Thermodynamics of Binding of Oxidized and Reduced Nicotinamide Adenine Dinucleotides, Adenosine-5′-diphosphoribose, and 5′-Iodosalicylate to Dehydrogenases†

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ABSTRACT: The thermodynamic parameters for the binding of NADH, ADP-ribose, and 5-iodosalicylate to some selected dehydrogenases measured calorimetrically at pH 7.6 and 25 °C are reported here. This study in conjunction with our previous work on NAD^+ binding (Subramanian S., & Ross, P. D. (1977) *Biochem. Biophys. Res. Commun. 78,* 461) permits a comparison of the binding characteristics of coenzymes and inhibitors in the so-called nucleotide-binding domain of the dehydrogenases as proposed from the high resolution x-ray diffraction studies. The thermodynamic parameters for the binding of NAD^+ and NADH to the enzymes are broadly similar. This reflects a possible extension of the structure–function correlation to the thermodynamics of binding coenzymes and coenzyme fragments as well. This relationship may be a consequence of the similarity of the super-secondary structure of the coenzyme-binding domains of these dehydrogenases and the presence of the conserved amino acid residues in the coenzyme-binding domains. In the case of horse liver alcohol dehydrogenase, the thermodynamic parameters for the binding of NAD^+ and NADH differ dramatically from those obtained with other dehydrogenases, which would be consistent with the occurrence of a conformational change in horse liver alcohol dehydrogenase when NAD^+ or NADH binds to this enzyme. The thermodynamic parameters for the binding of ADP-ribose are similar for all the enzymes including liver alcohol dehydrogenase thus indicating the requirement of the nicotinamide moiety for eliciting a conformational change in horse liver alcohol dehydrogenase. The binding of the inhibitor, 5-iodosalicylate, suggests a possible diversity in the composition of the hydrophobic pocket of the coenzyme binding site.

In recent years the three-dimensional structures of a number of NAD^+-dependent dehydrogenases have been described to high resolution by x-ray crystallographers and a unified concept has emerged (Rossman et al., 1975) regarding the architectural feature called the NAD^+-binding domain. The nucleotide binding domains, which have the common function of binding coenzyme molecules, exhibit fundamental similarities in their structure and also in their mode of coenzyme binding, while the "catalytic" domains, having different functions, have very different structures. The structural similarity of the nucleotide binding domains, which consist of six parallel strands of pleated sheet (βA to βF) and four helices (αB, αC, αE, and α1F) as described (Ohlsson et al., 1974) for LDH,[1] GAPDH, and liver ADH, suggests that the coenzyme domains were present in precellular evolution and have been conserved (Rossmann et al., 1974). In addition to the conservation of the three-dimensional structure of the domain, it has also been found that certain amino acid residues are conserved at the NAD^+ binding subsites of the enzymes. The coenzyme binds in the central region at the carboxyl ends of the β pleated sheets in an open conformation. Such a gross homology delineated in these enzymes by x-ray crystallography naturally led to work in probing the NAD^+ binding domain in solution. Comparative studies have been made on several dehydrogenases using circular polarization of fluorescence (Schlessinger et al., 1975), affinity chromatography (Thompson et al., 1975), and equilibrium and kinetic studies (Suhadolnik et al., 1977). Recently we reported (Subramanian & Ross, 1977) the thermodynamic parameters for the binding of NAD^+ to some dehydrogenases which suggested a structure–thermodynamics correlation. In this study we report the thermodynamic parameters for the binding of NADH, ADP-ribose and a competitive inhibitor 5-iodosalicylate to several dehydrogenases at 25 °C in an attempt to further characterize the similarities and differences of the NAD^+-binding domains. While thermodynamics is incapable of describing microscopic processes in detail, comparative studies can still be of use in understanding the homology described in the case of these dehydrogenases.

