Biophysical Chemistry 7 (1978) 375–378

THERMODYNAMICS OF THE GLUTAMATE DEHYDROGENASE CATALYTIC REACTION

S. SUBRAMANIAN

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, USA

Received 15 July 1977

The enthalpy change for the oxidative deamination of glutamate by $NADP^+$ catalyzed by bovine liver glutamate dehydrogenase has been determined calorimetrically. The ΔH^0 values are 64.6 ± 1.2 kJ/mol and 70.3 ± 1.2 kJ/mol at 25 and 35°C respectively. The equilibrium constants for the reaction at the two temperatures were determined spectrophotometrically. This enabled the determination of ΔG^0 and ΔS^0 of the reaction as well. ΔH^0 values were also determined for the reaction using an alternative coenzyme and the deuterated substrate.

1. Introduction

Free energy changes for enzyme-catalyzed reactions are fundamental biochemical parameters. Accurate determinations of these quantities are important for studies of the mechanisms of biological energy transformations and in conjunction with enthalpy changes, they provide a complete thermodynamic picture of the reactions of interest. While a large body of free energy data is available for many biochemical reactions, adequate information on the enthalpy changes is scarce. Besides, calorimetrically determined ΔH values are more reliable than those obtained from the temperature dependence of equilibrium constants. Brown [1] has compiled the enthalpy changes for some enzyme-catalysed reactions which have been studied calorimetrically. In this paper we report the calorimetric determination of the enthalpy change for the reaction,

$$NADP^+ + \text{L-glutamate} + H_2O = NADPH + \text{2-oxoglutarate} + NH_4^+ + H^+ \quad (1)$$

catalyzed by bovine liver glutamate dehydrogenase [L-glutamate-$NAD(P)^+$ oxidoreductase (deaminating), EC 1.4.1.3]. This enzyme has an essential function in the regulation of glutamate which is located at the crossroad between amino acid metabolism and the tricarboxylic acid cycle. This study forms part of the overall objective of characterizing the entire thermodynamic profile of the reaction along the reaction coordinate incorporating most of the active as well as abortive complexes of the enzyme with coenzymes, substrates and substrate analogs.

2. Materials and methods

2.1. Materials

Glutamate dehydrogenase was purchased from Sigma Chemical Co. as the type I crystalline ammonium sulfate suspension. The dialysis, treatment with Norit A and the assay were done as described previously [2]. $NADP^+$, NADPH, 3-acetylpyridine adenine dinucleotide and 2-oxoglutaric acid were purchased from Sigma. L-glutamic acid was a product of Mann Research Laboratories and L-[2-^{2}H] glutamic acid was purchased from Diaprep Inc., and purified as described [3]. The pH values of all solutions were determined with a Radiometer PHM 26 pH meter. The buffer used in all the experiments was 0.1 M potassium phosphate at pH 7.6.

2.2. Calorimetric measurements

The calorimetric measurements were carried out in an LKB 10700 batch microcalorimeter fitted with gold cells. Both the forward and reverse reactions were per-

formed in the calorimeter. In the reverse direction, over 99.9% of the NADPH would be oxidized upon attainment of equilibrium, under the experimental conditions. The extent of the forward reaction (reaction 1) was determined by measuring the concentration of NADPH formed (at the end of the calorimetric experiment) in a Zeiss PMQ II Spectrophotometer at the same temperature as in the calorimetric experiment. In general the concentrations were in the range 100–300 μM for coenzyme; 30 mM glutamate and 2 mM 2-oxoglutarate. The reaction was initiated by mixing the substrate(s) and coenzyme mixture (4.0 ml) in the larger compartment with enzyme in catalytic amounts (0.1 ml) in the smaller compartment in the sample cell. The heat of dilution of the reactants was blanked out by mixing corresponding volumes of reaction mixture and buffer in the reference cell. The heat of dilution of the enzyme was found to be negligible. Generally the reaction (and the heat effect) was complete in 15–20 minutes and a further mixing did not give any more heat effect. The calorimeter was calibrated by measuring the heat of dilution of sucrose. The signal from the calorimeter was amplified with a Keithley 149 microvolt amplifier and recorded on a Houston Omnigraphic 3000 recorder with a built-in integrator. The measured heats were of the order of 10–20 mcal.

2.3. Equilibrium measurements

Relatively large enzyme concentrations, up to 0.1 mg/ml, were added to reaction mixtures of NADP and glutamate in phosphate buffer to reach equilibrium quickly (10–15 min) and to minimize coenzyme breakdown. The reaction mixtures were made up in test tubes and incubated in a water bath at the required temperature, and transferred to quartz cells of optical path 1 cm and the absorbance at 340 nm was measured in a Zeiss PMQ II Spectrophotometer equipped with a temperature controller. The concentration of NADPH was calculated from the absorbance at 340 nm at equilibrium using a molar extinction coefficient $\epsilon = 6.22 \times 10^6$ cm^2/mol.

