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THERMODYNAMICS OF REDOX REACTIONS INVOLVING NICOTINAMIDE ADENINE DINUCLEOTIDE

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ABSTRACT

Literature data on the thermodynamics of redox nicotinamide adenine dinucleotide (NAD) dependent reactions have been analyzed. It has been established that for the redox reaction of NAD

$NAD^+ + H_{2(gas)} = NADH + H^+$

where all substances except H_2 are in the aqueous buffer with the ionization enthalpy equal to zero, the most reliable thermodynamic parameters should be considered as: ΔH (298.15 K; pH 7) = —27.4 ± 1.7 kJ mole^{-1}; ΔG (298.15 K; pH 7) = —17.8 kJ mole^{-1}. From the above thermodynamic parameters of the reaction ΔH, ΔG and ΔS for reactions of NAD with natural substrates, synthetic mediators and some inorganic compounds have been calculated.

INTRODUCTION

Over 250 nicotinamide adenine dinucleotide (NAD)-dependent enzymes catalyzing diverse redox conversions of organic compounds in living things are known at present. Quantification of such processes and a deeper insight into the role of these conversions in vital processes require reliable thermodynamic parameters of redox NAD-dependent reactions. NAD-dependent reactions can find practical application in industry [1,2], analytical chemistry, etc. Thermodynamic data are thus necessary to calculate optimal physico-chemical conditions of these processes.

Despite a great need for reliable thermodynamic parameters for reactions under study, the number of publications containing data reliable for thermodynamic calculations is insufficient. It is only recently that the number of publications on the determination of the heat and free energy of redox NAD-dependent reactions has somewhat increased [7,10,46].

The present review is devoted to analysis of papers on the thermochemistry of NAD-dependent redox reactions. Special emphasis is given to potentialities of calorimetric studies in this field of biothermochemistry.

THERMOCHEMISTRY OF NAD-DEPENDENT ENZYMATIC REACTIONS

The first thermochemical determination of the enthalpy of enzymatic reactions involving NAD was performed by Katz in 1955 [3] for the reduc-

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tion of sodium pyruvate by NADH in the presence of lactate dehydrogenase at 25°C. The reaction was initiated by introduction of 0.03 ml lactate dehydrogenase into the reaction solution (24.97 ml) containing sodium pyruvate and NADH in 0.15 M sodium phosphate buffer (pH 7.3). An overall heat yield was about 5—10 J. The calorimeter was a Dewar flask (25 ml) placed in an isothermic sheath (±0.001°C). The temperature rise was measured with a thermistor (32 kOm) with an accuracy of 0.00006°C. The amount of NADH in this and in all experiments described below, if not otherwise stated was determined spectrophotometrically at 340 nm; the experimental results were corrected for the ionization enthalpy of the buffer. NADH and other reagents of Sigma Chemical Co. * were used without further purification.

For the reaction

$$CH_3—CO—CO_2^- + NADH + H^+ = CH_3—\underset{\displaystyle OH}{\underset{|}{CH}}—CO_2^- + NAD^+ \qquad (1)$$

$\Delta H_{(1)} = -44.4 \pm 1.7$ kJ mole^{-1} [3,4] was obtained.

The enthalpy of this reaction, obtained by Donnovan et al. [5] at 25°C in 0.5 M potassium phosphate buffer, pH 7.50 ± 0.05 in 120 experiments, was equal to —61.9 ± 1.3 kJ mole^{-1}. The reaction was run in a glass calorimetric vessel (5 ml) enveloped with an adiabatic sheath (±0.0002°C). The calorimeter was equipped with a thermistor (2 kohm) to measure temperatures accurately to ±0.00005°C, a magnetic stirrer, a calibrated heater to maintain an even temperature in the adiabatic sheath and in the glass vessel, as well as a tubing to inject a thermostatted solution [6]. A calorimeter of this construction could be used for both calorimetric titration of sodium pyruvate by NADH and direct measurement of ΔH of the reaction. Before a thermochemical investigation of reaction (1), the calorimeter was tested by determining the enthalpy of the neutralization reaction (NaOH + HCl). The results of the determinations agreed well (within 1%) with reliable published data [18,19]. The authors [5] reported that the accuracy of the results is limited by a relative instability of NADH in the buffer solution used.

Enthalpies of reaction (1) reported in refs. 3 and 5 differ by ~17 kJ mole^{-1}, with an experimental error in both cases of ±1.3—1.7 kJ mole^{-1}. More reliable seems to be $\Delta H_{(1)} = -61.9 \pm 1.3$ kJ mole^{-1} reported by Donnovan et al. [5], in which the authors described an application of more up-to-date and reliable equipment, a great battery of experiments (120) by various tehcniques. Reliability of the calorimetric setup operation was controlled by the enthalpy determination of the neutralization reaction (HCl + NaOH).

