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Enthalpy and Heat Capacity Changes for the Reduction of Insulin†

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ABSTRACT: The enthalpy changes for the reduction of three disulfide bonds of insulin by dithiothreitol (DTT) were calorimetrically measured at various temperatures ranging from 289 to 308 K. The reduction was performed in three different buffer solutions of pH 9.6, and the observed heat changes were corrected for the ionization heats of the buffer components to obtain the net heats of reduction of insulin with DTT. By subtracting the enthalpy of DTT oxidation reported in the previous paper [Fukada, H., & Takahashi, K. (1980a) *J. Biochem. (Tokyo) 87*, 1105-1110], we determined the standard enthalpy of reduction of insulin to be ΔH_r = 93.4 ± 7.8 kJ (mol of insulin)$^{-1}$ at 298 K. The heat capacity change was $\Delta C_{p,r}$ = 3.2 ± 0.3 kJ mol^{-1} K^{-1}. Using the heat of oxidation of the cysteine residue, we estimated the enthalpy change for the conformational transition of insulin induced by the cleavage of three disulfide bonds to be ΔH_{conf} = 91 kJ mol^{-1} at 298 K. The heat capacity change was 2.1 kJ mol^{-1} K^{-1}. These results imply that the conformational transition taking place during the reduction of three disulfide bonds is thermodynamically of the same nature as the thermal denaturation observed for other globular proteins.

Many calorimetric measurements have been made to obtain the thermodynamic parameters for protein denaturation processes (Tsong et al., 1970; Privalov, 1974, 1979; Privalov & Khechinashvili, 1974; Pfeil & Privalov, 1976a-c). However, the data hitherto accumulated are limited to those for thermally and chemically induced unfolding and do not cover denaturation induced by the reduction of disulfide bonds. In our previous study (Fukada & Takahashi, 1980b), we have carried out calorimetric measurements on the reduction of insulin with dithiothreitol (DTT)[1] at 298 K and have shown that the enthalpy of the conformational change of an insulin molecule induced by the cleavage of disulfide bonds has a value very similar to those reported for the thermal or chemical denaturation of other globular proteins.

It has been shown that many reactions involving proteins are accompanied by large changes in the apparent heat capacity (Sturtevant, 1977). In this respect, it would be worthwhile to determine the heat capacity change as well as the enthalpy change also with the present system. In this study, further calorimetric measurements were made on the reduction of insulin with DTT to obtain the net heat of reduction of insulin at different temperatures, and the heat capacity change associated with the conformational transition of insulin taking place during the reduction of the three disulfide bonds was evaluated.

Materials and Methods

Bovine zinc insulin (molecular weight 5700) and DTT were purchased from Sigma Chemical Co. and were used without further purification. All other chemicals used were commercial preparations of reagent grade. Solutions were prepared with distilled and deionized water which was previously saturated with nitrogen to remove oxygen before use.

Crystalline insulin (zinc content 0.5%, lot no. 24C-3130) was dissolved in 0.01 N HCl to a concentration of about 10 mg cm^{-3} and dialyzed against the same acid solution at 5 °C to remove zinc according to the method described by Cunningham et al. (1955). The solution was then exhaustively dialyzed against 1 mmol dm^{-3} sodium carbonate, pH 9.6, buffer and was used as a stock solution. The final buffer pH was adjusted at the temperature of the measurements.

The insulin stock solutions were diluted with the appropriate buffer solution of pH 9.6 (0.4 mol dm^{-3} carbonate, glycine, or ammonium buffer, each containing 1 mmol dm^{-3} EDTA) to give an insulin concentration of 0.16 mmol dm^{-3} which was determined spectrophotometrically by using an absorption coefficient of 1.05 cm^2 mg^{-1} at 276 nm and at pH 7 (Frank & Veros, 1968).

The DDT solution was prepared in the same buffer as described in the previous paper (Fukada & Takahashi, 1980a). The DDT concentration in the reactant solution was 15 mmol dm^{-3}, being 94-fold molar excess over insulin.

