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THERMOCHEMISTRY OF THE REACTION CATALYZED BY LACTATE DEHYDROGENASE

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ABSTRACT

The thermochemistry of the reduction of pyruvate to lactate, in the presence of nicotinamide adenine dinucleotide (reduced form); catalyzed by the enzyme lactate dehydrogenase, has been studied. After approximately 120 experiments, a best value for the enthalpy of reaction has been determined to be -14.80 ± 0.30 kcal mol^{-1}. This reveals that the driving force for the reaction is almost completely enthalpic in nature. In addition, using the current methodology, it is possible to determine lactate dehydrogenase activity as low as 0.15 international units (325 Wroblewski units) per sample.

INTRODUCTION

Considerable interest has been expressed in thermal methods of analysis of late. Thermometric monitoring of reactions in solution is one of the brighter possibilities, for clinical analysis. In this regard, enzyme assay seems to be a perfect model to study the applicability of such methods.

This report concerns the determination of the ΔH of the reduction of pyruvate to lactate and the oxidation of NADH to NAD^+ as catalyzed by lactate dehydrogenase (LDH). Since future enzyme assays will require the interconversion of rates in terms of calories per unit time to products produced per unit time, the precise determination of the ΔH value is essential. This is the first step in developing a thermometric methodology for the assay of LDH and its various isoenzymes.

EXPERIMENTAL

Chemicals

Phosphate buffers (0.5 M), prepared by neutralization of reagent grade KH_2PO_4 and K_2HPO_4, were used throughout. All reactions were run at pH 7.50 ± 0.05 as determined with a Corning Research Model pH meter and commercial glass and calomel electrodes. Buffer solutions for standardization were saturated potassium bitartrate (pH 3.57) and a commercial phosphate buffer (pH 7.00).

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Sodium pyruvate and nicotinamide adenine dinucleotide-reduced form (NADH) were obtained from Sigma Chemical Co. (St. Louis). Both were used without further purification. The sodium pyruvate was analyzed by titration of pyruvic acid immediately after passage through a cation-exchange resin. It was found to be 97% pure. The main impurity is thought to be water. NADH was analyzed spectroscopically using the known molar absorptivity of 6.22×10^3 mol^{-1} cm^{-1} at 340 nm. It was found to be 81% pure. Concentrations of sodium pyruvate and NADH solution were calculated from the weight and purity of the material used for their preparation.

Isoenzyme preparations of lactate dehydrogenase (LDH; IUB No. 1.1.1.27) were obtained from several sources including Sigma Chemical Co. and Nutritional Biochemical Corp. (Cleveland). All were crystalline fractions suspended in ammonium sulfate with activities in the order of 600 International Units (IU) per milligram of protein. The isoenzymes studied were Beef Heart (BH), Beef Muscle (BM), and Rabbit Muscle (RM).

Methods

The calorimetric apparatus used has been previously described[1]. Two experimental approaches are possible with this set-up. The first is the normal thermometric titration mode[2]. In this case, buffered mixtures of pyruvate and enzyme were titrated with NADH. The second approach, closely akin to classical solution calorimetry called Direct Injection Enthalpimetry (DIE)[3], was also used. In this case, enzyme was injected into mixtures of NADH and pyruvate and temperature versus time curves were produced. In both cases, the overall temperature change could then be converted to a heat of reaction via expression 1. Typically, ΔT is expressed in recorder chart

$$-\Delta H(\text{cal mol}^{-1}) = \frac{(\Delta T)\,(\text{heat capacity})}{(\text{moles pyruvate consumed})} \qquad (1)$$

divisions while the heat capacity term has units of cal/chart div. in our experiments. (Note: 1 calorie = 4.184 Joules.) Appropriate corrections to ΔT were applied to eliminate heat effects not due to the chemical process of interest (i.e., heat of stirring, dilution, etc.)[2].

