Biophysical Chemistry 10 (1979) 129–141

THERMODYNAMIC COMPENSATION PROCESS IN INTERACTING PROTEIN SYSTEMS: DEFINITION OF THERMODYNAMIC COMPENSATORY TEMPERATURE, $\langle T_c \rangle$☆

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Received 26 September 1978

In our thermodynamic analysis of the non-linear Van 't Hoff expression as applied to several self-associating systems – specifically in the cases of bovine liver L-glutamate dehydrogenase (GDH), glucagon and S-carboxymethylated apo A-II protein from human high density lipoprotein – we have examined the interrelationships of a number of thermodynamic temperatures as they affect the association process. We found the principal determinants of the linear thermodynamic compensation process to be $\Delta S^\circ_{(T)}/\Delta C^\circ_{p(T)} = \langle \Delta T_c \rangle / \langle T_{exp} \rangle$, where $\langle \Delta T_c \rangle = (\langle T_c \rangle - T_{exp})$. We have defined the unique compensatory temperature, $\langle T_c \rangle$, for any interacting protein system, at which the contributions of enthalpy and entropy to the association process are balanced.

1. Introduction

A closer examination of the thermodynamics of several self-associating protein systems by analytical ultracentrifugation, molecular sieve chromatography and circular dichroic studies, over a wide range of temperatures, reveals that the association process is affected by several specific thermodynamic temperatures which we have designated $\langle T_c \rangle$, the temperature of compensation; T_H, the harmonious temperature at which the Gibbs free energy change is at a minimum; $T_{\phi h}$, the temperature at which the enthalpy of the solution becomes zero, and T_s, the temperature at which the entropy becomes zero.

In these studies, we closely examine the interrelationships of these thermodynamic temperatures as they affect a) bovine liver L-glutamate dehydrogenase [1], b) S-carboxymethylated apo A-II (Cm apo A-II) from the human high density lipoprotein complex [2], and c) glucagon [3].

Recent studies have reported that bovine liver L-glutamate dehydrogenase undergoes isodesmic association in 0.2 M sodium phosphate buffer, 1×10^{-3} M EDTA at pH 7.0 [1,4–7]. Glucagon, a 29-amino acid residue polypeptide hormone that is bound to target tissues and activates adenyl cyclase, undergoes monomer–trimer association in 0.2 M K_2HPO_4, pH 10.6 [3,8–11].

The reduced carboxymethylated form of apo A-II protein from human high density lipoproteins has a molecular weight of 8696 g/mole and undergoes monomer–dimer association in 0.01 M phosphate, pH 7.4 [2]. In each of these three protein systems, self-association appears to be endothermal at low and exothermal at high temperatures, with a resultant variation in heat capacity.

In considering a non-linear Van 't Hoff expression, then, the self-association reaction has a significant effect on the partial molar heat capacity of the interacting protein system, i.e., $d(\Delta H^\circ)/dT = dT\,d(\Delta S^\circ)/dT = \Delta C_p^\circ$. The heat capacity change is an essential

☆ This work was supported by National Science Foundation Grant PCM 76-04367 and in part by General Computer Research support, College of Arts and Sciences, University of Florida. Portions of this work have been presented at the 176th A.C.S. Meeting, "Symposium on Physical Aspects of Protein Interaction", Miami Beach, FL (1978) and the Biophysical Society Meeting, session T-PM-A10, Atlanta, GA (1979).
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thermodynamic quantity that expresses the interaction of the various hydrophobic groups with H_2O [13–19]. Perhaps the most striking aspect of the ordering of water molecules about these hydrophobic groups is the thermal lability, which imparts a large, anomalous heat capacity to aqueous solutions of hydrophobic or partially hydrophobic solute, as observed and explained by Edsall [20] and Frank and Evans [12].

The linear relationship between the enthalpy–entropy compensation has been recognized and formulated in some detail by Barclay and Butler [21], Everett and Wynne-Jones [23], Pitzer [22] and Lumry and Rajender [25]. Lumry and Rajender described the relationship by

$$\Delta H^\circ_{(T)} = \Delta G^\circ_{\langle T_c \rangle} + \langle T_c \rangle \Delta S^\circ_{(T)}.$$

The concept of such enthalpy–entropy compensation forms the basis for these studies of the non-linear Van 't Hoff plot [26–32].