Calorimetric Measurements. The flow microcalorimeter described previously (Fukada & Takahashi, 1980a) was used with further modification. The calorimeter cell consisted of 2 m of Teflon tubing instead of 1 m as in the original design to attain a longer residence time. The sensor unit, consisting of a set of 12 Sanyo thermocouple plates in the prototype, was replaced by four Melcor thermoelectric modules (C.P. 1.4-71-06L) to improve the time constant of heat exchange between the cell and the aluminum heat sink.

The calorimeter was calibrated by the heat of neutralization, by using ΔH = −56.58 kJ mol^{-1} at 298 K (Ackermann, 1958). For other temperatures, values were obtained by using Ackermann's equation.

The solutions were usually delivered at a flow rate of 0.2 cm^3 min^{-1} which was frequently determined by weighing the

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[1] Abbreviations: DTT, dithiothreitol; DTE, dithioerythritol; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin.

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Table I: Observed Heats for the Reaction of Insulin with DTT at pH 9.6

buffer	ΔH_i[a] (kJ mol^{-1})	ΔH_{obsd}[b] [kJ (mol of insulin)$^{-1}$] 289 K	293 K	298 K	303 K	308 K
carbonate	19.2	7.7 ± 3.6 (2)	7.5 ± 3.8 (4)	26.9 ± 3.8 (7)	33.3 ± 2.2 (5)	37.2 ± 0.6 (4)
glycine	44.5	−58.5 ± 0.7 (2)	−44.7 ± 1.0 (5)	−24.8 ± 5.3 (7)	−9.0 ± 1.9 (5)	−11.5 ± 0.6 (4)
ammonium	52.3	−80.2 (1)	−68.8 ± 2.5 (4)	−40.5 ± 7.7 (6)	−32.0 ± 4.2 (6)	−25.6 ± 0.5 (4)

[a] Enthalpy change for the ionization of buffer component. [b] Observed enthalpy change ± standard error. The values given in parentheses are the number of measurements.

Table II: Enthalpy Change and Calorimetrically Determined Proton Uptake during the Reaction of Insulin with DTT at pH 9.6[a]

T (K)	ΔH_0 [kJ (mol of insulin)$^{-1}$]	ΔH_{DTT} [kJ (mol of DTT)$^{-1}$]	ΔH_r [kJ (mol of insulin)$^{-1}$]	n_0	n_{DTT}	n_r
289	58.7 ± 9.4	−1.3 ± 2.7	62.6 ± 12.4	−2.7	−1.3	1.1
293	51.6 ± 8.1	−5.1 ± 2.6	66.9 ± 11.5	−2.3	−1.2	1.2
298	66.1 ± 0.3	−9.1 ± 2.6	93.4 ± 7.8	−2.0	−1.1	1.1
303	70.9 ± 10.0	−12.5 ± 2.5	108.4 ± 12.5	−1.9	−1.0	1.0
308	73.6 ± 1.0	−15.3 ± 2.4	119.5 ± 7.3	−1.9	−0.9	0.8

[a] Precision in standard error.

effluent. The residence time of the solution after mixing in the cell was 4 min at this flow rate. The calorimeter signal reached a steady state within 10 min and was recorded for at least 20 min.

With this system, a heat effect of 200 μJ s^{-1} could be evaluated with a precision of ±0.4%. In practical measurements, the observed heat effect was in the range of 5–200 μJ s^{-1}.

The heats of dilution of DTT and insulin solutions were separately measured and applied as corrections to obtain the net heat effect due to the reaction alone. The concentration of DTT was adjusted so that the reaction with insulin was completed within the residence time in the calorimeter cell.

During the measurements, the entire assembly was placed in a nitrogen atmosphere to prevent air oxidation of DTT. Also the exterior of the Teflon tubing used for introducing the solutions was flushed with nitrogen. Measurements were made at 289, 293, 298, 303, and 308 K as determined by a certified standard mercury thermometer. The pH of the solutions was checked before and after each run at the temperature of the experiment.

Results and Discussion

In Figure 1, typical recordings of the flow calorimeter signal during the mixing of insulin with DTT (A) and the dilution of DTT with buffer (B) obtained at 293 K are shown. The heat of dilution of insulin solution was found to be practically negligible.

