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THERMOCHEMISTRY OF THE REACTION CATALYZED BY MALATE DEHYDROGENASE*

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ABSTRACT

The heat of reaction for the process

$$H^+ + \text{oxalacetate}^{2-} + \text{NADH} \rightarrow \text{malate}^{2-} + \text{NAD}^+$$

has been determined to be -21.4 ± 0.2 kcal mol^{-1}. The similar reactions catalyzed by lactate dehydrogenase (IUB No. 1.1.1.27) and alcohol dehydrogenase (IUB No. 1.1.1.1) are compared with reference to their Gibbs–Helmholtz thermodynamic parameters.

INTRODUCTION

There exists a wealth of data on the kinetics of relatively well-known enzyme catalyzed systems. For a great many of these reactions, the equilibrium constants (and therefore ΔG values) are also known. At present there are few data available on the enthalpy (ΔH) and entropy (ΔS) changes for these processes[1]. While it is possible to obtain the missing ΔH and ΔS values from heats of formation or combustion, a survey of the literature shows that the more interesting biochemical substrates, more often than not, are not listed. It is thought that the major reason for this is the difficulty of obtaining the pure substrates for combustion studies.

Solution calorimetry, as described here, takes advantage of enzyme selectivity and therefore the purity of reagents is not as critical as above. The utility of solution calorimetry in enzyme studies has been well demonstrated[2–6]. In this report, the thermodynamic parameters of three closely related, enzyme catalyzed reactions are compared.

EXPERIMENTAL

The methodological approach and theory of the calorimetric system has been previously described[3,4]. No changes have been made in the experimental procedure or calculation of results.

Chemicals used were the purest available. Phosphate buffer (0.5 molar at

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pH 7.40±0.05) was prepared from reagent grade NaH_2PO_4 neutralized with a 50% NaOH solution to the required pH. All other solutions were prepared using the buffer as the solvent. Malate dehydrogenase enzyme (from pig heart), oxalacetic acid (90–95% pure) and nicotinamide adenine dinucleotide–reduced form (NADH) were obtained from Sigma Chemical Co. (St. Louis). These chemicals were used without further purification.

The reaction of interest (1), catalyzed by malate dehydrogenase,

$$H^+ + NADH + oxalacetate^{2-} \rightarrow malate^{2-} + NAD^+ \quad (1)$$

has an equilibrium constant of 1.55×10^{12} when oxalacetate is the substrate[7,8]. Assuming that the concentrations of NADH and oxalacetate are 0.01 M and have a pH of 7.4, the reaction will be 99.6% complete at equilibrium. If one of the reactants is in excess, the other will be even more completely utilized. As a result, no corrections for incomplete reaction are necessary.

Since NADH is readily measurable at 340 nm via spectrophotometric methods, it was used as the limiting reagent. Each experiment involved 6.8 m molar NADH and 9.2 m molar oxalacetate, along with a large amount of malate dehydrogenase (ca. 100 units) so that the reaction period would be short.

RESULTS AND DISCUSSION

Figure 1 is a tracing of an experimental curve obtained with this system. The rate of reaction is constant until more than 80% of the NADH is consumed. This is expected since the Michaelis constant is rather small[7].

Table 1 gives the results of five replicate determinations. All except one agree quite well. The one divergent value was rejected statistically and a pipetting error is suspected as the cause of the high result. A heat of reaction of -20.5 ± 0.2 kcal mol^{-1} is the best value for the ΔH. This value includes $+0.9$ k al mol^{-1} for the ionization of $H_2PO_4^-$ to H^+ and $HPO_4^=$, and the ΔH of reaction (1) is calculated as 21.4 ± 0.2 kcal mol^{-1}.

Since the free energy change (ΔG) may be calculated from the equilibrium constant, the entropy change (ΔS) for reaction (1) is readily evaluated. This is listed in Table 2 along with data for the alcohol dehydrogenase catalyzed reaction (2) and the lactate dehydrogenase system (3)

$$H^+ + acetaldehyde + NADH \rightarrow ethanol + NAD^+ \quad (2)$$

$$H^+ + pyruvate^- + NADH \rightarrow lactate^- + NAD^+ \quad (3)$$

Corrected data for the NADH/NAD half reaction (4) are also given[9,4].

$$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^- \quad (4)$$

Since the three substrates can be considered as a homologous series (each differs by a COO^- group) the data were inspected to see if any correlations were present.