Experimental Section

Materials. All the enzymes were purchased from Sigma Chemical Co. with the exception of pig heart mitochondrial malate dehydrogenase, which was obtained from Boehringer Mannheim. The enzymes, prior to use, were (dissolved and) dialyzed in the buffer exhaustively, treated with activated charcoal, and filtered through 0.45-μm Millipore filter disks to remove any bound nucleotides. Ligand concentrations were determined using the following absorption coefficients: NAD^+, 18.0×10^3 M^{-1} cm^{-1} at 260 nm; NADH, 6.22×10^3 M^{-1} cm^{-1} at 340 nm; ADP-ribose, 15.4×10^3 M^{-1} cm^{-1} at 259 nm; 5-iodosalicylate, 3.1×10^3 M^{-1} cm^{-1} at 312 nm. Enzyme concentrations were determined using the following absorption coefficients (cm^2 mg^{-1}) at 280 nm: liver ADH, 0.455; yeast ADH, 1.26; m-MDH, 0.28; rabbit muscle and beef heart LDHs, 1.4; and GDH, 0.93. The molecular weights used for the subunits of the enzymes were: liver ADH, 40 000; yeast ADH, 36 500; m-MDH, 34 000; rabbit muscle LDH and beef

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[1] Abbreviations used: ADH, alcohol dehydrogenase (EC 1.1.1.1); LDH, lactate dehydrogenase (EC 1.1.1.27); m-MDH, mitochondrial malate dehydrogenase (EC 1.1.1.37); GDH, glutamate dehydrogenase (EC 1.4.1.3); GAPDH, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ADP-ribose, adenosine-5′-diphosphoribose.

TABLE I: Thermodynamic Parameters for Binding of NADH (A), ADP-ribose (B), and 5-Iodosalicylate (C) to Dehydrogenases at 25 °C and pH 7.6.

Enzyme	K_b (M^{-1})	$-\Delta G°$ (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$-\Delta S°$ (cal K^{-1} mol^{-1})
A. Yeast ADH	0.04×10^6	6.3 ± 0.1	9.5 ± 0.2	10.7 ± 1.0
Beef heart LDH	2.85×10^6 [a]	8.8	9.7 ± 0.2	3.0 ± 1.0
Rabbit muscle LDH	0.28×10^6 [a]	7.4	6.9 ± 0.1	−1.7 ± 0.7
Pig heart m-MDH	1.00×10^6 [a]	8.2	12.1 ± 0.3	13.1 ± 1.3
Horse liver ADH	2.85×10^6 [a]	8.8	0 ± 0.4	−29.5 ± 1.7
B. Yeast ADH	1.66×10^3	4.4 ± 0.1	11.2 ± 0.4	22.8 ± 1.7
Beef heart LDH	5.15×10^3	5.0 ± 0.1	8.5 ± 0.2	11.7 ± 1.0
Rabbit muscle LDH	2.24×10^3	4.6 ± 0.1	7.6 ± 0.2	10.0 ± 1.0
Pig heart m-MDH	8.55×10^3	5.4 ± 0.1	9.2 ± 0.3	12.8 ± 1.3
Horse liver ADH	8.69×10^3	5.4 ± 0.1	6.0 ± 0.2	2.2 ± 1.0
C. Yeast ADH	0.79×10^3	3.9 ± 0.1	19.2 ± 2.0	51.3 ± 7.0
Beef heart LDH	2.98×10^3	4.7 ± 0.1	8.0 ± 0.8	11.1 ± 3.0
Rabbit muscle LDH	0.38×10^3	3.5 ± 0.1	21.7 ± 2.0	61.0 ± 7.0
Pig heart m-MDH	2.97×10^3	4.7 ± 0.1	12.1 ± 1.0	24.8 ± 3.7
Horse liver ADH	3.25×10^3	4.8 ± 0.1	12.5 ± 0.9	25.8 ± 3.3

[a] Values taken from Dalziel (1975).

heart LDH, 35 500; bovine liver GDH, 56 100. The ratio of the absorption of the enzymes at 280 nm to that at 260 nm (after charcoal filtration) was in the range 1.9 to 2.0 except for liver ADH in which case it ranged from 1.2 to 1.3. Enzymatic assays were routinely performed prior to use and found to be within the limits specified by the manufacturers. The buffer used in all the experiments was 0.1 M in sodium phosphate, pH 7.6. NAD^+, NADH, and ADP-ribose were all Sigma products of the highest purity available. They were used as such. 5-Iodosalicylic acid (technical grade) was obtained from Aldrich and recrystallized several times from 25% (v/v) acetone-water.