The heats of reaction of insulin with DTT, ΔH_{obsd}, at various temperatures in three different buffer solutions at pH 9.6 are summarized in Table I. This table also includes the enthalpy change for the ionization of each buffer component, ΔH_i, at 298 K (Watt & Sturtevant, 1969; Izatt & Christensen, 1970).

The time courses of reaction were examined by measuring changes in the optical rotation and in the absorbance of the reaction mixture prepared under the same conditions as those employed for the calorimetric measurements. The methods are described in the previous paper (Fukada & Takahashi, 1980b). Also the number of disulfide bonds reduced was determined as the amount of oxidized DTT by the method of Iyer & Klee (1973). It was found that all three disulfide bonds are reduced with 2–3 min at all the temperatures studied.

Figure 2 shows the relation between the observed heats and the heats of ionization of buffer components. The net enthalpy change of the reaction of insulin with DTT, ΔH_0, and the

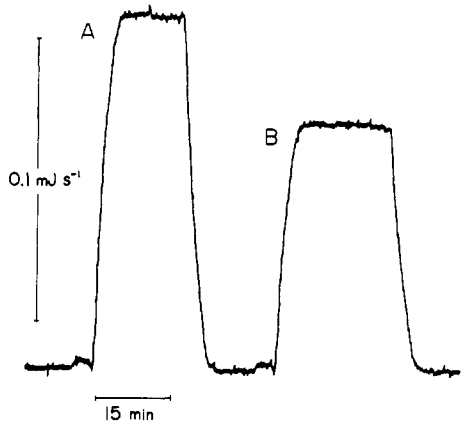

FIGURE 1: Calorimeter signal produced by (A) the reaction of insulin with DTT in ammonium buffer, pH 9.6, and (B) the dilution of DTT solution with the buffer at 293 K. The insulin and DTT concentrations were 0.156 and 15 mmol dm^{-3}, respectively. The solutions were pumped at flow rates of 0.198 cm^3 min^{-1} (insulin) and 0.206 cm^3 min^{-1} (DTT).

number of protons released from the buffer, n_0, were determined from the intercept and the slope of the plots according to the relation described in the literature (Dobry & Sturtevant, 1952; Hinz et al., 1971). In this procedure, it was assumed that the enthalpies of ionization of the buffers are independent of temperature.

The values of ΔH_0 and n_0 thus determined at the various temperatures are summarized in Table II. The enthalpy change of the reduction of insulin, ΔH_r, was obtained by subtracting that of DTT oxidation, ΔH_{DTT}, from the net enthalpy change of the reaction, ΔH_0. The values of ΔH_{DTT} and ΔH_r are given in the third and fourth columns, respectively. The enthalpy change of DTT oxidation was previously determined at 298 K (Fukada & Takahashi, 1980a). Values for other temperatures were estimated by using the van't Hoff relation on an assumption that the enthalpy changes for the oxidation of fully ionized DTT, ΔH_{int}^{DTT}, and for the first and second ionization of two sulfhydryls, ΔH_1^{DTT} and ΔH_2^{DTT}, are constant over the temperature range studied. The calculation was made in the following manner.

The enthalpy change of DTT oxidation at a given temperature, ΔH_{DTT}, is expressed as

$$\Delta H_{DTT} = \Delta H_{int}^{DTT} + n_1 \Delta H_1^{DTT} + n_2 \Delta H_2^{DTT} \quad (1)$$