In this communication, we report the thermodynamic analysis of the non-linear Van 't Hoff expressions of several self-associating protein systems, specifically in the cases of bovine liver L-glutamate dehydrogenase, glucagon and S-carboxymethylated apo A-II from the human high density lipoprotein complex. We have defined the unique compensatory temperature, $\langle T_c \rangle$, for any interacting protein system, at which the contribution of enthalpy and entropy are balanced. The principal determinants of the linear thermodynamic compensation process are found to be $\Delta S^\circ_{(T)}/\Delta C^\circ_{P(T)} = \langle \Delta T'_c \rangle / \langle T_{exp} \rangle$, where $\langle \Delta T'_c \rangle = (\langle T_c \rangle - T_{exp})$.

2. Theory

2.1. Thermodynamic analysis of the non-linear Van 't Hoff expression

The standard Gibbs free energy of association is expressed as a series expansion as a function of temperature, from the non-linear Van 't Hoff expression, as follows:

$$-RT \ln K_{eq} = \Delta G^\circ = \Delta H^\circ_0 - \alpha RT - lRT^2 - \gamma RT^3. \quad (1)$$

A linear, optimized regression analysis of this equation is performed in order to obtain the free energy values, a procedure which may be used with some success provided that the equilibrium constant and second virial coefficient, β, are precisely evaluated as a function of temperature at different concentrations.

The standard Gibbs free energy change (ΔG°) is at a minimum, i.e. $d\Delta G^\circ_{(T)}/dT = 0$, at a point we have designated as the harmonious temperature, T_H, where $T_H^2 + (2l/3\gamma)T_H + \alpha/3\gamma = 0$. The change in entropy of the interacting system may be determined from the expression

$$\partial \Delta G^\circ / \partial T = -\Delta S^\circ = -\alpha R - 2lRT - 3\gamma RT^2. \quad (2)$$

The entropic temperature, T_s, at which ΔS° becomes zero, is that point at which $T_s^2 + (2l/3\gamma)T_s + \alpha/3\gamma = 0$.

The derivative of eq. (2) as a function of temperature is

$$\frac{\partial(\Delta G^\circ/T)}{\partial(1/T)} = \Delta H^\circ = \Delta H^\circ_0 + RlT^2 + 2R\gamma T^3. \quad (3)$$

When $d(\Delta G^\circ/T)/d(1/T) = 0$, it is possible to evaluate the enthalpic temperature, $T_{\phi h}$, at which the enthalpy of the solution becomes zero, from

$$T^3_{\phi h} + (l/2\gamma)T^2_{\phi h} + \Delta H^\circ_0/2\gamma R = 0.$$

The derivatives of eq. (3) as a function of temperature may be evaluated for the change in heat capacity term,

$$\partial \Delta H^\circ / \partial T = \Delta C^\circ_P = 2\,RlT + 6R\gamma T^3. \quad (4)$$

It should be noted that in dealing with the derivative of a series expansion of standard free energy changes as a function of temperature, it is essential to perform residual error analysis at each step of the procedure in order to ascertain the validity of the resulting values. It is imperative to cover a temperature range in which there is adequate curvature in the dependence of the free energy of association as a function of temperature.

2.2. The definition of the thermodynamic compensatory temperature, $\langle T_c \rangle$, and the linear thermodynamic compensation process

It is possible to express the total gross change in the standard Gibbs free energy which is a result of all the processes contributing to any interacting system

for all experimental temperatures as follows:

$$\Delta G^\circ_{(T)\text{gross}} = \Delta H^\circ_{(T)\text{gross}} - T_{\text{exp}} \Delta S^\circ_{(T)\text{gross}}, \quad (5)$$

where

$$\Delta H^\circ_{(T)\text{gross}} = \Delta G^\circ_{\langle T_s \rangle} + \langle T_c \rangle \Delta S^\circ_{(T)\text{gross}} \quad (6)$$

(Lumry's expression). Substitution of eq. (6) into eq. (5) yields

$$\Delta G^\circ_{(T)\text{gross}} = [\Delta G^\circ_{\langle T_s \rangle} + \langle T_c \rangle \Delta S^\circ_{(T)}] - T_{\text{exp}} \Delta S^\circ_{(T)\text{gross}}, \quad (7)$$

that is

$$\Delta G^\circ_{(T)\text{gross}} = \Delta G^\circ_{\langle T_s \rangle} + [\langle T_c \rangle - T_{\text{exp}}] \Delta S^\circ_{(T)\text{gross}}. \quad (8)$$

Hence, for any system undergoing linear thermodynamic compensation,

$$T_{\text{exp}} = \langle T_c \rangle - \left(\frac{\Delta G^\circ_{(T)\text{gross}} - \Delta G^\circ_{\langle T_s \rangle}}{\Delta S^\circ_{(T)\text{gross}}} \right), \quad (9)$$

where

$$\lim_{T_{\text{exp}} \to T_s} \left(\frac{\Delta G^\circ_{(T)\text{gross}} - \Delta G^\circ_{\langle T_s \rangle}}{\Delta S^\circ_{(T)\text{gross}}} \right)$$

$$= \lim_{T_{\text{exp}} \to T_s} \left(\frac{-T_s \Delta S^\circ_{(T)\text{gross}}}{\Delta C^\circ_{P(T)\text{gross}}} \right) = 0,$$

since $\Delta C^\circ_{P(T)\text{gross}} \neq 0$ over the temperature range. Therefore, eq. (9) states that $\lim_{T_{\text{exp}} \to T_s} T_{\text{exp}} = \langle T_c \rangle$ and $\langle T_c \rangle = \langle T_H \rangle = \langle T_s \rangle$.

