STUDIES ON AN ENERGY STRUCTURE–FUNCTION RELATIONSHIP OF DEHYDROGENASES

II. Calorimetric investigations on the interaction of coenzyme fragments with pig skeletal muscle lactate dehydrogenase

H.-J. HINZ, G. STEININGER, F. SCHMID and R. JAENICKE

Institut für Biophysik und Physikalische Biochemie, FBB, Universität Regensburg, Universitätsstraße 31 D-8400, Regensburg, FRG

Received 27 December 1977

1. Introduction

The considerable differences in some physical chemical parameters of lactate dehydrogenases (turnover numbers, thermodynamic quantities [1–4], isoelectric points, and inhibition characteristics) have been rationalized in terms of a small number of amino acid exchanges in the vicinity of the active center [5]. The molecular origin of the similarities of lactate dehydrogenase isozymes and other dehydrogenases provides only a basis for a qualitative enumeration of possible differences in the interacting groups. Conclusions on the energetics of protein–ligand interaction drawn from sequence or structural data always require substantiation by direct energy determinations. Such energy parameters were furnished by calorimetric studies and measurements of the binding constants for binary complex formation between pig skeletal muscle lactate dehydrogenase and various coenzyme fragments.

2. Materials and methods

Preparation of the lactate dehydrogenase solutions and the calorimetric and fluorimetric measurements followed procedures in [1–4]. The enzyme showed spec. act. 600–670 units/mg in the standard test (Biochemica Information, Boehringer). Enzyme concentrations were determined using an extinction coefficient, $E_{280} = 1.4\ cm^2\ mg^{-1}$. Coenzymes and coenzyme fragments were dissolved in the last dialysate (0.2 M potassium phosphate buffer, pH 7.0). Their concentrations were determined spectrophotometrically using the absorption coefficients [6] given in part I of this study.

3. Results and discussion

The standard Gibbs free energy, ΔG°_B, derived from the fluorimetrically-determined binding constants, the association enthalpies, ΔH°_B, and the heat capacity changes, Δc_{pB}, obtained by direct calorimetric measurements, as well as the entropies of association, ΔS°_B, have been compiled in table 1. Inspection of the ΔG°_B values in table 1 shows that for both isoenzymes the Gibbs free energy is practically not affected by the introduction of the second phosphate group into the coenzyme fragment, while the difference in binding free energy between adenosine and AMP amounts to approx. 4 kJ/mol binding site. Extension of ADP by the ribose renders the ΔG°_B values more negative for either isoenzyme, however, less so in case of the skeletal muscle enzyme. Binding of the oxidized coenzyme is slightly favoured over that of ADP-ribose for the skeletal muscle type, while the association of NAD^+ with the heart muscle lactate dehydrogenase appears to be even slightly impaired in comparison to binding of ADP-ribose. A large decrease in the Gibbs free energy of binding reflects a stronger interaction of both isozymes with

Table 1
Apparent thermodynamic parameters for binary complex formation between pig skeletal muscle lactate dehydrogenase and various coenzyme fragments in 0.2 M potassium phosphate buffer, pH 7.0, 25°C

Ligand	$-\Delta G^\circ_B$ (kJ/mol)	$-\Delta H^\circ_B$ (kJ/mol)	$-\Delta S^\circ_B$ (J/K·mol)	$-\Delta c_{pB}$ (J/K·mol)
Adenosine				
Adenosine	(12.1 ± 0.2)	(25.5 ± 1.3)	(44.8 ± 5)	(−13 ± 40)
AMP	14.6 ± 0.4	16.9 ± 3	7.5 ± 11	787 ± 59
AMP	(16.7 ± 0.4)	(13.4 ± 0.8)	(−10.9 ± 4)	(431 ± 29)
ADP	14.5 ± 0.4	21.9 ± 2.4	24.4 ± 9	883 ± 63
ADP	(16.9 ± 0.3)	(24.7 ± 1.7)	(26.4 ± 6)	(310 ± 59)
ADPR	17 ± 0.2	32.6 ± 2.3	52.2 ± 8	1439 ± 67
ADPR	(20.8 ± 0.1)	(26.8 ± 0.8)	(20.5 ± 1.7)	(414 ± 33)
NAD^+	18.8 ± 0.3	27.6 ± 2.5	29.3 ± 13.4	510 ± 46
NAD^+	(19.9 ± 0.2)	(25.5 ± 0.8)	(18.8 ± 3)	(351 ± 33)
NADH	28.9 ± 0.3	31.6 ± 2.1	8.4 ± 8.4	1360 ± 42
NADH	(30.9 ± 0.3)	(44.4 ± 1.3)	(45.2 ± 5)	(699 ± 38)