Jespersen [7] gave for reaction (1) $\Delta G_{(1)} = -25.9$ kJ mole^{-1} (pH = 7), calculated from determinations of the equilibrium constant of reaction (1) in the aqueous buffer [9].

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* The reagents mentioned below, if not otherwise stated, were purchased from this Company and used without further purification.

The authors in ref. 7 described experiments in thermochemistry of reductions of oxalacetate to malate catalyzed by NAD-dependent malate dehydrogenase

$$^{-}O_2C{-}CH_2{-}CO{-}CO_2^{-} + NADH + H^{+} = {}^{-}O_2C{-}CH_2{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}CO_2^{-} + NAD^{+} \qquad (2)$$

Measurements were made with the equipment described above [6] at pH 7.40 ± 0.05 in the sodium phosphate buffer (0.5 M).

It should be noted that until the present there are no conventional "standard" conditions for studying the thermodynamic parameters of enzymatic reactions. This, in many cases, makes it difficult to compare the results of different authors.

For reaction (2) [7] $\Delta H_{(2)} = -89.5 \pm 0.9$ kJ mole^{-1} and $\Delta G_{(2)} = -29.7$ kJ mole^{-1} (pH 7).

THERMODYNAMICS OF NAD REDOX REACTION

A change in the free energy, G in the NAD redox reaction

$$NAD^{+} + H_{2(gas)} = NADH + H^{+} \qquad (3)$$

was reported by Burton and Wilson [8,10]. It was obtained from definition of equilibrium constants of NAD-dependent enzymatic reactions catalyzed by alcohol dehydrogenase. Burton and Wilson [8] described studies of NAD-dependent reduction of acetone to propane-2-ol and acetaldehyde to ethanol, $\Delta G_{(3)} = -18.1$ kJ mole^{-1} (pH 7). In ref. 10 Burton found $\Delta G_{(3)} = -17.8$ kJ mole^{-1} (pH 7) from determinations of the equilibrium constant of the NAD-dependent reduction of acetone to propane-2-ol. ΔG values found in refs. 8 and 10 agree well with each other; Burton [10] recommended $\Delta G_{(3)} = 17.8$ kJ mole^{-1}, as calculated using the precise values of free energies of formation of aqueous acetone and propane-2-ol solutions.

Values of $\Delta H_{(3)}$ obtained by methods other than calorimetry (by temperature dependence of the equilibrium constant) strongly differed from -29.3 kJ mole^{-1} [10] to -14.3 kJ mole^{-1} [11]. Poe et al. [12] were the first to determine $\Delta H_{(3)}$ calorimetrically. They used a differential calorimeter with the Dewar reaction and a comparison flask (50 ml) equipped with thermocouple batteries to measure a temperature drop between them (sensitivity 200 $V/I°C$), an oxygen electrode and a tube for drainage of solution by oxygen and introduction of solutions. A temperature rise, 0.05°C, was recorded with a precision of ±0.0005 + 0.0003°C. Operation of the calorimetric setup was tested through the enthalpy determination of the neutralization reaction (HCl + KOH).

NADH oxidation by oxygen dissolved in the buffer and in the presence of NADH-oxidase was measured directly in the calorimeter

$$2\,NADH + 2\,H^{+} + O_2 = 2\,NAD^{+} + 2\,H_2O \qquad (4)$$

The authors obtained the NADH-dehydrogenase by the method described

TABLE 1

Enthalpy [$\Delta H_{(3)}$] of the NAD redox reaction (pH 7; 25°C) [$NAD^+ + H_{2(gas)} = NADH + H^+$]. Hydrogen is in the gas form, other reactants are in the buffer with the ionization enthalpy equal to 0

ΔH_3 (kJ mole^{-1})	Method	Reactions	Refs.
—14.9	Determination of dE/dT	Oxidation—reduction NAD/NADH on electrode	11
—28.0 ± 5.4	Calorimetry	$2\ NADH + O_{2(aq.soln.)} + 2\ H^+ = 2\ NAD^+ + 2\ H_2O$	12
—27.4 ± 2.5	Calorimetry	Unpublished results by Scott and Sturtevant (cited from ref. 10)	10
—29.2	Determination of $d \log K/dT$	Propane-2-ol + NAD^+ = acetone + NADH + H^+	10
—27.6 ± 1.7	Calorimetry	$HCOO^- + NAD^+ + H_2O = HCO_3^- + NADH + H^+$	14, 15
—27.2 ± 1.7	Calorimetry	$NAD^+ + H_{2(aq.soln.)} = NADH + H^+$	14, 15

