CALORIMETRIC INVESTIGATION OF NAD BINDING TO SOME DEHYDROGENASES

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SUMMARY: The thermodynamic parameters for the binding of NAD to some dehydrogenases have been determined calorimetrically at 25° and pH 7.6. Except for liver alcohol dehydrogenase (LADH) the ΔG^{o}, ΔH^{o} and ΔS^{o} values for NAD binding to the dehydrogenases are very similar pointing out a possible structure - thermodynamics correlation. The large deviation observed in the case of LADH would be consistent with the occurrence of a conformational change in this enzyme upon binding NAD.

Recent X-ray crystallographic studies of lactic dehydrogenase (1), liver alcohol dehydrogenase (2), malate dehydrogenase (3) and glyceraldehyde-3-phosphate dehydrogenase (4) have shed some light on the basic structure-function relationships within the family of dehydrogenases. The coenzyme binding domains in all these enzymes exhibit fundamental similarities in their unique folding as well as in their mode of coenzyme binding (5). Some details of the coenzyme interactions with the amino acid residues are also remarkably similar.

It is interesting to see if these structural similarities extend to the energetics of interaction of the coenzyme with the enzymes since the mode of coenzyme binding and the structural details of the coenzyme binding sites are known. In this paper, results are reported from direct calorimetric measurements on the binding of NAD to some dehydrogenases. Interestingly the thermodynamic parameters of binding correlate with the structural similarities in a broad sense, even though some small differences are noted. In addition, our calorimetric

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Abbreviations used:

YADH, yeast alcohol dehydrogenase (EC 1.1.1.1); BHLDH, beef heart lactic dehydrogenase (EC 1.1.1.27); RMLDH, rabbit muscle lactic dehydrogenase (EC 1.1.1.27); m-MDH, pig heart mitochondrial malate dehydrogenase (EC 1.1.1.37); LADH, horse liver alcohol dehydrogenase (EC 1.1.1.1).

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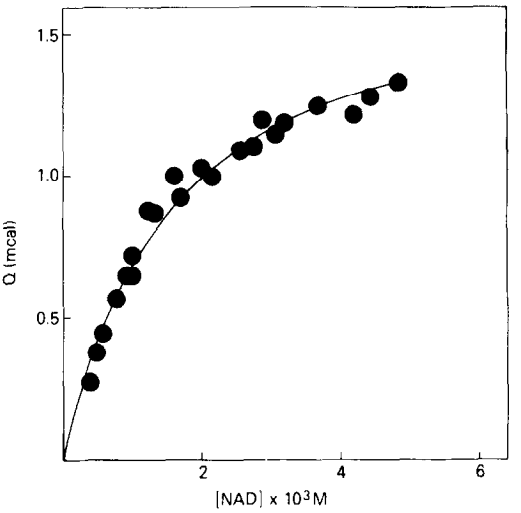


(1) Thermal titration of rabbit muscle lactic dehydrogenase with NAD at 25° in 0.1 M sodium phosphate buffer at pH 7.6. The enzyme concentration was 46.95 X 10^{-6} M in subunit.

measurements suggest the occurrence of a coenzyme-induced conformational change in the case of liver alcohol dehydrogenase (6).

MATERIALS AND METHODS

All the enzymes were purchased from Sigma Chemical Co. with the exception of pig heart mitochondrial malate dehydrogenase, which was obtained from Boehringer. The enzymes, prior to their use, were dialyzed in the buffer exhaustively, treated with activated charcoal and filtered through 0.45 μ Millipore filter disks to remove any bound nucleotides. NAD was a Sigma product (Grade V). The buffer used in all the experiments was 0.1 M in sodium phosphate, pH 7.6 except for LADH-NAD binding in which case 0.1 M imidazole at the same pH was used, for reasons explained later.

Calorimetric measurements were made at 25° using an LKB batch mirco-calorimeter equipped with gold cells. Typically 2 ml of the enzyme solution was mixed with 4 ml of the NAD solution in the sample chamber and 2 ml of buffer was mixed with 4 ml of the NAD solution in the reference chamber. Heats measured ranged from 0.4 to 4.0 mcal. Heats of dilution of the enzymes were measured and found to be negligible at the concentrations ($\sim$ 150 X 10^{-6} M) used.

RESULTS AND DISCUSSION

Figure 1 shows the experimental heat of binding of NAD to RMLDH at 25°C as a function of NAD concentration. The heat evolved in the binding process (0.3 to 1.3 mcal) follows a regular saturation curve. For all the enzymes the data were treated assuming independent and identical binding sites for NAD and that each enzyme subunit binds one NAD molecule. The binding constants, K_b, and

TABLE 1

Thermodynamic parameters for binding of NAD^+ to Dehydrogenases at 25° and pH 7.6