Methods. Calorimetric measurements were made at 25 °C and pH 7.6 using a LKB batch microcalorimeter equipped with gold cells. In a typical experiment 2 mL of the enzyme solution ($\sim 150 \times 10^{-6}$ M) was mixed with 4 mL of the ligand solution in the sample chamber and 2 mL of buffer was mixed with 4 mL of the ligand solution in the reference chamber. The heats of dilution of the ligands were usually less than 1 mcal and were eliminated by the above procedure. Heats of dilution of the enzymes were measured separately and the measured heats were accordingly corrected. In most cases the heats of dilution of the enzymes were found to be negligible at the concentrations used. The heats of binding measured were usually in the range 0–3 mcal.

The heat outputs from the calorimeter were appropriately amplified and recorded. The thermoelectric elements of the calorimeter were connected to a Peltier-Seebeck equilibrator designed by F. W. Noble of the Laboratory of Technical Development, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Md. The feedback control voltage was recorded. The use of this device sped up the thermal response of the calorimeter approximately threefold, thereby shortening both the calorimetric experiment and the equilibration time of the instrument between successive heat measurements.

Electrical calibration of the calorimeter was checked by measuring the heat of dilution of sucrose (Gucker et al., 1939). The binding of each ligand to all the enzymes was studied at several (between 10 and 30) concentrations for each ligand in order to obtain typical saturation curves and to extract the binding constant and enthalpy of binding. In general the saturation curves covered 10–90% saturation approximately, and in no case was it less than 50%. Saturation curves obtained were similar to the one shown in the earlier study (Subramanian & Ross, 1977) and showed no sigmoidal behavior. For NADH binding to some enzymes where it is known that the binding is very tight, only saturation values of heats of binding (several determinations) were determined and the dissociation constants were obtained from the literature as indicated in Table I.

Results

For all the ligand binding reactions, the heat data were treated assuming independent and identical binding sites for NAD^+, NADH, ADP-ribose, and 5-iodosalicylate and a stoichiometry of 1 mol of ligand per mol of enzyme subunit. The binding constants, K_b, and the enthalpies of binding, ΔH, were obtained by nonlinear least-squares fitting of the equation

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

to the experimental data (usually 10 to 30 points) for Q as a function of L_t where Q is the heat evolved, L_t is the total ligand concentration, E_t is the total enzyme concentration (in subunits), and V is the total volume of the calorimetric solution. The thermodynamic parameters obtained for all the binding reactions studied are listed in Table I, A–C. The uncertainties listed reflect the standard errors of the parameters, K_b and ΔH, resulting from the fit of the equation to the experimental data. It should be remembered that the parameters represent 1 mol of ligand complexed with 1 mol of enzyme subunit. In order to make a comparative evaluation visually perceptible the $\Delta G°$, $\Delta H°$, and $\Delta S°$ are also pictorially represented in Figure 1.

In general, for the binding of each ligand to all the dehydrogenases, the free energies of binding are very similar; the variation in the free energies is usually of the order of 1 kcal mol^{-1} for any one ligand. In all cases the binding affinity of the ligands decreased in the order NADH > ADP-ribose > NAD^+. The enthalpies of binding of these three ligands vary within ±2 kcal mol^{-1} for the enzymes (except liver ADH), the magnitudes following the order m-MDH > yeast ADH ≈ beef heart LDH > rabbit muscle LDH. The binding affinities of