Thermodynamic calculations require $\Delta H_{(3)}$(298.15 K; pH 7) = —27.4 ± 1.7 kJ mole^{-1} [14, 15]; $\Delta G_{(3)}$(298.15 K; pH 7) = —17.8 kJ mole^{-1} [10].

by Crane et al. [13]. Measurements were made at 25°C in various 0.10 M buffers — citrate, glycine-glycine-potassium phosphate and "tris" (tris-oxymethyl-aminomethane + HCl) — at various values of pH (6.4, 6, 9, 7.4, 7.9, 8.4). The mean $\Delta H_{(4)}$ reported by Poe et al. [12] was —257.7 ± 5.4 kJ mole^{-1} of NAD, which corresponded to $\Delta H_{(3)}$ = —28.0 ± 5.4 kJ mole^{-1}.

Direct calorimetric determination of $\Delta H_{(3)}$ was made by Rekharsky et al. [14,15] in the LKB-2107-III microcalorimeter [16,17] *. Reduction of NAD by hydrogen dissolved in the buffer was catalyzed by NAD-dependent hydrogenase at 25°C in 0.05 M potassium phosphate buffer at pH 7.2. This resulted in $\Delta H_{(3)}$ = —27.2 ± 1.7 kJ mole^{-1}. In the same calorimeter the authors [14,15] determined the enthalpy of NAD reduction by sodium formate catalyzed by NAD-dependent formate dehydrogenase (pH 7).

$$NAD^+ + HCOO^- + H_2O = NADH + HCO_3^- + H^+ \quad (5)$$

The resultant $\Delta H_{(5)}$ = 6.9 ± 1.0 kJ mole^{-1} and the literature data for the enthalpies of formation of aqueous solutions of formate ion, carbonic acid and its dissociation products [18,19] were used to calculate $\Delta H_{(3)}$ = —27.6 ± 1.7 kJ mole^{-1}, which coincided with the result of the direct determination.

NAD samples (Reanal, Hungary) used by Rekharsky et al. [14,15] were purified before the calorimetric determinations according to the method described elsewhere [21]; hydrogenase and formate dehydrogenase were isolated according to the technique described previously [32,33]. The method of hydrogenase isolation is described by Schneider and Schlegel [45]. Results of $\Delta H_{(3)}$ determination reported by various authors are collected in Table 1.

* No description of the microcalorimetric setup is given. It can be found in the original literature and in catalogues of companies.

THERMODYNAMICS OF NAD REACTIONS WITH NATURAL COENZYMES AND SYNTHETIC MEDIATORS

Parallel with NAD, other coenzymes participate in enzymatic redox reactions; these are nicotinamide adenine dinucleotide phosphate (NADP), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), etc. It should be noted that natural coenzymes could be substituted in some cases in enzymatic reactions by synthetic mediators: methylviologen (MV), benzylviologen (BV), sodium dithionite, etc. The rate of enzymatic reactions on substitution of a synthetic mediator for a natural coenzyme grows in some cases [20]. Such a substitution can be promising in large-scale industrial enzymatic processes [1]. We should also emphasize that some synthetic mediators react with natural cofactors in the absence of enzymes. This effect can be used in practice for a chemical regeneration of cofactors [2,20]. All the afore-mentioned accounts for the interest of researchers in investigations into the thermodynamics of redox reactions of natural cofactors and synthetic mediators.

Here we shall consider the thermodynamics of NADP cofactor oxidation—reduction, with its physico-chemical properties similar to NAD. Engel and Dalziel [22] compared the thermodynamics (determined equilibrium constants at various temperatures) for reactions of glutamic acid with NADP and NAD. They thus found differences in ΔG and ΔH between the redox reactions of NADP and NAD. For the reaction

$$NADPH + NAD^+ = NADP^+ + NADH \qquad (6)$$

it was calculated that $\Delta G_{(6)} = -3.0$ kJ mole^{-1} and $\Delta H_{(6)} = -2.5 \pm 1.3$ kJ mole^{-1}. The authors [22] pointed out the resultant $\Delta H_{(6)}$ should be considered as highly approximated.

Burton [10] reported unpublished results of Scott and Sturtevant on two independent batteries of calorimetric experiments with the resultant $\Delta H_{(6)}$ equal to -3.8 ± 1.3 kJ mole^{-1} and -4.3 ± 1.7 kJ mole^{-1}. Experimental details were not given.

