 6.5 μ M under energy-linked conditions. Thus, energized membranes might provide for a special interaction of the transhydrogenase with NADP (possibly involving the guanido group of the essential arginyl residue and the 2'-phosphate of NADP to form enzyme-arginyl . . . 2'-phospho-NAD), which will allow the nucleotide to unfold, be better accommodated at the enzyme active site in relation to NADH or NADPH, and allow its nicotinamide C-4 to become a better hydride ion acceptor than when it is in close proximity of the negatively charged 2'-phosphate. This mechanism can also allow for consumption of stoichiometric amounts of ATP during energy-linked transhydrogenation (23). It also agrees with the significant fact that in the absence of an energy supply, submitochondrial particles can still catalyze NADH \rightarrow NADP transhydrogenation with appreciable rates when the assay pH is lowered to pH \leq 6.0 (27), and the protonated state of the phosphate groups of the nucleotide substrates is favored.

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