5-iodosalicylate to all the enzymes are comparable to those of NAD^+. For the binding of each ligand to all the enzymes the enthalpies are large negative values and so are the entropies, typical of enthalpy–entropy compensation. The only apparent exception to this behavior is liver ADH forming complexes with NAD^+ and NADH. In these two cases the enthalpies of binding were not very different from zero and as can be seen from Table I of our previous study (Subramanian & Ross, 1977) and Table IA of this work, the near total contribution to the free energy of binding is entropic. The thermodynamic parameters for the binding of ADP-ribose and 5-iodosalicylate to liver ADH are similar to those for the other dehydrogenases. By far, the largest negative enthalpies of binding are obtained for the binding of 5-iodosalicylate and with this ligand the intragroup variations in the thermodynamic parameters are also pronounced (Table IC and Figure 1C). In addition to the binding of NAD^+ to the enzymes reported by us earlier (Subramanian & Ross, 1977), we also studied the binding of NAD^+ to bovine liver GDH and the thermodynamic parameters at 25 °C are as follows: $\Delta G° = -3.7$ kcal mol^{-1}; $\Delta H° = -15 \pm 0.5$ kcal mol^{-1}; and $\Delta S° = -38.0 \pm 2.0$ cal K^{-1} mol^{-1}. These values were calculated from the heat data assuming that GDH binds one NAD^+ molecule per enzyme subunit (Dalziel & Egan, 1972).

Discussion

NAD^+ and NADH. In terms of the free energies of binding, the $\Delta G°$ values for NADH binding to all the dehydrogenases studied are 2 to 4 kcal mol^{-1} more negative than the values for NAD^+ binding to the same enzymes. The tighter binding of NADH (over NAD^+) to the dehydrogenases is generally understood to result from the absence of the positive charge on the nitrogen in the nicotinamide ring of NADH; the different geometrical configuration of the reduced nicotinamide does not seem to be of any great significance. The hydrophobicity of the nicotinamide binding sites in the enzymes (Rossmann et al., 1975) would be expected to favor the binding of the neutral reduced nicotinamide moiety of the coenzyme. The free energies of binding demonstrate a superficial similarity, the nature of which can be understood better by knowing the enthalpic and entropic components of the free energies. The complete thermodynamic parameters for the binding of NADH to the enzymes listed in Table IA are reported for the first time by us and hence no comparison with literature values is possible. Calorimetric studies of the binding of NAD^+ to pig heart muscle LDH (Schmid et al., 1976) and NADH to pig skeletal muscle LDH (Hinz & Jaenicke, 1975) have been reported and the ΔH values of -6.1 kcal mol^{-1} and -7.5 kcal mol^{-1}, respectively, at 25 °C are in close agreement with the values we have obtained for the rabbit muscle LDH and beef heart LDH. The minor interspecific variations in the enzymes may be responsible for the small differences. The ΔH of binding of NADH to rabbit muscle LDH has been reported (Czerlinski & Schreck, 1964) to be -8.1 kcal mol^{-1} from temperature-jump experiments and our value, -6.9 kcal mol^{-1}, is not very different from this.

Except for horse liver ADH, the binding of NAD^+ to the enzymes (Subramanian & Ross, 1977) is accompanied by fairly large negative enthalpies partly compensated by negative entropies of binding. Of the enzymes, yeast ADH, beef heart LDH, rabbit muscle LDH, pig heart m-MDH, and bovine liver GDH, GDH uses either NAD^+ or $NADP^+$ as the coenzyme (Smith et al., 1975). The enthalpy of binding of NAD^+ to GDH is -15.0 kcal mol^{-1} and is much greater than the other $\Delta H°$ values for NAD^+ binding to the other enzymes and may specifically reflect the characteristics of the ambivalent nature

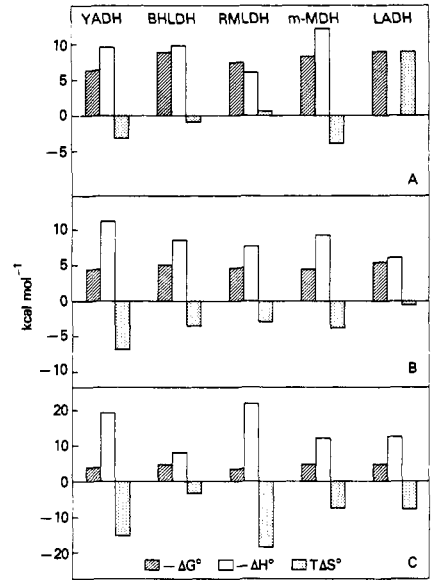

FIGURE 1: Diagrammatic representation of $\Delta G°$, $\Delta H°$, and $\Delta S°$ of binding NADH (A), ADP-ribose (B), and 5-iodosalicylate (C) to dehydrogenases at 25 °C and pH 7.6. The abbreviations used in this figure are: YADH, yeast alcohol dehydrogenase; BHLDH, beef heart lactic dehydrogenase; RMLDH, rabbit muscle lactic dehydrogenase; m-MDH, pig heart mitochondrial malate dehydrogenase; LADH, horse liver alcohol dehydrogenase.