Beavdette and Langermann [23] determined the enthalpy of FMN redox in a Beckman-190 flow microcalorimeter [24]. The latter was specially modified for work with solutions under oxygen-free conditions. To this end all the tubings to supply solutions to the calorimeter were placed inside wider tubings filled with nitrogen prepurified of oxygen. Such a construction completely ruled out penetration of oxygen into the solution in junctions of the tubings, as well as diffusion of oxygen into the solution through the walls of the tubings. They studied directly in the microcalorimeter the following reaction

$$FMNH_2 + 2[Fe(CN)_6]^{3-} = FMN + 2[Fe(CN)_6]^{4-} + 2\,H^+ \qquad (7)$$

Measurements were made in the potassium phosphate buffer (0.2 M), pH 7.0. FMN was purified before the experiments by paper chromatography [25]; potassium ferricyanide was a product of Fischer Scientific Co. and was used without further purification. The microcalorimetric setup was tested by determining the published [26] ionization enthalpy of the "tris" buffer.

$\Delta H_{(7)} = -164.0 \pm 1.7$ kJ mole^{-1} was obtained. Using $\Delta H_{(7)}$ and the enthalpy of the redox reaction of ferricyanide ion equal to -111.1 ± 1.3 kJ mole^{-1} [27], the authors calculated the enthalpy of the redox reaction FMN

$$FMN + H_{2(gas)} = FMNH_2 \quad \Delta H_{(8)} = -59.4 \pm 2.9 \text{ kJ mole}^{-1} \qquad (8)$$

It should be noted that in the equations of the present review we used conventional symbols for ionic forms of cofactors in aqueous solutions. It should be borne in mind, however, that FMN and $FMNH_2$ at neutral pH in aqueous solutions are, in fact, in the form of negatively (—2) charged ions analogous with the symbol NAD^+ corresponding to NAD ion having a charge of —1, and NADH ion with a charge of —2. It is also noteworthy that the FMN redox reaction (8) is written in a simplified form because at pH 7 both $FMNH_2^{2-}$ and $FMNH^{3-}$ ions ($pK_a = 6.72$) are present in solution [23]. There are no published data on ΔH of $FMNH_2^{2-}$ ionization, which would facilitate a more correct calculation of $\Delta H_{(8)}$.

Until now the published data on dissociation constants and ionization enthalpies of natural redox cofactors and synthetic mediators have been scarce. In particular, no information is available on the ionization enthalpies of NAD, NADH, FMN, $(FMNH)_2$, FAD, etc., which makes thermodynamic calculations difficult.

A change in the free energy of reaction (8) can be calculated from the electrode potential [28]; so for the reaction

$$FMNH_2 + NAD^+ = FMN + NADH + H^+ \qquad (9)$$

we find $\Delta H_{(9)} = +32.2 \pm 3.3$ kJ mole^{-1} and $\Delta G_{(9)} = +18.8$ kJ mole^{-1} at pH 7.

Watt and Burns [29] studied redox reactions involving natural cofactors and synthetic mediators, such as methylviologen, benzylviologen, FMN, FAD, and $Fe(CN)_6^{3-}$, microcalorimetrically. They used a Beckman-190 flow current microcalorimeter [24] modified for work with solutions under oxygen-free conditions in the same way as in the work of Beavdette and Langermann [23]. Sodium dithionite (98% pure) purchased from Associated Chemical Co., U.K., was stored in an atmosphere of dried ($CaCl_2$ as desiccant) argon; purity of the preparation was verified by amperometric and calorimetric titration.

Experiments were run at pH 7, 7.25 and 8 in 0.05 M or 0.1 M buffers, such as phosphate, acetate, cacodelate, "tris" (tris-oxymethylaminomethane) and "tes" (tris-hydroxymethyl-methyl-2-aminoethanesulfonic acid). Reactants were quantified in the buffers spectrophotometrically [30,31]. Ten reactions between various pairs of cofactors and mediators listed above were studied directly in the calorimeter. $\Delta H_{(8)}$ obtained in ref. 29 (-54.8 ± 2.9 kJ mole^{-1}) agreed with the data of Beavdette and Langermann [23]. The enthalpy of the ferricyanide ion redox reaction

$$[Fe(CN)_6]^{3-} + \tfrac{1}{2} H_2 = [Fe(CN)_6]^{4-} + H^+ \quad \Delta H_{(10)} = -110.9 \pm 2.1 \text{ kJ mole}^{-1} \qquad (10)$$

also agreed with literature data [27].