Due to the nature of the results obtained (vide, infra.), the heat capacity values obtained for use in expression (1), via classical electrical methods, were checked by a chemical reaction with a well known ΔH. The reaction was the neutralization of HCl with NaOH with a ΔH of -13.34 kcal mol^{-1} (ref. 4). Our calculated value from these experiments was within 1% of the theoretical, validating our heat capacity determination methods, while giving added confidence to the heats of reaction reported below.

RESULTS

Using expression (1), overall heats of reaction are determined for the process:

$$\text{NADH} + \text{H}_2\text{PO}_4^- + \text{pyruvate}^- \underset{\longleftarrow}{\overset{\text{LDH}}{\longrightarrow}} \text{NAD}^+ + \text{HPO}_4^{2-} + \text{lactate}^- \qquad (2)$$

Results in Table 1 give these ΔH values as determined with solutions of various pyruvate concentrations. It is obvious that the precision obtained is not the best, as expected from DIE data[3]. Results for other DIE determinations are given in Table 2, where different LDH isoenzymes are used.

TABLE 1

ΔH VALUES DETERMINED BY DIE AS PYRUVATE CONCENTRATION IS VARIED[a]

Pyruvate (mM)	$\Delta H_{calc.}$ *(kcal per mole pyruvate)*	*Average dev.*	*No. of determinations*
2.0	−13.3	0.4	5
3.0	−14.5	0.8	5
4.0	−14.7	0.3	5
5.0	−14.0	0.7	5
6.0	−13.5	0.5	5
Mean	−14.0	0.5[b]	25

[a] Other conditions; 10 mM NADH; approximately 2 IU LDH (RM) per experiment; 25 °C. [b] Calculated from the means of the 5 concentrations reported; overall AD is 1.0.

TABLE 2

ΔH VALUES OBTAINED AT 25 °C USING DIE USING VARIOUS ISOENZYME PREPARATIONS[a]

Isoenzyme (approx. 2 IU)	$\Delta H_{calc.}$ *(kcal per mole pyruvate)*	*Average dev.*	*No. of determinations*
Rabbit muscle (I)[b]	−14.0	1.0	25
Rabbit muscle (II)	−13.9	0.8	50
Beef heart (I)	−14.0	1.2	10
Beef heart (II)	−13.7	0.9	25

[a] (I) denotes Sigma Chemical Co. as source; (II) denotes Nutritional Biochemical Corp. as source. [b] From Table 1.

Since the imprecision is appreciable in the above results (5.7–7.2%) thermometric titration experiments were tried in order to improve them. Relatively large concentrations of LDH are needed to keep the reaction suitably fast compared to the titration rate[5]. As a consequence, runs were made which involved 2 and 16 IU of LDH. Figure 1 illustrates the differences obtained, due to the variations of LDH. No matter what the enzyme concentration was, the precision was increased by a factor of 3 as shown in Table 3.

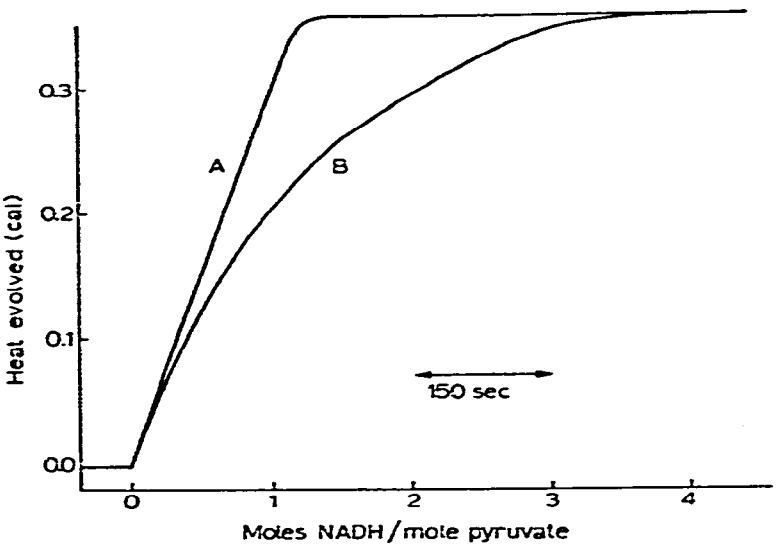

Fig. 1. Thermometric titration curves for the reaction of 0.0050 M pyruvate with 0.1 M NADH titrant in the presence of rabbit muscle LDH isoenzyme. Curve A, 16 IU enzyme; Curve B, 2 IU enzyme. Titration rate, 0.001487 ml sec^{-1}.