of the coenzyme binding site in GDH. Excluding GDH and horse liver ADH, the range for $\Delta H°$ and $\Delta S°$ parameters of binding to the other four enzymes is significantly narrow either for NAD^+ or NADH. The enthalpies of binding NAD^+ to the NAD^+-specific dehydrogenases (except for horse liver ADH, which we shall discuss separately) vary within ± 2 kcal mol^{-1} which is about the energy of an average hydrogen bond in aqueous solution. The entropy changes also correspond to this. The large negative magnitudes of the ΔH and ΔS values indicate, generally, that ionic and hydrogen bond interactions prevail over hydrophobic interactions at 25 °C. Similarly for the binding of NADH to these four enzymes the enthalpies again vary within a range of ± 2 kcal mol^{-1}. The magnitudes of the enthalpies are very similar for NAD^+ and NADH binding to these enzymes. The ΔS values are less negative for NADH binding and the tighter binding of NADH may arise from this source.

Several generalities have been noted in the NAD^+-binding domain of the dehydrogenases. In a comparative study of the LDH, s-MDH, GAPDH, and liver ADH (Rossmann et al., 1975), it has been pointed out that: (a) the coenzyme binds to these enzymes in an open conformation; (b) the adenine ring binds in a pocket lined with hydrophobic residues; (c) the foldings of the coenzyme binding domains are similar; and (d) some amino acid residues are conserved at supposedly crucial regions in the nucleotide-binding domain. In view of all these similarities one would expect that the energetics of interaction also follow some particular pattern. The features outlined above might broadly govern the overall energetics of the binding of ligands to the enzyme. The small differences noted in the ΔH–ΔS values may reflect some specific details of the coenzyme–enzyme interaction. These may include the positioning of a particular charged residue on the enzyme at an optimum distance from a charged group on the coenzyme or the formation (or the absence thereof) of a particular hydrogen bond. Considering these factors it is quite significant that the extension of the structure–function correlation is manifested in the realm of energetics too. If a large number of NAD^+-

enzymes are studied, it is possible that the thermodynamic parameters may vary over a large range. The thermodynamic parameters for NAD^+ binding to GDH reported here ($\Delta H^\circ = -15.0$ kcal mol^{-1}; $\Delta S^\circ = -38$ cal K^{-1} mol^{-1}) and to yeast GAPDH ($\Delta H^\circ = -12.4$ kcal mol^{-1}; $\Delta S^\circ = -16.8$ cal K^{-1} mol^{-1}) reported in an earlier study (Velick et al., 1917) are two cases wherein the variation from the rest of the dehydrogenases becomes obvious. Since it has been recognized lately that the so-called dinucleotide fold is more universal than originally thought, no strong case can be made either for structure-function correlation or the extension of the structure-function correlation to include energetics. However, within the framework of the dehydrogenases that have been studied extensively by x-ray crystallography, the striking pattern of the thermodynamic parameters is remarkable and reflects the structural similarities of the domains.