From the data of Watt and Burns [29] we calculated the thermochemical parameters of NAD interaction with the natural cofactors and synthetic

TABLE 2

Thermodynamic parameters of redox reactions of NAD with natural cofactors and synthetic mediators (pH 7, 25° C, the ionization enthalpy of the buffer is equal to zero)

No.	Reaction	ΔH (kJ mole^{-1})	ΔG (kJ mole^{-1})	ΔS (J mole^{-1} K^{-1})
	$NAD^+ + H_{2(gas)} = NADH + H^+$	−27.4 ± 1.7	−17.8	−32.2
1.	$FMNH_2 + NAD^+ = FMN + NADH + H^+$	+29.7 ± 2.9	+19.2	+35.1
2.	$2\ FMNH_2 + NAD^+ = (FMNH)_2 + NADH + H^+$	+2.1 ± 4.2	−6.3	+28.0
3.	$NADPH + NAD^+ = NADP^+ + NADH$	−4.2 ± 1.7	−2.9	−4.2
4.	$S_2O_4^{2-} + NAD^+ + 2\ H_2O = 2\ SO_3^{2-} + NADH + 3\ H^+$	+1.7 ± 2.1	−36.4	+127.6
5.	$2[Fe(CN)_6]^{4-} + NAD^+ + H^+ = 2[Fe(CN)_6]^{3-} + NADH$	+194.6 ± 4.2	+128.9	+220.5
6.	$2\ MV + NAD^+ + H^+ = 2\ MV^+ + NADH$	−61.9 ± 2.1	−23.0	−130.5
7.	$2\ BV + NAD^+ + H^+ = 2\ BV^+ + NADH$	−73.2 ± 2.1	−7.5	−220.5
8.	$FADH_2 + NAD^+ = FAD + NADH + H^+$	+29.3 ± 2.9	+19.2	+33.5

mediators in question. Changes in the free energies of the reactions were calculated using data from ref. 31. Calculations are collected in Table 2. Data from Table 2 allow thermodynamic calculations for a host of redox biochemical reactions.

THERMODYNAMIC PARAMETERS OF NAD-DEPENDENT REACTIONS CALCULATED ACCORDING TO HESS' LAW

Poe et al. [12] and Subramanian [34] studied the thermochemistry of redox enzymatic reactions with no NAD involved (for instance, reaction of oxygen with succinate [12]). From the data of these works and those of Table 2 one can calculate the thermochemical parameters for reactions of natural metabolites studied [12,34] with NAD.

Poe et al. [12] studied, in a differential calorimeter described above, an interaction between oxygen dissolved in the buffers and succinate (reaction 11).

$$2\ ^-O_2C—CH_2—CH_2—CO_2^- + O_2 = 2\ ^-O_2C—CH = CH—CO_2^- + 2\ H_2O \qquad (11)$$

Experiments were run at pH 7.4 (25° C) in 0.10 M phosphate buffer and in 0.10 M "tris" buffer. The average for $\Delta H_{(11)}$ was found to be −151.5 ± 5.10 kJ mole^{-1} succinate; $\Delta G_{(11)}$ = −149.4 kJ mole^{-1}.

TABLE 3

Thermodynamic parameters of NAD reactions with organic compounds of particular biochemical interest (pH 7; 25° C, the ionization enthalpy of the buffer is equal to zero)

No.	Reaction	ΔH (kJ mole^{-1})	ΔG (kJ mole^{-1})	ΔS (J mole^{-1} K^{-1})
A				
1.	$^{-}O_2C-\underset{\underset{OH}{|}}{CH}-CH_3 + NAD^+ = {}^{-}O_2C-CO-CH_3 + NADH + H^+$ (lactate) (pyruvate)	+61.9 ± 1.3	+25.9	+120.5
2.	$^{-}O_2C-CH_2-\underset{\underset{OH}{|}}{CH}-CO_2^- + NAD^+ = {}^{-}O_2C-CH_2-CO-CO_2^- + NADH + H^+$ (malate) (oxalacetate)	+89.5 ± 0.8	+29.7	+200.8
3.	$^{-}O_2C-CH_2-CH_2-CO_2^- + NAD^+ = {}^{-}O_2C-CH=CH-CO_2^- + NADH + H^+$ (succinate) (fumarate)	+106.7 ± 5.4	+73.6	+110.9
4.	$^{-}O_2C-CH_2-CH_2-\underset{\underset{NH_3^+}{|}}{CH}-CO_2^- + NAD^+ + H_2O = {}^{-}O_2C-CH_2-CH_2-CO-CO_2^-$ (glutamic acid) (ketoglutarate) $+ NADH + H^+ + NH_4^+$	+60.2 ± 2.1	+32.2	+94.1