In order to validate eq. (9), the derivatives of eq. (7) with respect to T_{exp} should result in zero values, i.e. $d\Delta G^\circ_{(T)\text{gross}}/dT_{\text{exp}} = 0$.

$$\frac{d\Delta G^\circ_{(T)\text{gross}}}{dT_{\text{exp}}} = \left(\frac{d\Delta G^\circ_{\langle T_s \rangle}}{dT_{\text{exp}}} \right) + \langle T_c \rangle \frac{d\Delta S^\circ_{(T)\text{gross}}}{dT}$$

$$- T_{\text{exp}} \left(\frac{d\Delta S^\circ_{(T)\text{gross}}}{dT_{\text{exp}}} \right) = 0,$$

$$\langle T_c \rangle \frac{\Delta C^\circ_{P(T)\text{gross}}}{T_{\text{exp}}} - \Delta S^\circ_{(T)\text{gross}} - T_{\text{exp}} \frac{\Delta C^\circ_{P(T)\text{gross}}}{T_{\text{exp}}} = 0,$$

$$\langle T_c \rangle \Delta C^\circ_{P(T)\text{gross}} - T_{\text{exp}} \Delta S^\circ_{(T)\text{gross}}$$

$$- T_{\text{exp}} \Delta C^\circ_{P(T)\text{gross}} = 0,$$

$$\langle T_c \rangle \Delta C^\circ_{P(T)\text{gross}} - T_{\text{exp}} [\Delta S^\circ_{(T)\text{gross}} + \Delta C^\circ_{P(T)\text{gross}}] = 0.$$

Thus the compensatory temperature may be defined by the equation

$$\langle T_c \rangle = T_{\text{exp}} + T_{\text{exp}} [\Delta S^\circ_{(T)\text{gross}} / \Delta C^\circ_{P(T)\text{gross}}] \quad (10)$$

and as $T_{\text{exp}} \to T_s$, $\Delta S^\circ_{(T)\text{gross}} \to 0$. Theoretically, therefore, $\langle T_c \rangle = \langle T_s \rangle = \langle T_H \rangle$.

In Lumry's expression, when $\Delta H^\circ_{(T)\text{gross}}$ is plotted against $\Delta S^\circ_{(T)\text{gross}}$, at $\Delta S^\circ_{(T)\text{gross}} = 0$, $\Delta G^\circ_{\langle T_s \rangle} = \Delta H^\circ_{(T)\text{gross}}$ and at $\Delta H^\circ_{(T)\text{gross}} = 0$, $\langle T_c \rangle = (-\Delta G^\circ_{\langle T_s \rangle} / \Delta S^\circ_{T_{\text{oh}}})$. The resulting linear function confirms the principle of linear thermodynamic compensation operating in each of the associating systems we examined.

In order to prove that the compensatory and experimental temperatures are indeed equivalent ($\langle T_c \rangle = T_{\text{exp}}$), it is necessary to take the derivatives of $\Delta G^\circ_{(T)\text{gross}}$ with respect to $\Delta S^\circ_{(T)\text{gross}}$ from eq. (7), which yield

$$\frac{d\Delta G^\circ_{(T)\text{gross}}}{d\Delta S^\circ_{(T)\text{gross}}} = \frac{d\Delta G^\circ_{T_s}}{d\Delta S^\circ_{(T)\text{gross}}} + \langle T_c \rangle - T_{\text{exp}}$$

$$- \Delta S^\circ_{(T)\text{gross}} \frac{dT_{\text{exp}}}{d\Delta S^\circ_{(T)\text{gross}}}, \quad (11)$$

$$\frac{-\Delta S^\circ_{(T)\text{ gross}}}{\Delta C^\circ_{P(T)\text{gross}}/T_{\text{exp}}}$$

$$= \langle T_c \rangle - T_{\text{exp}} - T_{\text{exp}} \left[\frac{\Delta S^\circ_{(T)\text{gross}}}{\Delta C^\circ_{P(T)\text{gross}}} \right] \quad (12)$$

Hence, from eq. (12), it may be seen that $\langle T_c \rangle = T_{\text{exp}}$.