Horse Liver ADH. The free energy of binding NAD^+ to horse liver ADH is not significantly different from that for the other dehydrogenases studied (Subramanian & Ross, 1977). The same is true for NADH too. But in contrast to the other enzymes, horse liver ADH binds NAD^+ with a $\Delta H^\circ = -1.0$ kcal mol^{-1} and NADH with a $\Delta H^\circ = 0$. In both cases the binding is predominantly entropy-driven at 25 °C. It is surprising that the horse liver ADH is so different (in binding the coenzyme) from not only LDH and MDH but also yeast ADH. The ΔH° and ΔS° values for NAD^+ and NADH binding to yeast ADH are in line with those for NAD^+ and NADH binding to LDH and MDH. Except for the rabbit muscle LDH-NADH complex, in all other cases the entropies of binding are largely negative. Only for the binding of NAD^+ and NADH to horse liver ADH, large positive entropies are obtained. This large difference in ΔS° values between horse liver ADH-coenzyme complex and the complexes formed with the other enzymes cannot be accounted for in terms of differences, if any, in solvation and desolvation of the coenzyme binding site of the enzyme since, in all cases, the binding of the coenzyme involves the transfer of NAD(H) from the aqueous phase to the supposedly similar coenzyme binding sites. This large positive entropy change on binding the coenzyme is unusual but not without precedent. In the case of octopine dehydrogenase (Luisi et al., 1975) it was found that the binding (constant) of NAD^+ or NADH to the enzyme was temperature independent ($\Delta H = 0$) from 2.5 to 40 °C and hence the binding was totally entropy driven. It has been suggested that the binding of NAD(H) to horse liver ADH is accompanied by conformational change in the enzyme (Branden et al., 1975; Czeisler & Hollis, 1973). In this respect the positive entropy changes observed on binding NAD(H) to the horse liver ADH would be compatible with the occurrence of a conformational change. Hollis (1967) studied the coenzyme proton resonances of the coenzymes when bound to yeast ADH and horse liver ADH and concluded that both the adenine and nicotinamide moieties were bound firmly to the horse liver ADH, whereas only the adenine moiety was preferentially bound to yeast ADH while the nicotinamide moiety was relatively free. The binding constants of the two coenzymes to the two enzymes also tend to support such a conclusion. The big difference in the ΔH° and ΔS° parameters of binding NAD(H) between yeast ADH and horse liver ADH would also be consistent with a conformational change in horse liver ADH but not in yeast ADH upon binding the coenzyme. This conclusion is further strengthened by the results for ADP-ribose binding to these enzymes detailed below.

ADP-Ribose. The binding of ADP-ribose to the enzymes shown in Table IB is weak but stronger than that of NAD^+ in most cases. The ΔG° values are 1-2 kcal mol^{-1} more negative than those for NAD^+ binding but 2-3 kcal mol^{-1} less negative than those for NADH binding. ADP-ribose lacks only the nicotinamide moiety from NADH, and so the free energies of binding ADP-ribose to the enzymes indicate that, while the unfavorable interaction of the positively charged nicotinamide in NAD^+ has been eliminated, the neutral reduced nicotinamide ring can favorably contribute to the free energy of binding as seen for NADH binding. The enthalpies and entropies of binding ADP-ribose to beef heart LDH, rabbit muscle LDH, pig heart m-MDH, and horse liver ADH are all similar. The enthalpies vary within a range of ± 1.6 kcal mol^{-1} and the entropies are all negative. Once again the ΔH°-ΔS° values represent a manifestation of the similarity of the nucleotide domain in these enzymes in terms of the interaction of segments of the coenzyme with amino acid residues at the coenzyme-binding site. Two further important points are obvious from looking at Table IB and Figure 1B: (1) the binding of ADP-ribose to horse liver ADH is enthalpically driven (unlike that of NAD(H)) and the magnitude of the ΔH° and ΔS° values is similar to that for the other dehydrogenases, i.e., the anomalous nature of horse liver ADH noticed upon coenzyme binding vanishes. (2) The thermodynamic parameters for the binding of ADP-ribose to yeast ADH deviate slightly from the values for other enzymes. These two features constitute a reversal of the trend found for the NAD(H) binding. Taken in conjunction with the results of NAD(H) binding, the thermodynamic parameters of binding ADP-ribose to horse liver ADH indicate that the presence of the nicotinamide moiety in the reduced or oxidized coenzyme is responsible for turning an enthalpy-driven binding (ADP-ribose) into an entropy-driven[2] binding (NAD(H) binding). Shore et al. (1977) studied the temperature dependence of the equilibrium binding of NAD^+ and ADP-ribose and reached a similar conclusion. It is very likely that the change-over is the result of a conformational change of the enzyme induced by the nicotinamide moiety of the coenzyme. The process can be explained by the following equations:

$$E + L \overset{\Delta H_1}{\rightleftharpoons} EL \overset{\Delta H_2}{\rightleftharpoons} (EL)' \tag{2}$$

$$\Delta H_1 < 0; \Delta H_2 > 0 \tag{3}$$

where E is the enzyme and L the ligand. With ADP-ribose the process may stop at the formation of EL, which may be characterized by an exothermic process. With NAD(H), the ADP-ribose segment may still be responsible for the formation of EL (exothermic) but the nicotinamide segment may induce (upon binding) an isomerization of the EL complex to EL (endothermic). If the exothermic and endothermic heats are of almost equal magnitude, the conditions for an entropically driven process (as in the binding of NAD(H)) are fulfilled.

The deviation in the ΔH° and ΔS° values for the binding of ADP-ribose to yeast ADH as compared with the other enzymes including horse liver ADH is not easy to explain. The difference could reflect the differences in the hydrophobic nature of the adenine binding site in the yeast ADH and the other enzymes. This is illustrated to a certain extent in the binding of 5-iodosalicylate to these dehydrogenases.

5-Iodosalicylate. The binding of 5-iodosalicylate to horse liver ADH has been studied (Einarsson et al., 1974) by fluo-

[2] It must be emphasized here that the terms "enthalpy-driven" and "entropy-driven" are of limited utility and if the heat capacities of binding are large, an "enthalpy-driven" reaction can become an "entropy-driven" reaction and vice versa over a moderate range of temperature (cf. Velick et al., 1971). We have used the terms here in the limited sense as they apply to the binding of NAD(H) and ADP-ribose to horse liver ADH at 25 °C.

rescence and x-ray crystallographic methods and it has been shown that this molecule binds in the hydrophobic pocket in an almost identical mode corresponding to the binding of the adenine part of the coenzyme molecule. This pocket has been recognized in all the dehydrogenases as a general-purpose hydrophobic cavity capable of binding aromatic molecules usually with a negatively charged group attached to the ring system. In this context we probed this hydrophobic pocket by studying the thermodynamics of binding of 5-iodosalicylate to the enzymes listed in Table I. On the basis of the similarity of the supersecondary structure and the hydrophobic patch, one would expect to see a distinct pattern in the binding parameters for the interaction of 5-iodosalicylate with these dehydrogenases. As for the free energies of binding, they are all very similar but they do not reveal much information. The $\Delta H°$ and $\Delta S°$ values are by far the most widely varying of all the ligands we have studied. Only pig heart m-MDH and horse liver ADH are identical in the thermodynamic parameters and the beef heart LDH is closely similar to these two enzymes. Yeast ADH and rabbit muscle LDH give similar but large negative enthalpies and entropies of binding. The large negative enthalpies and entropies of binding are not unusual in view of the polarizability of iodine atom and the nature of the hydrophobic pocket. The binding of iodide ion to serum albumin has been shown (Lovrien & Sturtevant, 1971) to have an enthalpy of binding of -17.4 kcal mol^{-1} and an entropy of binding of -42 cal K^{-1} mol^{-1}. Such large negative values are characteristic of electron-dense atoms and molecules binding to proteins. The widely varying $\Delta H°$ and $\Delta S°$ values obtained for the binding of 5-iodosalicylate to these enzymes are probably characteristic of the particular hydrophobic pocket in each enzyme. While it is true that the pockets are all lined up with hydrophobic amino acid residues, their compositions may differ and the strength of van der Waals interactions of the hydrophobic ligand with the residues will depend upon the nature and number of such residues.

In conclusion, the thermodynamic parameters for the binding of coenzymes and ADP-ribose (measured under identical conditions) to the dehydrogenases are broadly similar following the features of the super-secondary structure of the NAD$^+$ domain. Small but significant differences are noted. These, most likely, reflect the specific details of the individual interactions. Horse liver ADH is markedly different from the others with respect to the thermodynamic parameters for the binding of coenzymes which is in accord with the suggestion that horse liver ADH undergoes a conformational change upon binding the coenzyme. The binding of the inhibitor, 5-iodosalicylate, suggests a possible diversity in the composition of the hydrophobic pocket while the general environment of the pocket could still be described as largely nonpolar.

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