5.	$HCOO^- + NAD^+ + H_2O = HCO_3^- + NADH + H^+$ (formate)	−6.9 ± 1.0	−14.6	+26.0
6.	$CH_3{-}CH_2OH + NAD^+ = CH_3{-}CHO + NADH + H^+$	+50.6 ± 1.7 *	+23.8	+90.0
B				
7.	$CH_3OH + 2\,NAD^+ + H_2O = HCOO^- + NADH + 3\,H^+$ (methanol) (formate)	+51.0	−15.1	+221.8
8.	$CH_3{-}\underset{\displaystyle OH}{\underset{\vert}{CH}}{-}CH_3 + NAD^+ = CH_3{-}CO{-}CH_3 + NADH + H^+$ (propane-2-ol) (acetone)	+81.6	+6.7	+251.0
9.	$CH_3{-}CHO + NAD^+ + H_2O = CH_3{-}CO_2^- + NADH + 2\,H^+$ (acetaldehyde) (acetate)	−19.2	−50.2	+103.8
10.	$^-O_2C{-}CH{=}CH{-}CO_2^- + NAD^+ + 2\,H_2O = {}^-O_2C{-}CO{-}CH_3 + HCO_3^- + NADH + H^+$ (fumarate) (pyruvate)	+33.5	−0.8	+115.1
11.	$^-O_2C{-}CO{-}CH_3 + NAD^+ + 2\,H_2O = {}^-O_2C{-}CH_3 + HCO_3^- + NADH + 2\,H^+$ (pyruvate) (acetate)	−37.7	−67.4	+99.6
12.	$^-O_2C{-}\underset{\displaystyle NH_3^+}{\underset{\vert}{CH}}{-}CH_3 + NAD^+ + H_2O = {}^-O_2C{-}CO{-}CH_3 + NADH + NH_4^+ + H^+$ (alanine) (pyruvate)	+84.5	+38.5	+154.4

* Unpublished data of J. Jordan and W. Brattlie, Pennsylvania State University.

From the data of Poe et al. [12], for the interaction between NAD and succinate

$$^{-}O_2C—CH_2—CH_2—CO_2^{-} + NAD^{+} = {}^{-}O_2C—CH=CH—CO_2^{-} + NADH + H^{+} \quad (12)$$

the following were calculated: $\Delta H_{(12)} = +106.7 \pm 5.4$ kJ mole^{-1}; $\Delta G_{(12)} = +73.6$ kJ mole^{-1} (pH 7.0).

Subramanian [34] studied the thermodynamics of glutamic acid oxidation accompanied by NAD reduction. The reaction was catalyzed by glutamine dehydrogenase [35]. The experiments were run in the LKB-10700 (batch) microcalorimeter equipped with golden cells. Potassium phosphate buffer (0.1 M, pH 7.6) was used in the work.

For the reaction

$$NADP^{+} + {}^{-}O_2C—CH_2—CH_2—\underset{\displaystyle NH_3^{+}}{\underset{|}{CH}}—CO_2^{-} + H_2O$$

$$= NADPH + {}^{-}O_2C—CH_2—CH_2—CO—CO_2^{-} + H^{+} + NH_4^{+} \quad (13)$$

they found: $\Delta H_{(13)} = +66.4 \pm 1.3$ kJ mole^{-1}, $\Delta G_{(13)} = +35.1 \pm 0.4$ kJ mole^{-1}.

Subramanian [34] studied both the direct and reverse reactions [eqn. (13)]. Using $\Delta H_{(13)}$ and $\Delta G_{(13)}$ and the results of Table 2 we calculated $\Delta H = +60.2 \pm 2.1$ kJ mole^{-1} and $\Delta G = +32.2$ kJ mole^{-1} (pH 7) for the NAD-dependent oxidation of glutamic acid.

It is also possible to calculate according to Hess' Law the thermodynamic parameters for the NAD reaction with organic and inorganic compounds undergoing redox processes in aqueous solutions, for which reliable thermodynamic parameters have been determined. Such a calculation requires ΔH_f and ΔG_f values both for oxidized and reduced forms of the appropriate compounds and ions.

Reactions of NAD with inorganic compounds (except the afore-mentioned reactions of NAD with ferricyanide ion and dithionite ion) are not discussed in this review. If necessary, ΔH and ΔG can be calculated for a number of such reactions using thermodynamic parameters of inorganic compounds in aqueous solution [18,19].