2.3. Computational analysis of thermodynamic parameters

2.3.1. Evaluation of the weight fraction of interacting monomer from the weight average molecular weight (M_w) as a function of concentration

The evaluation of the weight fraction of a monomer undergoing isodesmic association has been previously described [6,7,34,35]. For isodesmic association, the non-ideality term BM_1 (in ml/g where β is mole ml/g) is evaluated from the slope of a plot of $(M_1/M_{\text{wapp}}) - [\sqrt{f_1}/(2 - \sqrt{f_1})]$ versus C [35]. In the case of bovine liver L-glutamate dehydrogenase, data for the temperature-dependent molecular weight dis-

tribution at different concentrations were taken from Reisler and Eisenberg [1].

2.3.2. Evaluation of the weight fraction of monomer from the mean residue ellipticity ($[\theta]_\lambda$) as a function of concentration at a given temperature

In evaluating equilibrium constants and stoichiometry in cases of two-species association such as that between the glucagon monomer–trimer or apo A-II monomer–dimer, the mean residue ellipticity or weight average ellipticity is related to the weight fraction of monomer of each interacting species as a function of concentration, at a given wavelength, i.e.,

$$[\theta]_\lambda = \sum_i f_i(\theta_i + \beta_i C_i), \tag{13}$$

where $\Sigma_i f_i = 1$, the weight fraction of the ith species, and β_i is the second virial coefficient of ith species. The weight fraction of monomer is related to the equilibrium constant, K, by the expression (7):

$$K_i = (Cf_i/C_1^i) - Cf_i/(Cf_i)^i = f_i/(f_1^i C^{i-1}). \tag{14}$$

Once the second virial coefficient, β in deg-cm^2-ml/decimole/mg, is evaluated from

$$[\theta]_\lambda = f_1[\theta]_1 + f_i[\theta]_i + \beta C_T, \tag{15}$$

the three interaction parameters, the weight fraction of monomer, equilibrium constant and second virial coefficient, are used in the following expression to regenerate the original data points by an SAS least-square curve-fitting procedure (Barr et al. [36], Statistical Analysis System, Northeast Regional Data Center, University of Florida). The contribution of the third virial coefficient was too small to be considered.

Using the parameters obtained from the first fitting procedure, we evaluate the equilibrium constant from:

$$[\theta]_\lambda = f_1\theta_1 + K_i f_1^i \theta_i C_T^{i-1} + \beta C_T, \tag{16}$$

where

$$[\theta]_\lambda C_T = \sum_i K_i \theta_i C_1^i + \beta C_T^2.$$

The modes of association for both glucagon and apo A-II protein (Cm apo A-II) have been determined by analytical ultracentrifugation. Data for the temperature-dependent mean residue ellipticity as a function of concentration for these two systems were taken from Osborne et al. [2] and Formisano et al. [3].

2.3.3. Methods of computation

All calculations in the computation of the Van 't Hoff expression were done using the general linear models (SAS GLM procedure, Barr et al. [36]), which use the principle of least squares to fit a fixed-effect linear model to virtually any type of univariate and multivariate analysis, including simple linear regression,

Table 1
Tabulation of the weight fraction of monomer of bovine liver L-glutamate dehydrogenase as a function of temperature (Isodesmic association model)

Temp. K	$\hat{k}$ (ml/mg)	$C = 0.5$	$C = 1.0$	$C = 1.5$	$C = 2.0$	$C = 2.5$	$C = 3.0$	$C = 3.5$	$C = 4.0$
283	1.29	0.4782	0.3299	0.2556	0.2099	0.1789	0.1563	0.1389	0.1252
288	1.62	0.4273	0.2867	0.2189	0.1783	0.1509	0.1312	0.1162	0.1045
293	1.95	0.3873	0.2542	0.1921	0.1553	0.1309	0.1134	0.1004	0.0898
298	2.10	0.3717	0.2419	0.1820	0.1469	0.1235	0.1069	0.0943	0.0845
303	2.05	0.3767	0.2459	0.1852	0.1496	0.1258	0.1089	0.0962	0.0862
308	1.82	0.4021	0.2660	0.2018	0.1636	0.1381	0.1198	0.1059	0.0950
313	1.50	0.4444	0.3009	0.2309	0.1886	0.1600	0.1393	0.1235	0.1111
BM_1 (ml/mg)		140.0	24.90	3.67	3.99	4.04	5.24	6.46	6.48

The concentration, C, is expressed as $\times 10^{-3}$ g/ml. The weight fraction of monomer is computed from the following expression: $f_i = (i/\hat{k}c)[1 + (1/2\hat{k}c)(1 - \sqrt{1 + \hat{k}c})]^i$. The correlation coefficient in all cases was found to be 0.9999. $\hat{k}$ values were taken from Reisler and Eisenberg, Biochemistry 10 (1971) 2659). Detailed computation and analysis of the data for glutamate dehydrogenase appear in an earlier paper (Chun and Kim, Biochemistry 8 (1969) 1633; Chun et al., Biopolymers 11 (1972) 197).

multiple linear regression, analysis of variance, analysis of covariance, and partial correlation analysis.