3. Results and discussion

The equilibrium constant, K_c, for reaction (1) is given by

$$K_c = \frac{[\text{2-oxoglutarate}]\,[NH_4^+]\,[H^+]\,[\text{NADPH}]}{[\text{L-glutamate}]\,[\text{NADP}^+]\,[H_2O]}.$$

Taking into account that the standard state for water is pure liquid (55.56 M) and the stoichiometric equivalence of 2-oxoglutarate, NH_4^+ and NADPH the thermodynamic equilibrium constant, K_a, can be written as

$$K_a = \frac{[\text{NADPH}]^3[H^+]}{([\text{glutamate}]_i - [\text{NADPH}])([\text{NADP}]_i - [\text{NADPH}])},$$

where $[\text{glutamate}]_i$ and $[\text{NADP}]_i$ represent initial concentrations. Therefore, to calculate the equilibrium constant of the reaction, K_a, only the equilibrium concentration of NADPH and equilibrium pH need to be known.

Several determinations of the equilibrium constants have been made and their mean values along with the free energies, ΔG^0, are listed in table 1. The enthalpy values for the reaction (1) appropriately corrected for the heat of proton uptake by the phosphate buffer [4] are also listed in table 1. The ΔH^0 values are average values of ΔH^0 measured in the forward as well as reverse directions. The differences in the two measurements were within the experimental error. Using the ΔG^0 values from the equilibrium measurements, the ΔS^0 values were also calculated and given in table 1. Also shown in table 1 are literature values for the equilibrium constant for purposes of comparison. As pointed out by Engel and Dalziel [5] the equilibrium constant is largely dependent on the ionic strength of the medium. Under comparable conditions the agreement in ΔG^0 values is good. The only available literature value for ΔH^0, 73.2 kJ/mol obtained [5] from the temperature dependence of the equilibrium constant representing a higher ionic strength ($I = 0.47$) and covering a temperature range of 25–38°C differs from our calorimetric values significantly. Since the calorimetric value had concordance in both forward and reverse directions the calorimetric value is more dependable than the van't Hoff one.

The enthalpy change for the reaction (1) using 3-acetyl pyridine adenine dinucleotide (3-APAD) as the coenzyme was also determined. L-[2-^{2}H] glutamate was also used as an alternative substrate. Table 2 lists the values obtained at 25° and 35°. Both normal glutamate and L-[2-^{2}H] glutamate produced same ΔH^0 val-

Table 1
Thermodynamic parameters for reaction (1) catalyzed by L-glutamate dehydrogenase

Authors	Temp. (°C)	pH	Ionic strength	10^{14} K_a a) (M^2)	ΔG^0 b) (kJ/mol)	ΔH^0 b) (kJ/mol)	ΔS^0 c) J/mol.K
this work	25	7.6	0.305	7.3	75.0 ± 0.4	64.6 ± 1.2	−35 ± 5
	35	7.6	0.305	16.0	75.5 ± 0.4	70.3 ± 1.2	−17 ± 5
Olson and Anfinsen [6]	27	6.4–7.5	0.47	9.9	74.7	–	–
Engel and Dalziel [5]	25		0	0.9	80.1		
	25	6.9–7.1	0.1	4.8	76.0	73.2	
	27		0	1.1	80.2	from van 't Hoff plot	–
	27		0.47	12.0	74.2	$f \approx 0.47$ $T = 25–38°C$	
	38		0.25	24.9	75.0		

a) The thermodynamic equilibrium constant, K_a, is defined in the text.
b) The stated uncertainties are standard deviations of the mean.
c) The uncertainties in ΔS^0 follow from the uncertainties in ΔG^0 and ΔS^0.

Table 2
Enthalpy and heat capacity changes for reaction (1) for different coenzyme-substrate combinations

		ΔH^0 (kJ/mol)		
Coenzyme	*Substrate*	*25°C*	*35°C*	ΔC_p (J/mol.K)
NADP	L-glutamate	64.6	70.3	570
NADP	L-[2-^{2}H] glutamate	–	70.3	–
3-APAD a)	*L-glutamate*	*46.0*	*52.3*	*630*
3-APAD a)	*L-[2-^{2}H]-glutamate*	–	51.5	–

a) 3-APAD = 3-acetyl pyridine adenine dinucleotide.

ues within experimental error, for the reaction (1) for each coenzyme. This indicates the similarity of the character of the C–H bond that is broken in glutamate and the one that is formed in the reduced coenzyme. With 3-APAD as the coenzyme, the ΔH^0 value is about 18 kJ/mol *less* endothermic than that for the reaction with NADP as the coenzyme. The difference between the ΔH values for NADP reaction and NAD reaction is only 2.5 kJ/mol, the NAD reaction being less endothermic [5]. The 18 kJ/mol difference observed in the 3-APAD reaction must be largely due to the fact that while in NADP the $-C\overset{=O}{\underset{NH_2}{}}$ group is coplanar with the pyridine ring, in 3-APAD, the $-C\overset{=O}{\underset{CH_3}{}}$ group is *not* coplanar with the pyridine ring. The strain energy in 3-APAD may be contributing to the lower endothermic heat for the reaction. The change in the heat capacity, ΔC_p, for the reaction is 570 J/mol.K for NADP as the coenzyme and 630 J/mol.K for 3-APAD as the coenzyme at 30°C. These two values are not significantly different.

The data obtained in this study should help in a better understanding of the shape of the thermodynamic profile for the overall catalytic reaction, when thermodynamic data for the various complexes of the enzyme in the reaction path become available.

Acknowledgement

I am grateful to Dr. H. Fisher (Veterans Administration Hospital, Kansas City, Missouri) in whose laboratory part of this work was done. I thank Dr. P.D. Ross for suggestions regarding the manuscript and Mrs. Barbara De Larco for her help in preparing this manuscript.

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