The published data on the thermodynamic parameters of organic compounds in aqueous solution are insufficient, especially the data on ΔH of formation of aqueous solutions of organic compounds. The most important thermodynamic parameters (ΔH, ΔG and ΔS) from the available publications can be calculated only for a few reactions of NAD with organic compounds. Table 3 collects ΔH, ΔG and ΔS values calculated for these reactions.

It is noteworthy that ΔH values taken from experimental works (Table 3A) have estimated errors; Table 3B does not give errors for the listed ΔH values because the errors cannot be calculated from the available published data. These values (Table 3B) should, therefore, be considered rough (preliminary). The least reliable value seems to be ΔH of reaction (8) (Table 3B), calculated from the data published in ref. 4. It does not agree with the experimental value found by Burton [10]. Table 3 shows that the entropy factor for all listed reactions is conducive to the shift of reactions towards the formation of NADH.

We should emphasize that using Table 3 one can obtain thermodynamic parameters for some other biochemically important reactions. For instance, combination of eqns. (3) and (10) (Table 3) gives thermodynamic parameters for the reaction of NAD-dependent decarboxylation of succinate with the formation of pyruvate, NADH and bicarbonate ion. The data from Tables 2 and 3 can also be used to calculate thermodynamic parameters of redox reactions of organic compounds given in Table 3 with natural cofactors and synthetic mediators given in Table 2.

The data collected in this review permit calculations of the enthalpies for over 100 biochemically important redox reactions. It has already been noted that this review considers in detail only NAD-dependent reactions for which ΔH_r is determined calorimetrically or can be calculated.

CONCLUSIONS

First, it should be noted that the published experimental data on the thermochemistry of redox NAD-dependent reactions are insufficient. This is principally because of the extremely involved technique of obtaining such data. A researcher should have at his disposal highly sensitive microcalorimetric equipment and fairly pure samples of coenzymes, substrates and enzymes. The modern commercially available microcalorimeters afford determination of the enthalpies of enzymatic reactions in stationary and flow current runnings when both soluble and immobilized enzymes are used (see, for instance, ref. 44). This may well be the reason for the fact that a somewhat greater number of recent publications dealt with determination of the enthalpies of enzymatic reactions.

Second, we should emphasize that at present no conventional "standard" conditions have been elaborated in the thermochemistry of enzymatic reactions. Recommendations have not been developed for running thermochemical experiments, as those accepted by the Commission on Biothermodynamics for the determination of biochemically important equilibrium constants [40—42]. This leads to the fact that researchers run thermochemical experiments under different physico-chemical conditions (pH, ionic strength, buffer, etc.). Conversion of ΔH values for other conditions is difficult and sometimes impossible because of the lack of necessary data. Some authors reported that the thermodynamics of enzymatic reactions are strongly dependent (a difference is sometimes equal to several kcal $mole^{-1}$) on change in the ionic strength [23] or on the buffer used [29,12].

We find it expedient to accept the following conditions as standard: temperature, 298.15 K, pressure 1 atm; potassium or sodium phosphate buffer (0.05 M) at pH 7. The enthalpy and free energy of ionization are reliably determined (see, for instance, ref. 43), which is important for follow-up thermodynamic calculations of thermodynamic parameters. Besides, equilibrium constants and changes in free energies are mostly determined under these physico-chemical conditions (see, for instance, ref. 39). We thus obtain a comprehensive thermodynamic characterization of enzymatic reactions (ΔH_r, ΔG_r, ΔS_r), all the parameters referring to identical physico-chemical conditions.

We believe it is not expedient to use a pure aqueous solution or hypothetical solutions with pH 0 as standards. In practice, enzymatic reactions cannot be run under such conditions, whereas an accurate thermodynamic conversion of ΔH, ΔG, and ΔS for these conditions from experimental ones requires many additional experimental data.

The data given in this review can also be used in practice, i.e., for thermodynamic calculations of optimal physico-chemical conditions of redox enzymatic reactions.