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. The values in brackets refer to the corresponding reactions of the pig heart muscle enzyme and have been taken from [7]

the reduced coenzyme than with all other fragments or NAD^+. One contributing factor to the weaker binding of NAD^+ as compared to NADH may be the presence of the positively charged Arg 109 in the active center of lactate dehydrogenase [5]. Gln 31 is in the heart enzyme and Ala 31 in the muscle enzyme [5]. It was implied that the glutamine could form a hydrogen bond with the nicotinamide phosphate, thus increasing the energy of binding for the heart isozyme. On the basis of the free energy values, at 25°C, the Gibbs free energies of binding are more negative by 1–2 kJ/mol binding site for all coenzyme fragments having the nicotinamide phosphate. However, in as far as a hydrogen bond is considered as an energetic contribution, the picture is not so clear. At 25°C the enthalpy involved in binding ADP to the heart isozyme is more negative than for the corresponding reaction with the muscle enzyme. However, with ADP-ribose and NAD^+ the situation is reversed. NADH exhibits again an enthalpy, which is more negative for complex formation with the heart than with the muscle isozyme.

Due to the marked temperature dependence of the reaction enthalpies and the rather temperature-insensitive Gibbs free energies of binding, the enthalpic contributions will not always be the dominant term.

One of the evident differences between pig heart and pig skeletal muscle enzyme is the magnitude of the heat capacity change associated with complex formation. For each reaction the decrease in the heat capacity is by far more pronounced with the skeletal muscle than with the heart muscle enzyme.

A comparison of the individual Δc_{pB} values reveals only minor differences in the trend of the heat capacity changes when going from adenosine to NADH. The heart muscle isozyme shows a further decrease of the heat capacity associated with binding of NADH as compared to ADP-ribose, whereas the Δc_{pB} value of the reaction between the skeletal muscle enzyme and NADH or ADP-ribose is practically the same. While the heat capacity change concomitant with binding of ADP to the heart muscle isozyme appears to be smaller than that involved in AMP binding, the corresponding quantities referring to the reactions

with the skeletal muscle enzyme show the opposite trend.

A comparison of the thermodynamic binding parameters of liver alcohol dehydrogenase with those of the lactate dehydrogenase isozymes shows, that, altogether, the differences appear to be greater than the similarities. This conclusion is supported by direct comparison of corresponding quantities as well as by the trend, which these values exhibit within the series of fragments. That becomes particularly evident on inspection of the reaction enthalpies and the heat capacity changes. While one can speak of a similar trend in the ΔH values for binding of corresponding fragments to the LDH isozymes, this trend is completely absent in the reactions of the analogous fragments with LADH. On the contrary, the binary complex formation with NADH and NAD^+, which was enthalpy determined at 25°C in the reactions with the lactate dehydrogenases, is distinctly entropy controlled for LADH over the whole temperature range investigated.