The statistical analysis system language, interfaced with PL/1, was utilized for all routines (GLM 127, GLM 131). The stepwise regression procedure (STE 251, Barr et al. [36]) which was applied to our analysis of thermodynamic parameters includes five techniques to find that variable of a collection of independent variables which is most likely to be included in a regression model. This method of computation was extremely useful for data screening, permitting some insight into the relative strengths of the relationship between proposed independent variables and dependent variables (largest R^2 statistic).

The five-part procedure includes: 1) a forward selection technique which finds first the single-variable model, 2) a backward elimination technique which is first performed for a model including all the independent variables, 3) stepwise modification of the forward selection, 4) the maximum R^2 improvement and 5) minimum R^2 improvement. The fourth and fifth procedures produce models which fit the data equally well in our computation.

3. Results

3.1. Bovine liver L-glutamate dehydrogenase association; molecular weight distribution as a function of temperature

In a recent publication, Reisler and Eisenberg [1] and Eisenberg et al. [4] have found by light scattering measurements of different concentrations of bovine L-glutamate dehydrogenase that the equilibrium constant varies as a function of temperature. Using their data and our molecular sieve chromatography data, we have recalculated the distribution of the weight

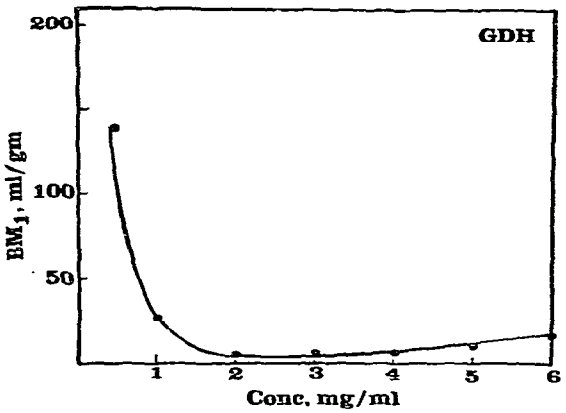


Fig. 1. Variation of BM_1 (ml/gm, β = (ml-mole/gm^2) as a function of concentration (GDH in 0.2 M phosphate buffer containing 1×10^{-4} M EDTA at 20°C), computed from a plot of the weight fraction of monomer [35] using combined molecular sieve and molecular weight data. Molecular weight data were taken from Reisler and Eisenberg [1]. The evaluation of confidence limits of each BM_1 value were within the limit of a correlation coefficient of 0.9999.

Table 2
Thermodynamic parameters of bovine liver L-glutamate dehydrogenase as a function of temperature (non-ideal case)

Temp, K	$\Delta G^\circ_{(T)}$ (kcal/mole)	$\Delta H^\circ_{(T)}$ (kcal/mole)	$\Delta S^\circ_{(T)}$ (kcal/mole-deg)	$\Delta C^\circ_{P(T)}$ (kcal/mole-deg)	$T_{exp}\Delta S^\circ_{(T)}/\Delta C^\circ_{P(T)}$
283	−7.27	12.55	0.0701	−0.420	−46.204
288	−7.53	10.21	0.0616	−0.484	−36.645
293	−7.77	7.59	0.0524	−0.541	−28.391
298	−7.95	4.68	0.0424	−0.600	−21.062
303	−8.06	1.48	0.0315	−0.660	−14.461
308	−8.12	−2.04	0.0198	−0.722	−8.436
318	−8.14	−5.87	0.0073	−0.785	−2.893

$\Delta G^\circ = A + BT + CT^2 + DT^3$, where $A = -50.02$, $B = 0.80$, $C = -39 \times 10^{-3}$, $D = 55 \times 10^{-6}$, and $C/D = 709$. Mean residue square = 0.997 and mean square of error = 5.2×10^{-5}. The sum of the square of the deviation = 0.006.

fraction of monomer undergoing isodesmic association as a function of temperature, as shown in table 1. The correlation coefficient of each value at each concentration was found to be 0.9999.

