STUDIES ON AN ENERGY STRUCTURE–FUNCTION RELATIONSHIP OF DEHYDROGENASES

I. Calorimetric investigations on the interaction of coenzyme fragments with horse liver alcohol dehydrogenase

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1. Introduction

The extensive characterization of the primary and tertiary structures of dehydrogenases [1–6] has raised discussions on possible correlations between obvious similarities in the three dimensional structure and the similar biological function of this class of enzymes. Therefore it appears to be an intriguing problem to see whether this structure–function relationship can be rationalized quantitatively by determining the characteristic thermodynamic reaction parameters for the binding of the coenzyme and coenzyme fragments to typical dehydrogenases. The studies on the interaction with the fragments are intended to sort out the energetic contributions of the various groups of the coenzyme involved in the interaction with the enzyme as detected by the X-ray investigations. Liver alcohol dehydrogenase and lactate dehydrogenase have been investigated since both enzymes exhibit typical three dimensional building principles of dehydrogenases although lactate dehydrogenase is a tetramer, while liver alcohol dehydrogenase is a dimer. The alcohol dehydrogenase has a reaction mechanism different from that of LDH due to the presence of a zinc atom in the catalytic center. The results obtained with liver alcohol dehydrogenase will be presented in this communication and discussed in connection with the studies on lactate dehydrogenase in the accompanying paper.

2. Materials and methods

Horse liver alcohol dehydrogenase (EC 1.1.1.1.), pig skeletal muscle lactate dehydrogenase (EC 1.1.1.27.), NADH, NAD^+ (both grade I), ADP-ribose, ADP and adenosine were purchased from Boehringer, Mannheim. The alcohol dehydrogenase solution was prepared by dialysing under nitrogen a sample of the crystal suspension at 4°C against 1000 vol. potassium phosphate buffer, pH 7.0, ionic strength 0.1 M. The buffer solution was exchanged three times. The resulting enzyme solutions showed spec. act. 5.0–5.7 U/mg, using the initial slope of the assay curve. The enzyme activity was determined in pyrophosphate/glycine buffer, pH 8.7, 22°C according to [7]. The A_{280}/A_{260} was 1.40–1.44. Enzyme concentration was based on an extinction coefficient E_{280} 0.455 $cm^2 . mg^{-1}$ [8] and mol. wt 40 000/subunit. Coenzymes and coenzyme fragments were dissolved in the last dialysate. Their concentrations were determined spectrophotometrically using the following extinction coefficients:

NADH: $6.23 \cdot 10^3\ M^{-1}\ cm^{-1}$ at 340 nm

NAD^+: $18.0 \cdot 10^3\ M^{-1}\ cm^{-1}$ at 260 nm

ADP-ribose: $15.3 \cdot 10^3\ M^{-1}\ cm^{-1}$ at 260 nm

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ADP: $15.4 \cdot 10^3$ M^{-1} cm^{-1} at 260 nm

AMP: $15.3 \cdot 10^3$ M^{-1} cm^{-1} at 260 nm

Adenosine: $14.9 \cdot 10^3$ M^{-1} cm^{-1} at 260 nm [9]

The equilibrium constant of the ADH–NADH complex was derived from fluorimetric measurements, employing the increase of the coenzyme fluorescence at 410 nm (excitation at 330 nm) when the coenzyme binds [10]. All other equilibrium constants were determined fluorimetrically, using the competitive displacement of NADH in the presence of varying concentrations of NAD^+ or the respective coenzyme fragments. The calorimetric measurements followed procedures in [11].

3. Results

The Gibbs free energy of binding, ΔG_B, the enthalpy of binding, ΔH_B, the heat capacity changes, Δc_{pB}, determined by direct calorimetric measurements and the resulting entropy changes on binary complex formation have been summarized in table 1.

A comparison of the thermodynamic parameters for binary complex formation between alcohol dehydrogenase and the various coenzyme fragments shows that the Gibbs free energy values of AMP, ADP and ADP-ribose binding at 25°C are the result of a compensation between comparable contributions of reaction enthalpies and entropies. Association of NAD^+ or NADH appears to be predominantly entropy controlled in the physiological temperature range, even if one takes the strong temperature dependence of the ΔH values into consideration. A similar phenomenon has recently been reported [13] for NAD^+ binding to ADH at 25°C in the presence of imidazole. The largest heat capacity decrease occurs with association of NADH, the next largest decrease in heat capacity is associated with binding of AMP, whereas binding of ADP, ADP-ribose or NAD^+ results in pronouncedly smaller heat capacity changes. Judging from the heat capacity changes associated with the binding of the coenzyme fragments, the introduction of the nicotinamide moiety, as long as it is positively charged, does not seem to exert any particular influence. However, the enthalpy and entropy values are markedly altered. It is worth mentioning that the slightly less negative ΔG_B value for ADP binding as compared to that for AMP binding is paralelled by a minutely less negative ΔH_B and more negative ΔS_B value, but by a pronouncedly less negative Δc_{pB}. The binding enthalpy of ADP-ribose is smaller than the corresponding ΔH values for AMP and ADP association. This result suggests, that there is no additional energetic contribution of the ribose moiety to the binding enthalpy when compared to the ΔH values for AMP and ADP binding. Thus, the assessment of the importance of the sugar for potential hydrogen bond formation cannot be based on enthalpic grounds.

Table 1
Apparent thermodynamic parameters for binary complex formation between horse liver alcohol dehydrogenase and various coenzyme fragments in potassium phosphate buffer, 0.1 M ionic strength, pH 7.0, 25°C

Ligand	$-\Delta G_B^\circ$ (kJ/mol)	$-\Delta H_B^\circ$ (kJ/mol)	$-\Delta S_B^\circ$ (J/K·mol)	$-\Delta c_{pB}$ (J/K·mol)
AMP	23.39 ± 0.58	42.7 ± 3.3	64.8 ± 13	1234 ± 134
ADP	20.38 ± 0.29	40.2 ± 2.5	66.5 ± 9.2	556 ± 96
ADP-ribose	25.48 ± 0.63	36.8 ± 1.3	38.1 ± 6.3	661 ± 54
NAD^+	21.55 ± 0.17	3.6 ± 0.9[a]	−60.2 ± 4	452 ± 40[a]
NADH	37.11 ± 0.63	−2.5 ± 3.3	−116 ± 13	1695 ± 126

[a] From [12]

All values refer to 1 mol binding site and are reported with standard errors. The error limits of the ΔS_B° values pertain to the situation when the errors of ΔG_B° and ΔH_B° go into opposite directions

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