where n_1 is the mole fraction of nonionized DTT at a given

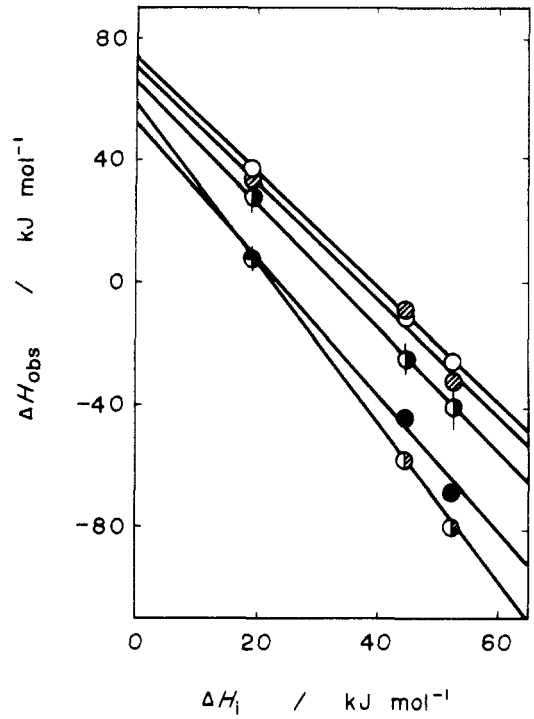

FIGURE 2: Plots of the observed heat for the reaction of insulin with DTT vs. the ionization heat of the buffer at pH 9.6 and various temperatures (K): (⊘) 289; (○) 293; (◑) 298; (●) 303; and (⊕) 308.

pH and n_2 is the mole fraction of singly ionized DTT, the mole fractions being given by the following equations:

$$n_1 = \frac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (2)$$

$$n_2 = \frac{[H^+]^2 + K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (3)$$

where K_1 and K_2 are the ionization constants of the first and second ionizable sulfhydryl groups, respectively.

pK_1 and pK_2 are determined to be 9.19 and 10.13, respectively, at 298 K (Fukada & Takahashi, 1980a). K_1 and K_2 for other temperatures were obtained with the van't Hoff relation by using the following equations:

$$pK_1 = \frac{\Delta H_1^{DTT}}{2.303R}\left(\frac{1}{T} - \frac{1}{298}\right) + 9.19 \quad (4)$$

$$pK_2 = \frac{\Delta H_2^{DTT}}{2.303R}\left(\frac{1}{T} - \frac{1}{298}\right) + 10.13 \quad (5)$$

For the calculation, the values of ΔH_1^{DTT} = 47.9 kJ mol^{-1}, ΔH_2^{DTT} = 21.2 kJ mol^{-1}, and ΔH_{int}^{DTT} = −37.6 kJ mol^{-1} which were reported in the previous paper were used.

The corresponding proton uptakes, n_{DTT} and n_r, are also included in Table II. The value of ΔH_r at 298 K is higher than the value observed earlier, 78.8 ± 7.9 kJ mol^{-1}, which was determined with an isoperibol calorimeter (Fukada & Takahashi, 1980b). In the previous measurements, it was impossible to remove completely the air from the calorimeter cell, and the DTT molecules which were present in excess and remained unreacted with insulin were slowly oxidized, resulting in a heat effect which was recorded as a linear exothermic process. For separation of the heat of reaction of insulin with DTT, subtraction of the heat effect due to this DTT oxidation was made by a simple extrapolation of the calorimeter recordings. A systematic error may have been involved in this correction procedure and may be the cause of the discrepancy.

As shown in Table II, over the temperature range studied the reduction of insulin is accompanied by a large positive change in enthalpy, the value of which is strongly dependent on temperature. The heat capacity change of the insulin reduction as estimated from the temperature dependence of ΔH_r is $\Delta C_{p,r}$ = 3.2 ± 0.3 kJ mol^{-1} K^{-1} or 0.56 ± 0.05 J g^{-1} K^{-1}.

The protons taken up by insulin in the reduction are considered to be absorbed by the protonation of cysteinyl residues formed in the reduction. However, since the insulin molecule contains one lysyl and four tyrosyl residues which further dissociate to release protons upon the conformational change during the reduction, it is probable that additional protons are actually taken up by the cysteinyl residues. The number of protons released from two residues, Δn, can be calculated by using their known pK values on the basis of the following equation:

$$\Delta n = 4\left(\frac{[H^+]}{[H^+] + K_N^{Tyr}} - \frac{[H^+]}{[H^+] + K_U^{Tyr}}\right) + \left(\frac{[H^+]}{[H^+] + K_N^{Lys}} - \frac{[H^+]}{[H^+] + K_U^{Tyr}}\right) \quad (6)$$

where K_N^{Tyr} and K_N^{Lys} are the dissociation constants of tyrosyl and lysyl residues in native insulin and K_U^{Tyr} and K_U^{Lys} are the dissociation constants of tyrosyl and lysyl residues in the unfolded insulin, respectively.