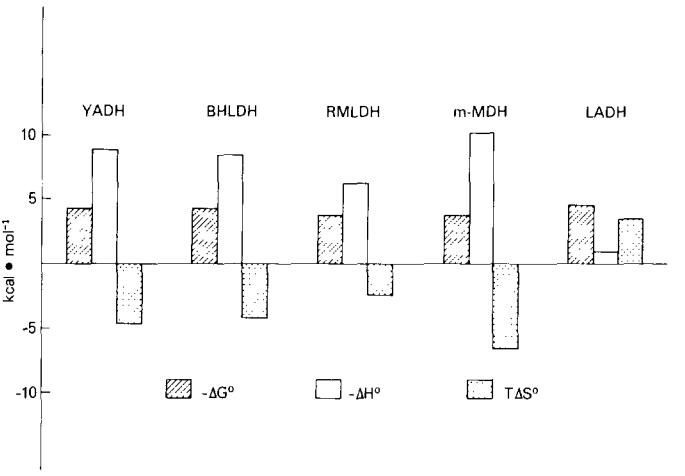
Enzyme	$10^3 K_b$ M^{-1}	$-\Delta G°$	$-\Delta H°$	$-\Delta S°$
		kcal·mol^{-1}		cal·K^{-1}·mol^{-1}
Yeast ADH	1.44	4.3 ± 0.1	8.9 ± 0.4	15.4 ± 1.5
Beef Heart LDH	1.35	4.3 ± 0.1	8.5 ± 0.4	14.2 ± 1.4
Rabbit Muscle LDH	0.65	3.8 ± 0.1	6.3 ± 0.2	8.3 ± 1.0
Pig Heart m-MDH	0.53	3.7 ± 0.1	10.2 ± 0.7	21.7 ± 2.7
Horse Liver ADH	2.32[a]	4.6 ± 0.1[a]	1.0 ± 0.2	-12.1 ± 1.0

[a] Value taken from Ref. 8.

the enthalpies of binding, ΔH, were obtained by least squares fitting of the equation

$$Q = (\Delta H)\cdot V\cdot \left\{ \frac{(E_t + L_t + 1/K_b) - \sqrt{(E_t + L_t + 1/K_b)^2 - 4\,E_t\,L_t}}{2} \right\} \qquad (1)$$

to the experimental data for Q as a function of L_t where Q is the heat evolved, L_t is the total NAD concentration, E_t is the total enzyme concentration (in subunit) and V is the total volume of the calorimetric solution. The thermodynamic parameters obtained for all the enzymes are listed in Table 1 and diagrammatically represented in Figure 2. Before we discuss the magnitudes of the thermodynamic parameters, the use of imidazole buffer in the case of LADH has to be explained. LADH is known to release protons upon binding NAD which is thought to arise from the dissociation of a proton from the zinc-bound water molecule (7). Hence, the observed heat of complexation of NAD with LADH is a composite of the heat of dissociation of proton from the zinc-bound water, the heat of proton abstraction by the buffer and the heat of binding of NAD itself, besides a heat of conformational change that occurs (6) when the coenzyme binds.



(2) Diagrammatic representation of ΔG°, ΔH° and ΔS° of binding of NAD to dehydrogenases at 25° and pH 7.6. The buffer was 0.1 M sodium phosphate in all cases except LADH in which case it was 0.1 M imidazole. For each enzyme the thermodynamic parameters were obtained from the heats of binding data similar to those shown in Figure 1 by the appropriate treatment outlined in the results and discussion section.

These aspects are under extensive investigation in our laboratory. However, in the presence of imidazole, the water coordinated by the zinc is replaced by imidazole and as a result there is no proton release upon NAD binding and the pH dependence of NAD binding to LADH is eliminated (8). Such a behavior of pH-independence of NAD binding is in line with that of other dehydrogenases.

The total spread of the free energies of binding of NAD to the various dehydrogenases, shown in Figure 2 and Table 1, is within 1 kcal/mol indicating an apparent similarity of interactions. However, an examination of the ΔH° and ΔS° values is more revealing since the free energy changes per se do not provide much of an insight into the nature of the interactions. Except for LADH, the enthalpies of binding vary within a range of ±2 kcal/mol which is of the order of the energy of an average hydrogen bond in aqueous solution. The large negative enthalpies are compensated by large negative entropies resulting in smaller negative free energies. The signs and magnitudes of the enthalpies and entropies of binding indicate that, in general, ionic and hydrogen bond interactions prevail over hydrophobic interactions resulting in net negative enthalpies

and entropies when NAD binds to these enzymes at 25°C. In view of the structural similarities of the coenzyme binding domains of these enzymes and the identical mode of binding of NAD, the extension of the similarity to the energetics of interaction of NAD with the enzymes is significant. The small differences in the parameters, of course, reflect the nature of the specific details of the interaction with each enzyme.

The free energy of binding NAD to LADH is not significantly different from that for the other dehydrogenases as shown in Table 1. In contrast to YADH, BHLDH, RMLDH and m-MDH, the binding of NAD to LADH results in a very small exothermic enthalpy, with the bulk of the contribution to the free energy stemming from a large positive entropy. The difference in the entropy of binding NAD to LADH versus other dehydrogenases (+12 cal. K^{-1}-mol^{-1} for LADH and a mean value of -15 cal. K^{-1}-mol^{-1} for the others) is significant and can not be accounted for in terms of differences, if any, in solvation and desolvation accompanying the binding of NAD since, in all cases, the binding of NAD involves the transfer of NAD from the aqueous phase to the supposedly similar coenzyme binding sites. We have also found (9) that the binding of NADH to LADH is totally entropy-driven at 25° while for all the other enzymes it is largely enthalpy-driven-entropy-compensated. While thermodynamics is incapable of describing processes in terms of molecular details this significant difference in the thermodynamic behavior suggests a large, conformational change taking place in LADH during the binding of NAD and NADH. A similar conclusion of a conformational change in octopine dehydrogenase has been reached to account for the athermic binding of NAD and NADH to that enzyme (10). The occurrence of conformational change in LADH upon binding NAD or NADH has been indicated previously (6,11). Our thermodynamic study lends further support to this hypothesis. The abnormality of the thermodynamics of binding NAD to LADH with respect to the other dehydrogenases vanishes (9) in the binding of ADP-ribose, which lacks the nicotinamide moiety of NAD, suggesting that the nicotinamide end of the coenzyme is responsible in inducing the

conformational change in LADH.

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