The non-ideality term BM_1 is also evaluated from molecular weight and partition data at each concentration. It is apparent from this table that distribution of the weight fraction monomer at a given concentration first decreases, then increases. Thus it appears that variation of the equilibrium constant is influenced not only by concentration, but also by non-ideality and temperature. Typical variation in BM_1 as a function of concentration is shown in fig. 1.

The thermodynamic parameters of bovine liver L-glutamate dehydrogenase given in tables 2 and 3 indicate that at the enthalpic temperature ($T_{\phi h}$) of 303 K, the enthalpy of the solution becomes zero. At higher temperatures, the enthalpy has a negative value, as shown in fig. 2.

The entropy of the solution reaches zero at 313 K, while the harmonious temperature (T_H), at which point the standard Gibbs free energy change is at a minimum, occurs at 302 K. Values for the thermodynamic temperatures of bovine liver L-glutamate dehydrogenase and other associating systems which we examined are summarized in table 3.

Initially, then, this isodesmic association is characterized by a positive enthalpy change and a larger

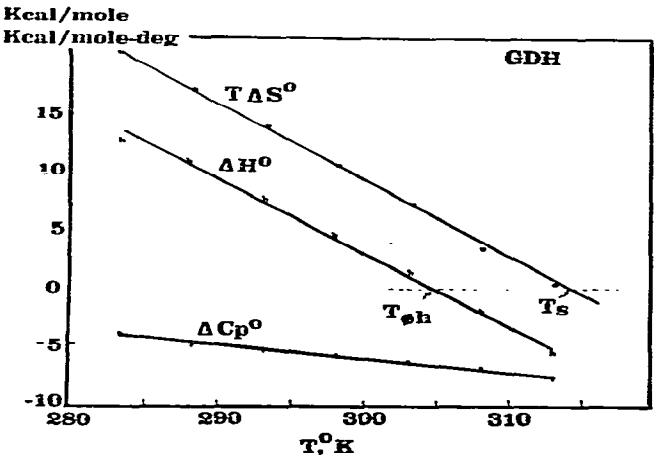


Fig. 2. Thermodynamic plot of bovine liver L-glutamate dehydrogenase (GDH) association as a function of temperature, within thermodynamic temperatures indicated on the plot. $\Delta S^\circ \times 10^3$ (kcal/mole-degree), $\Delta C_p^\circ \times 10$ kcal/mole-deg.). Each data point was evaluated with interpolation of F-statistics. Sum of square residuals is close to zero.

Table 3
Thermodynamic temperatures of interacting protein systems. $\langle T_c \rangle$ obtained from the plot of $\Delta H^\circ_{(T)\text{gross}}$ versus $\Delta S^\circ_{(T)\text{gross}}$

Temp, K	GDH	Glucagon	Cm Apo A-II protein
T_H	302	287	299
$T_{\phi h}$	303	285	298
T_s	313	295	303
$\langle T_c \rangle$	293	290	300
SEM, K	± 2.0	± 2.0	± 2.0

$\langle T_c \rangle$ obtained from the plot of T_{exp} versus $T_{exp}\Delta S^\circ_{(T)\text{gross}}/\Delta C^\circ_{P(T)\text{gross}}$

Cm Apo A-II protein	303	monomer ⇔ dimer
Glucagon	290	monomer ⇔ trimer
GDH	313	isodesmic association
SEM, K	± 0.1	

positive entropy change, while the Gibbs free energy change of the reaction is negative. As the solution approaches 303 K, the enthalpy changes are small, while the major contributor to the Gibbs free energy of interaction is the $T\Delta S^\circ$ term.

As may be seen in table 2, the minimum Gibbs free energy value is reached at T_H, 302 K. Over the temperature range studied, ΔC_p° varied from −430 to −785 cal/mole-degree.

The large changes in the BM_1 value (seen in fig. 1) and the distribution of the weight fraction of monomer (from table 3) strongly suggest that the effects on non-ideal behavior of the solute are much greater at low temperature than at high temperature.

3.2. Glucagon association: The mean residue ellipticity as a function of temperature

The thermodynamic parameters of glucagon association, which exists in an equilibrium between monomer and trimer, have been reevaluated from the data of Formisano et al. [3], 1A, 1B as shown in tables 2 and 4. After evaluating the equilibrium constants as a function of temperature and the second virial coefficient, we regenerated a theoretical curve of the

Table 4
Thermodynamic parameters of glucagon association (non-ideal case). All calculations were made at a glucagon concentration of 3.132 mg/ml