TABLE 3

THERMOMETRIC TITRATION RESULTS FOR THE REACTION OF PYRUVATE–LDH MIXTURE WITH NADH[a]

Isoenzyme	ΔH *(kcal per mole pyruvate)*	*Average dev.*	*No. of determinations*
Rabbit muscle (II)[b]	−13.59	0.30	3
Beef heart (I)[b]	−13.84	0.31	3
Beef heart (II)[b]	−13.85	0.25	4
Beef muscle (I)[b]	−13.95	0.20	4

[a] Conditions: pyruvate, 5.0 mM; NADH, 0.1 M; titration rate 0.001487 ml sec^{-1}; enzyme conc., 2–16 IU per 5 ml of sample. [b] (I) denotes Sigma Chemical Co. as source; (II) denotes Nutritional Biochemical Corp. as source.

DISCUSSION

Since the reaction of interest is

$$NADH + H^+ + \text{pyruvate}^- \underset{\longleftarrow}{\xrightarrow{LDH}} NAD^+ + \text{lactate}^- \qquad (3)$$

a correction must be made for the ionization of phosphate which participates as given in eqn (2). We have chosen +0.98 kcal mol^{-1} as the "best" value[6] for this process although values as low as 0.90 are reported[7]. The reason for this is that 0.98 is obtained from equilibrium measurements whereas the calorimetric determination (0.90) is the difference between two large heats of reaction (i.e., the ionization of water and the neutralization of $H_2PO_4^-$).

Correction for buffer ionization yields a "best" value of -14.80 kcal mol^{-1} pyruvate for reaction (3), assuming that the mean of the data in Table 3 is the "best" for process (2). The heat of reaction determined here differs greatly from that determined by Katz[8] (-10.6 kcal per mole of NADH). However, it is important to note that the relatively unstable NADH was the limiting reactant in that study.

Incorporation of the heat of reaction determined here into the Gibbs free energy expression gives the following values:

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

$$(-15.55 \text{ kcal}) = (-14.80 \text{ kcal}) - (298 \text{ K})\,(2.3 \text{ cal mol}^{-1} \text{ deg}^{-1})$$

As can be readily observed, the driving force of the reaction is almost totally enthalpic in nature. The individual half reactions[8] show, however, that both ΔH and ΔS are relatively large fractions of the ΔG values.

Analytical applications for the estimation of total LDH activity seem to be good. Considering that rates of heat evolution in the order of 0.002 cal min^{-1} are readily obtained from a 5.00-ml reaction volume, this would correspond to the consumption of 1.5×10^{-7} moles of pyruvate per minute. Therefore an activity of 0.15 IU of LDH per sample will yield good results. This corresponds to 325 Wroblewski Units. "Normal Values" in human sera[9] are thought to be around 300 Wroblewski Units ml^{-1} while abnormal concentrations due to various disease states are typically five to ten times greater. A very rapid test for elevated levels of LDH would use 0.5 ml of serum with observable temperature changes being readily apparent within 1 min. "Normal" levels of LDH would not be detected. A more sensitive apparatus as recently described by Smith et al.[10], could be readily used to determine normal levels of LDH activity.

CONCLUSION

The heat of reaction for the process catalyzed by LDH has been determined with a precision of $\pm 2\%$. Little variation is found between different crystalline isoenzyme fractions, under a wide variety of conditions. Using the ΔH from this work, it is observed that the driving force of the reaction is basically enthalpic in nature. In addition, clinical application of the thermometric methodology to LDH assay was evaluated.

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