The pK value of the lysyl residue in native insulin is reported to be 11.2 at 293 K (Bradbury & Brown, 1977). The value pK_N^{Lys} = 11.06 for 298 K was obtained by using the ionization enthalpy, ΔH_i^{Lys} = 43.9 kJ mol^{-1} (Shiao & Sturtevant, 1976), and the van't Hoff relation. The pK value of the tyrosyl residues in the native insulin molecule was estimated from the absorbance measurement of the insulin solution at λ = 295 nm by using the known absorption coefficient for the ionized tyrosyl residue (Tachibana & Murachi, 1966; Markland, 1969). Since there are four tyrosyl residues involved, this pK value is the apparent one corresponding to the average of the four residues. The pK value thus determined was pK_N^{Tyr} = 10.5 at 298 K. As for the pK value of the two residues in the unfolded insulin, the values reported by Shiao & Sturtevant (1976) which were determined on the normally ionizing groups in three proteins were used. By use of these values, the number of protons released from the tyrosyl and lysyl residues was calculated to be Δn = 0.51 + 0.17 = 0.68. If it is assumed that no other amino acid residue is responsible for proton uptake or release, the total number of protons taken up by cysteinyl residues at 298 K becomes

$$n_r + \Delta n = 1.8$$

Since, during the reduction, the six cysteinyl residues are formed, the pK value of cysteine is estimated as

$$pK^{Cys} = pH - \log\frac{6 - 1.8}{1.8} = 9.22$$

which is in good agreement with the value generally accepted for the cysteinyl residue. This fact strongly suggests that the above value for the number of protons taken up during the insulin reduction obtained in the present study is a reasonable one.

The reaction of insulin with DTT may be thought of as involving two successive processes, the reduction of disulfide bonds by DTT and the resulting conformational change of insulin. It has been shown that reduced insulin is approxi-

mately a random coil (Markus, 1964). We also have found from the circular dichroism and optical rotatory measurements that insulin undergoes a large conformational change upon the cleavage of three disulfide bonds. In order to separate the enthalpy change of these two processes and to determine the thermodynamic parameters for the conformational change, an attempt was made to estimate the enthalpy change in the reduction of cystine by DTT by using data reported in the literature.

Rupley and his co-workers (Lapanje & Rupley, 1973; Johnson et al., 1978) have made calorimetric measurements on the reduction of oxidized glutathione and of denatured (unfolded) proteins by DTT or DTE at neutral and alkaline pHs. According to their results, the apparent heats of reduction of unfolded BSA and unfolded lysozyme are essentially the same as that found for the reduction of oxidized glutathione by DTT, indicating that the formation of disulfide bonds introduces no remarkable strain into the unfolded proteins (Johnson et al., 1978). Accordingly the enthalpy change of these processes is considered to be mainly due to the heat of reduction of cystine by DTT or DTE.

The enthalpy change of the reduction of cystine by DTT, ΔH_{ex}, is given as the difference in the oxidation enthalpies of DTT (ΔH_{DTT}) and cysteine (ΔH_{Cys}):

$$\Delta H_{ex} = \Delta H_{DTT} - \Delta H_{Cys} \quad (7)$$

ΔH_{ex} is pH dependent and is further given as

$$\Delta H_{ex} = \Delta H_{DTT} - \Delta H_{int}^{Cys} - 2\Delta H_{i}^{Cys}\frac{[H^+]}{[H^+] + K^{Cys}} \quad (8)$$

where ΔH_{int}^{Cys} is the intrinsic enthalpy change for the oxidation of the fully ionized cysteinyl residue and ΔH_{i}^{Cys} and K^{Cys} are the ionization enthalpy and the ionization constant of cysteine sulfhydryl, respectively.

ΔH_{DTT} has been estimated as a function of pH in our previous paper (Fukada & Takahashi, 1980a). ΔH_{int}^{Cys} and K^{Cys} were determined by the method of successive calculation by using eq 8 and the values for ΔH_{ex} at three different pHs reported by Lapanje & Rupley (1973) and Johnson et al. (1978).[2] The value of ΔH_{int}^{Cys} was practically constant over the pH range studied.