The heat capacity data render a similar picture. There is some analogy within the reactions involving the LDH isozymes, but the trend of the Δc_p values in going from AMP to NADH binding is not comparable. The most prominent difference exists in the magnitude of the Δc_p value for AMP association. The value of −1234 J/K·mol for AMP binding to LADH is surprisingly large, if compared to the −556 J/K·mol for ADP binding, especially in view of the similar magnitude of the Δc_p values for the corresponding reactions with the LDH isozymes. Since it has been demonstrated, that heat capacity changes are diagnostic of structural changes in reactions involving enzymes [3,4,10–12] the difference in the Δc_p value for binding of AMP and ADP, respectively, to LADH, can be taken to reflect real differences in the extent of structure changes induced by the two reactions. A tentative explanation of this effect can be based on the following consideration. It has been pointed out [9] that the AMP phosphate residue forms an ion pair with Arg 47 when binding to the enzyme, and that this interaction plays an important role in the overall interaction between LADH and the coenzyme. The difference in the heat capacity change could stem from the introduction of 2 negative charges by the AMP phosphate in contrast to the 1 charge carried by the same phosphate residue in ADP, ADP-ribose and the reduced and oxidized coenzyme. The extra charge can be pictured to lead to stronger interaction and concomitantly to more extensive structural alterations. It should be emphasized, however, that these alterations do not only refer to the three dimensional structure of the macromolecule as detected by the X-ray analysis, but to the whole system in the thermodynamic sense of the term.

Summarizing one may state that the similarities in the tertiary structure of liver alcohol dehydrogenase and the lactate dehydrogenase isozymes extend only qualitatively into the energetics of the reactions of the LDH isozymes, but cannot be detected in the energetics of the LADH reactions. Actually such a correspondence between the tertiary structure and the energy parameters of the reaction should not be expected, since the resolution of the macromolecular structures is not better than 2–3 Å. Uncertainties in distances of that magnitude may result, however, in large errors of the estimates of the interaction energies. In addition, estimates of the free energy values on the mere basis of structural data are very difficult due to the completely unknown entropic contributions.

References

[1] Hinz, H.-J. and Jaenicke, R. (1973) Biochem. Biophys. Res. Commun. 54, 1432–1436.
[2] Hinz, H.-J. and Jaenicke, R. (1975) Biochemistry 14, 24–27.
[3] Schmid, F., Hinz, H.-J. and Jaenicke, R. (1976) Biochemistry 15, 3052–3059.
[4] Hinz, H.-J., Schmidt, R., Scheurmann, W. and Jaenicke, R. (1977) Eur. J. Biochem. 80, 543–550.
[5] Eventoff, W., Rossman, M. G., Taylor, S. S., Torff, H.-J., Meyer, H., Keil, W. and Kiltz, H.-H. (1977) Proc. Natl. Acad. Sci. USA 74, 2677–2681.
[6] Dawson, R. M. C., Elliot, D. C., Elliot, W. H. and Jones, K. M., eds. (1969) Data for Biochemical Research 2nd ed, Oxford Univ. Press.
[7] Hinz, H.-J. and Schmid, F. (1977) 2nd Int. Symp. Pyridine Nucleotide-Dependent Dehydrogenases, Konstanz (Sund, H. ed) de Gruyter, Berlin, in press.
[8] White, J. L., Mackert, M. L., Buehner, M., Adams, M. J., Ford, G. C., Lentz, P. L., jr, Smiley, I. R., Steindel, S. J. and Rossman, M. G. (1976) J. Mol. Biol. 102, 759–779.

[9] Eklund, H., Nordström, B., Zeppezauer, B., Söderlund, G., Ohlson, J., Boiwe, T., Söderburg, B. O., Tapia, O., Bränden, C. J. and Akeson, A. (1976) J. Mol. Biol. 102, 27–59.
[10] Hinz, H.-J., Shiao, D. D. F. and Sturtevant, J. M. (1971) Biochemistry 10, 1347–1352.
[11] Velick, S. F., Baggott, J. P. and Sturtevant, J. M. (1971) Biochemistry 10, 779–786.
[12] Hinz, H.-J., Weber, K., Flossdorf, J. and Kula, M.-R. (1976) Eur. J. Biochem. 71, 437–442.