Temp, K	β (deg-cm^2-ml / decimole-mg)	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (kcal/mole-deg)	ΔC_P° (kcal/mole-deg)	$T_{\exp}\Delta S^\circ_{(T)}/\Delta C^\circ_{P(T)}$
283	−1029	−6.34	0.04	0.0263	−1.0440	−7.130
288	−1092	−6.44	−5.16	0.0081	−1.0364	−2.246
293	−1050	−6.64	−10.31	−0.0097	−1.0280	2.760
298	−1020	−5.99	−15.43	−0.0279	−1.0186	7.899
303	−1004	−5.87	−20.50	−0.0439	−1.0083	13.182
308	−770	−5.70	−25.52	−0.0603	−0.9972	18.618
313	−479	−5.56	−30.48	−0.0762	−0.9851	24.224
318	−257	−4.82	−35.37	−0.0918	−0.9721	30.014

$A = 216, B = 1.79, C = -4.4 \times 10^{-3}, D = 3.0 \times 10^{-6}$ and $C/D = 1467$. The mean residue square = 0.9929, mean square of error = 0.047. The sum of the square deviation = 0.008. The data shown represent a single concentration and are merely a single example of the volume of data analyzed in determining the thermodynamic parameters of this protein in the non-ideal case.

mean residue ellipticity as a function of concentration.

We found that the mean residue ellipticity of monomer varied from −4550 ± 30 at 285 K ($T_{\phi h}$) to −5710 ± 32 at 318 K, and for the trimer from −6570 ± 54 at 285 K ($T_{\phi h}$) to −10 530 ± 206 degrees cm^2/decimole at 318 K. The second virial coefficient is seen to vary from −1030 to −250 deg-cm^2-ml/decimole-mg over the temperature range studied, as shown in fig. 3. Although the variation in the second virial coefficient as a function of temperature was quite pronounced between the ideal and non-ideal case, the overall variation in the thermodynamic parameters of glucagon at a concentration of 3.132 mg/ml was only 5.6 percent. As may be seen in tables 2 and 4, the enthalpy change, ΔH°, is small and positive at 283 K, but becomes negative and continues to increase in negativity as a function of temperature over the range studied.

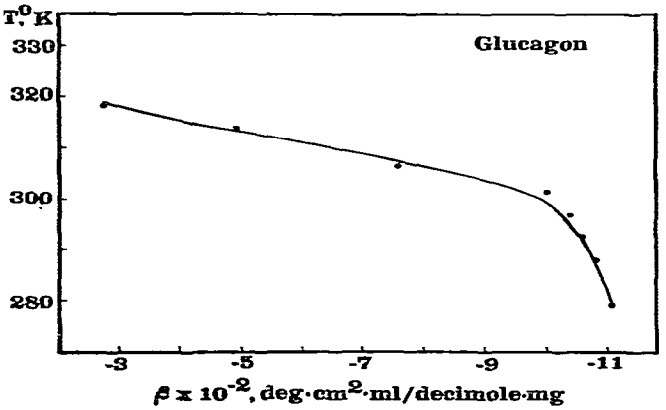


Fig. 3. Second virial coefficient as a function of temperature in the glucagon monomer–trimer equilibrium (ascertained by analytical equilibrium sedimentation). β = deg-cm^2-ml/decimole-mg. Our data were fitted equally well to a model describing isodesmic association.

The characteristic feature of this monomer–n-mer association appears to be the domination of the free energy change by the $T\Delta S^\circ$ term at temperatures below 290 K, where the enthalpy term is positive. At higher temperatures, the association is more distinctly driven by enthalpy. The Gibbs free energy of association is at a maximum at 287 K (T_H).

The heat capacity change, ΔC_P°, is seen to decrease gradually from −1044 cal/mole-degree at 283 K to −972 cal/mole-degree at 318 K. There is a strong correlation between the change in heat capacity and the corresponding alteration in the second virial coefficient, suggesting that there is a significant change in structural conformation over this temperature range upon association of the monomeric units into trimer.

3.3. Apo A-II association; the mean residue ellipticity as a function of temperature

The thermodynamics of the non-linear Van 't Hoff expression of the monomer–dimer association of the

Table 5
Thermodynamic parameter of S-carboxymethylated Apo A-II (cm Apo A-II) association. Cm Apo A-II concentration of 0.1373 mg/ml (non-ideal case)

Temp, K	$\beta\left(\frac{\text{deg-cm}^2\text{-ml}}{\text{decimole-mg}}\right)$	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (kcal/mole-deg)	ΔC_P° (kcal/mole-deg)	$T_{\exp}\Delta S^\circ_{(T)}/\Delta C^\circ_{P(T)}$
280	−1482	−5.40	18.40	0.0846	−0.9829	−24.113
285	−1336	−5.79	13.48	0.0670	−1.0071	−18.970
290	−1280	−6.03	8.38	0.0493	−1.0316	−13.861
295	−1204	−6.30	3.16	0.0315	−1.0563	−8.787
300	−1121	−6.44	−2.18	0.0135	−1.0812	−3.746
305	−1066	−6.41	−7.65	−0.005	−1.1064	1.262
310	−1061	−6.36	−13.24	−0.023	−1.1317	6.238
315	−1050	−6.21	−18.97	−0.041	−1.1574	11.182
320	−1023	−5.97	−24.81	−0.060	−1.1932	16.096