For this calculation, ΔH_{i}^{Cys} was taken to be 25.9 kJ mol^{-1}, which was determined for the ionization of glutathione sulfhydryl (Jagt et al., 1972). The values obtained were $\Delta H_{int}^{Cys} = -17.0$ kJ mol^{-1} and $pK^{Cys} = 9.25$ at 298 K. It should be noted that the value of pK^{Cys} obtained here is in good agreement with the value described above which was estimated from the analysis of proton uptake during the reduction.

ΔH_{Cys} at pH 9.6 was calculated at various temperatures by using the van't Hoff relation and the values of pK^{Cys}, ΔH_{int}^{Cys}, and ΔH_{i}^{Cys}. We assumed that the value of $-\Delta H_{Cys}$ thus obtained is the same as the enthalpy of reduction by DTT of the disulfide bonds in insulin and estimated the enthalpy of the conformational change of insulin associated with the disruption of its three disulfide bonds, ΔH_{conf}, by the relation $\Delta H_{conf} = \Delta H_r + 3\Delta H_{Cys}$. The values obtained are shown in Table III together with the enthalpies of insulin reduction, ΔH_r, and of cystine reduction, $-\Delta H_{Cys}$. The value of $-\Delta H_{Cys}$ changes from negative to positive values as the temperature goes up, depending on the ionization state of the sulfhydryls formed. It should be noted that the value for ΔH_{conf} per gram of protein is quite similar to the thermal denaturational enthalpy of globular proteins (Privalov & Khechinashvili, 1974; Pfeil & Privalov, 1976b; Privalov, 1979).

Table III: Enthalpy Changes of the Reduction of Insulin, the Reduction of Cystine, and the Conformational Change of Reduced Polypeptide Chain and Heat Capacity Change of the Conformational Change at pH 9.6

T (K)	ΔH_r (kJ mol^{-1})	ΔH_{Cys} (kJ mol^{-1})	ΔH_{conf} kJ mol^{-1}	ΔH_{conf} J g^{-1}	$\Delta C_{p,conf}$ kJ mol^{-1} K^{-1}	$\Delta C_{p,conf}$ J g^{-1} K^{-1}
289	62.6	−2.8	71.0	12.5		
293	66.9	−1.2	70.5	12.4		
298	93.4	0.9	90.7	15.9	2.1 ± 1.2	0.35 ± 0.21
303	108.4	2.9	99.7	17.5		
308	119.5	4.5	106.0	18.6		

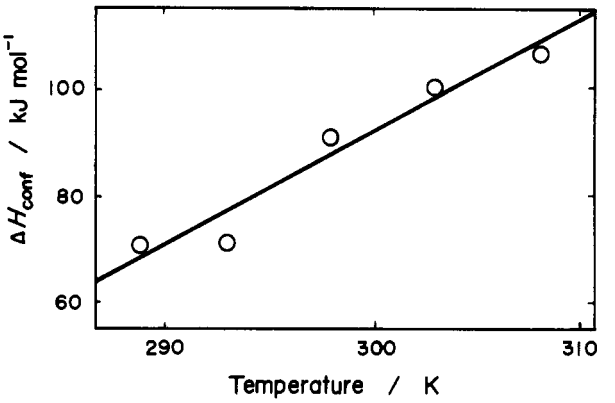

FIGURE 3: Temperature dependence of the enthalpy of the conformational change of insulin.

The protein undergoes spontaneous unfolding when the disulfide bonds no longer exist. This means that the unfolding of the reduced polypeptide chains is accompanied by an increase in conformational entropy which is large enough to overcome the energetic contributions of various intramolecular interactions such as hydrogen bonds, hydrophobic effects, etc., which stabilize the native structure of the protein. The enthalpy change associated with the cleavage of three disulfide bonds is, however, very much smaller in magnitude than the enthalpy of unfolding at any temperature studied. It is interesting to note that although the involvement of three disulfide bonds is essential to maintain the three-dimensional structure of the protein, the energetic contribution introduced by their formation is only a part of the entire enthalpy increase in going from the folded to the unfolded protein.