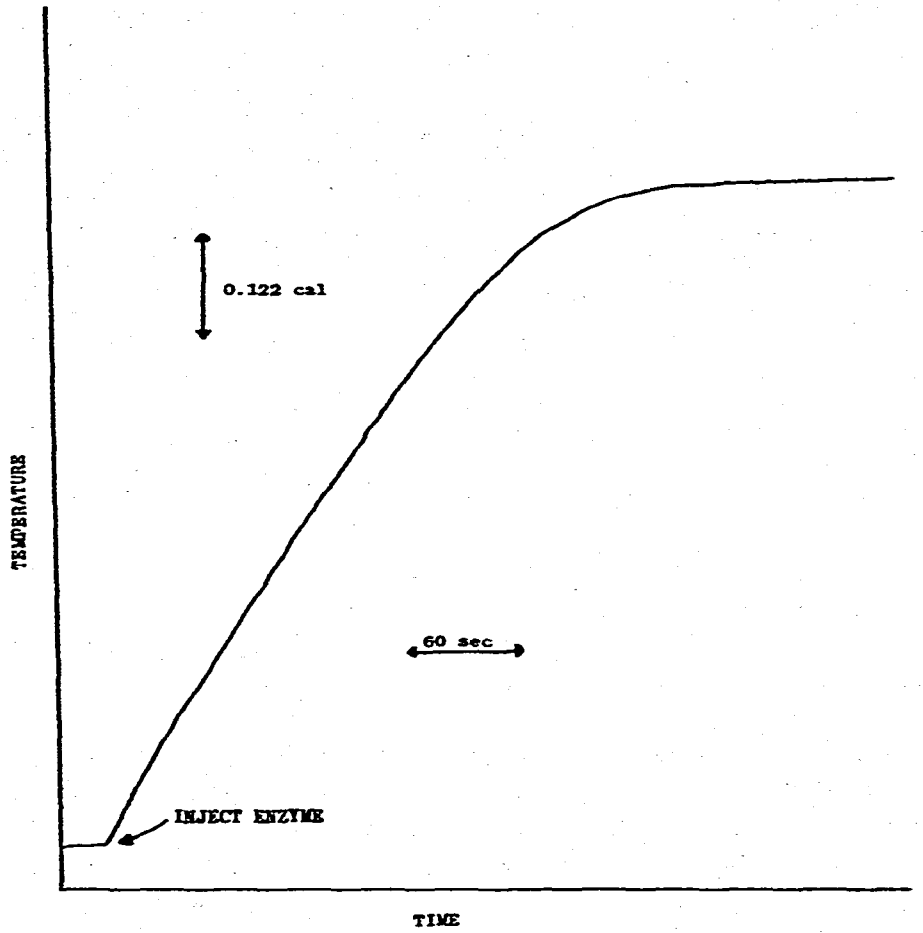

Fig. 1. Tracing of a typical curve obtained for the reaction of oxalacetate with NADH, catalyzed by malate dehydrogenase.

TABLE 1

DATA FOR THE CALCULATION OF THE HEAT OF THE REACTION
$H_2PO_4^- +$ oxalacetate + NADH → malate + NAD + $HPO_4^{=}$

Trial	*NADH used (μmoles)*[a]	*Conc. oxalacetate (m molar)*[b]	*Cal evolved*	*ΔH (kcal mol⁻¹)*	*Comments*
1	33.9	9.23	0.688	20.3	
2	33.5	9.23	0.687	20.5	
3	33.9	9.23	0.743	21.9	rejected
4	33.9	9.23	0.702	20.7	
5	34.3	9.23	0.698	20.4	
Mean	33.9	9.23	0.694	20.5	Avg. Dev. 0.14

[a] Measured photometrically before and after enzymatic reaction. [b] Assuming 90% purity.

TABLE 2

THERMODYNAMIC VALUES FOR REACTIONS RELATED TO THE MALATE DEHYDROGENASE SYSTEM

Rxn catalyzed by	*Equilibrium const.*[a]	ΔG (*kcal mol*$^{-1}$)	ΔH (*kcal mol*$^{-1}$)	ΔS (*kcal mol*$^{-1}$ *deg*$^{-1}$)
Alcohol dehydrogenase	1.25×10^{11} [b]	−15.1	−11.5[c]	+12.1
Lactate dehydrogenase	3.7×10^{11} [d]	−15.7	−14.8[e]	+3.2
Malate dehydrogenase	1.55×10^{12} [f]	−16.6	−21.4	−16.0
NADH/NAD[g]		−5.4	+5.3	+36.1

[a] Formulated from reaction written in the "reverse" direction (i.e., similar to equations 1–3). [b] Calculated from ref. 11. [c] Calculated from ref. 1, p. 307. [d] Calculated from ref. 10. [e] From ref. 4. [f] Calculated from refs. 7 and 8. [g] The half reaction $NADH + H^+ \rightarrow NAD + 2H^+ + 2e^-$ (refs. 9, 4).

A plot of ΔH vs. ΔS for the three reactions in Table 2 gives a straight line with a slope of 346 K. This behavior is well known and is called "enthalpy–entropy compensation." An excellent review of the subject is given by Lumry and Rajender[12]. In most cases, this behavior is taken to indicate that the ΔH and ΔS values for the "chemical" reactions are similar while the "non-chemical" processes differ. In aqueous solution, hydration and dehydration may be considered as "non-chemical."

For the systems listed, the "chemical" process is the conversion of a carbonyl to a hydroxyl group, while the "non-chemical" process would involve all other effects, primarily hydrogen bonding. The results are reasonable in that a larger increase in hydrogen bonding would be expected in the malate system than in the ethanol system since two fully charged carboxyl groups are exposed in the product. Lactate with one carboxyl group is at the intermediate position.

CONCLUSIONS

The enthalpy of the reaction catalyzed by malate dehydrogenase has been determined as -21.4 ± 0.2 kcal mol^{-1}. Comparing similar dehydrogenase processes, a regular change in enthalpy and entropy is found although there is little variation in the free energies of reaction. This behavior, known as enthalpy–entropy compensation, indicates that the major reason for the change in the thermodynamic parameters is solvation effects.

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