REFERENCES

1 Advances in Biochemical Engineering, Vol. 12, Immobilized Enzymes, II, Springer Verlag, Berlin, 1979.
2 K.J. Skinner, Chem. Eng. News, 33 (1975) 23.
3 S. Katz, Biochem. Biophys. Acta, 17 (1955) 226.
4 H.D. Brown (Ed.), Biochemical Microcalorimetry, Academic Press, New York, 1969.
5 L. Donnovan, K. Barclay, K. Otto and N. Jespersen, Thermochim. Acta, 11 (1975) 151.
6 N.D. Jespersen and J. Jordan, Anal. Lett., 3 (1970) 323.
7 N. Jespersen, Thermochim. Acta, 17 (1976) 23.
8 K. Burton and T.H. Wilson, Biochem. J., 54 (1953) 86.
9 R.A. Stinson and J.J. Holbrook, Biochem. J., 131 (1973) 719.
10 K. Burton, Biochem. J., 143 (1974) 365.
11 F.L. Rodkey, J. Biol. Chem., 234 (1959) 188.
12 M. Poe, H. Gutfreund and R.W. Estabrook, Arch. Biochem. Biophys., 122 (1967) 204.
13 F.L. Crane, J.L. Glenn and D.E. Green, Biochim. Biophys. Acta, 22 (1956) 475.
14 M.V. Rekharsky, A.M. Egorov, G.L. Gal'chenko and I.V. Berezin, Dokl. Akad. Nauk S.S.S.R., 249 (1979) 1156.
15 M.V. Rekharsky, A.M. Egorov, G.L. Chal'chenko and I.V. Berezin, Zh. Obshch. Khim., 10 (1980) 2365.
16 P. Monk and I. Wadso, Acta Chem. Scand., 22 (1968) 1842.
17 I. Wadso, Acta Chem. Scand., 22 (1968) 927.
18 V.P. Glyshko (Ed.), Termicheschie Konstantu Vechestv, Vols. 1—9, VINITI, Moscow, 1965—1978.
19 Natl. Bur. Stand. (U.S.), Tech. Note No. 270.
20 A.P. Osipov and A.M. Egorov, in V.L. Kretovich and I.V. Berezin (Eds.), Biologicheskay Khimia, Vol. 12, VINITI, 1978, pp. 153—209.
21 K. Balziel, J. Biol. Chem., 238 (1963) 1538.
22 O.C. Engel and K. Dalziel, Biochem. J., 105 (1967) 691.
23 M.V. Beavdette and N. Langermann, Arch. Biochem. Biophys., 161 (1974) 125.
24 J.M. Sturtevant and P.A. Lyons, J. Chem. Thermodyn., 1 (1969) 201.
25 R.D. Draper and L.L. Ingraham, Arch. Biochem. Biophys., 125 (1968) 802.
26 R.G. Bates and H.B. Hetzer, J. Res. Natl. Bur. Stand. 65 (1961) 667.
27 G.I.H. Hanania, O.H. Irvine, W.A. Eaton and P. Geirge, J. Phys. Chem., 71 (1967) 2022.
28 H.J. Lowe and W.M. Clark, J. Biol. Chem., 221 (1956) 983.
29 G.D. Watt and A. Burns, Biochem. J., 152 (1975) 33.
30 L. Yu and M.J. Wolin, J. Bacteriol., 98 (1968) 51.
31 H.A. Soler (Ed.), Handbook of Biochemistry, Marcel Dekker, Cleveland, 1968, p. 29.
32 E.E. Pinchykova, S.D. Varfolomeev and I.V. Berezin, Dokl. Akad. Nauk S.S.S.R., 236 (1977) 1253.
33 Yu.V. Rodionov. Yu.A. Avilova, A.M. Egorov and I.V. Berezin, Biokhimiya, 42 (1977) 1896.

34 S. Subramanian, Biophys. Chem., 7 (1978) 375.
35 O.G. Cross and H.F. Fisher, J. Biol. Chem., 245 (1970) 2612.
36 S.J. Phillips and R.C. Phillips, Chem. Rev., 66 (1966) 501.
37 J.J. Christensen, R.M. Izatt and L.D. Hansen, J. Phys. Chem., 89 (1967) 213.
38 H.A. Krebs and H.L. Kornberg, Energy Transformation in Living Matter, Springer-Verlag, Berlin, 1957.
39 T.E. Barman, Enzyme Handbook, Vols. 1—2, Springer-Verlag, Berlin, 1969.
40 Mol. Cell. Biochem., 15 (1977) 225.
41 Biochem. J., 153 (1977) 1.
42 Biochem. Biophys. Acta, 451 (1977) 1.
43 J.J. Christensen, L.D. Hansen and R.M. Izatt, Handbook of Proton Ionization Heats, Marcel Dekker, New York, 1976.
44 M.V. Rekharsky, A.M. Egorov and G.L. Gal'chenko, Sci. Tools, 27 (1980) 51.
45 K. Schneider and H.G. Schlegel, Biochim. Biophys. Acta, 452 (1976) 66.
46 S. Subramanian, Trends Biochem. Sci., 4 (1979) 102.