Figure 3 shows a plot of conformational enthalpy vs. temperature. It was found that a linear relationship holds, and the corresponding heat capacity change was calculated and is given in the fifth column of Table III. The value of $\Delta C_{p,conf}$ thus determined is again very close to that for the thermal denaturation of other proteins (Privalov, 1979).

The above finding that the two thermodynamic parameters ΔH_{conf} and $\Delta C_{p,conf}$ resemble those found for the unfolding transitions of other globular proteins strongly suggests that the conformational change in insulin associated with the cleavage of three disulfide bonds is thermodynamically of the

[2] In our preliminary measurements, the heat of oxidation of DTE was determined to be −7.7 ± 1.9 kJ mol^{-1} at pH 9.5 and 298 K, which are the same conditions as employed by Johnson et al. (1978) for their calorimetric measurements. Since this value is very close to that for DTT oxidation (−5.7 ± 2.6 kJ mol^{-1} at pH 9.5 and 298 K), it was assumed that the enthalpy change for the reduction of cysteine by DTT is essentially the same as that for the reduction of cysteine by DTE. A manuscript of the calorimetric study on DTE oxidation together with its pH titration is under preparation.

same nature as protein thermal denaturation.

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Interdependence of Neurophysin Self-Association and Neuropeptide Hormone Binding As Expressed by Quantitative Affinity Chromatography†

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ABSTRACT: The reciprocal modulation of neurophysin self-association and noncovalent peptide–protein interaction between neurophysin and the hormones oxytocin and vasopressin has been assessed by quantitative affinity chromatography. Competitive elutions of radiolabeled bovine neurophysin II (NPII) from the affinity matrices Met-Tyr-Phe-ω-(aminohexyl)- [and (aminobutyl)-] agarose were performed with increasing concentrations of either of the soluble ligands oxytocin or lysine-vasopressin. Also, the dependence of NPII retardation by the same adsorbents on the concentration of applied protein was investigated in the absence of soluble ligand. The affinity constant of NPII for the immobilized peptide increased markedly with increasing amounts of applied protein and with the addition of small amounts of soluble ligand, the latter being more pronounced at higher protein concentrations. The affinity constant of the protein for the soluble ligand showed a smaller increase. The variation of $1/(V - V_0)$ (where V = the NPII elution volume and V_0 = the elution volume of noninteracting control protein) with soluble ligand concentration was linear except near [ligand] = 0. The quantitative affinity chromatographic results on the tripeptidyl affinity columns are consistent with the view that NPII exists in a monomer ⇌ dimer equilibrium, with the dimer exhibiting a stronger interaction with both neuropeptide and tripeptide analogues. The data also indicate that the self-associated protein dimer itself exhibits cooperativity, that is, stronger binding of the immobilized ligand at one site when a second site is occupied with a molecule of the soluble ligand than when no soluble ligand is bound. The deduction from the above of ligand-induced dimerization is evident also in the increased retardation of NPII on neurophysin–Sepharose when the eluting buffer contains soluble peptide hormone.

Neurophysins, a class of disulfide-rich acidic proteins, are synthesized in the hypothalamus and then transported via axons to the posterior pituitary, in neurosecretory granules, as parts of noncovalent complexes with the peptide hormones oxytocin (OXT)[1] and VP (Seif & Robinson, 1978; Acher, 1979; Breslow, 1979). It has become apparent from biosynthesis studies (Sachs et al., 1969; Russell et al., 1980; Nicolas et al., 1980b; Schmale & Richter, 1981; Chaiken et al., 1981) that the origin of neurophysins and the associated peptide hormones is coordinate and involves precursor molecules which

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[1] Abbreviations: NPI and -II, bovine neurophysins I and II; Nps, *p*-nitrophenylsulfenyl; OXT, oxytocin; LVP, 8-lysine-vasopressin; VP, vasopressin; Met-Tyr-Phe-AH-A and Met-Tyr-Phe-AB-A, Met-Tyr-Phe immobilized through the α-carboxyl to (aminohexylamino)- and (aminobutylamino)agarose, respectively.

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