$A = 138.9$, $B = 0.88$, $C = 1.1 \times 10^{-3}$, $D = 7.8 \times 10^{-7}$ and $C/D = 1222$. Mean residue of square = 0.9995. Mean square of error = 0.047 and the sum of the square deviation = 0.005.

reduced carboxymethylated form of apo A-II protein from human high density lipoproteins have been described for the ideal case [2]. Using the experimental data for the effect of temperature on the mean residue ellipticity of apo A-II at 220 nm (presented by Osborne et al. in fig. 1 of their 1976 publication), we have extended these thermodynamic considerations of the non-ideal case. Although it is usually necessary to change the pressure, pH, salt or solvent composition with more stable proteins to observe the difference in heat capacity (ΔC_P°) change in the equilibration between native and denatured states (two-state model), the free energy of association of Cm apo A-II can be measured over a wide range of temperatures without modifying the solvent [16,25, 27–29,37,38], making it an excellent choice for thermodynamic studies.

The thermodynamic parameters of self-associating S-carboxymethylated apo A-II protein in the non-ideal case at a low concentration of 0.137 mg/ml are shown in tables 2 and 4. The mean residue ellipticity of monomer varied from −4320 ± 50 at 283 K to −7450 ± 50 at 320 K, and for the dimer from −13 150 at 283 K to −12 850 at 320 K. The second virial coefficient, as seen from tables 4 and 5 and fig. 4, varied from −1481 to −1023 deg-cm^2-ml/decimole-mg, decreasing exponentially over the temperature range of 280 ~ 320 K. ΔC_P° varied from −983 to −1193 cal/mole-degree in this temperature range.

3.4. Determination of the compensatory temperature

As seen from table 3, we observed a variation of some three degrees Kelvin between values of T_H, the harmonious temperature, and T_S, the entropic temperature, in each of the three associating systems we examined. By definition, however, the two temperatures are theoretically equivalent, since $d\Delta G^\circ/dT = 0$ or $-d\Delta G^\circ/dT = \Delta S^\circ = 0$ where $T_H^2 + (2I/3\gamma)T_H + \alpha/3\gamma = 0$ or $T_S^2 + (2I/3\gamma)T_S + \alpha/3\gamma = 0$. For $T_{\phi h}^3 +$

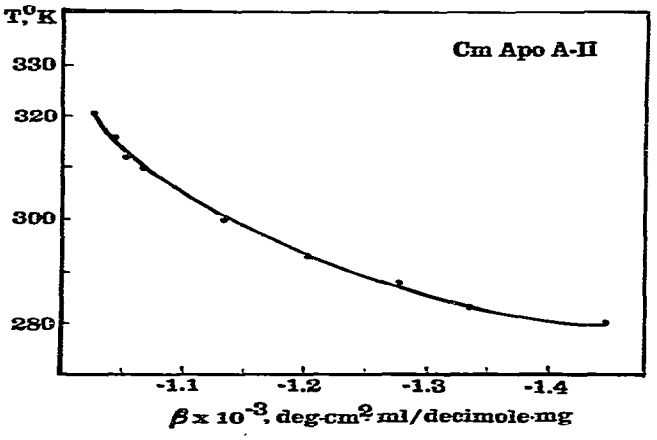
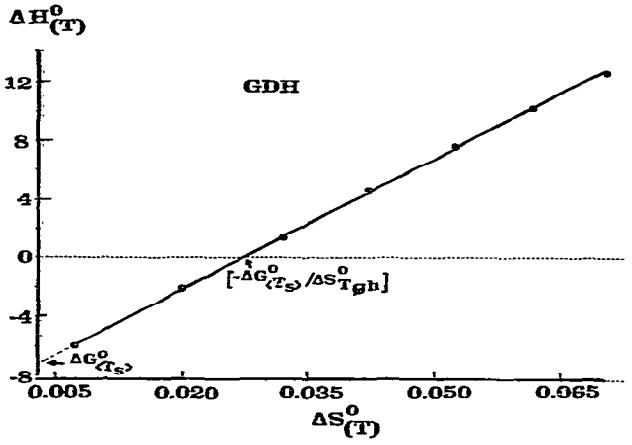


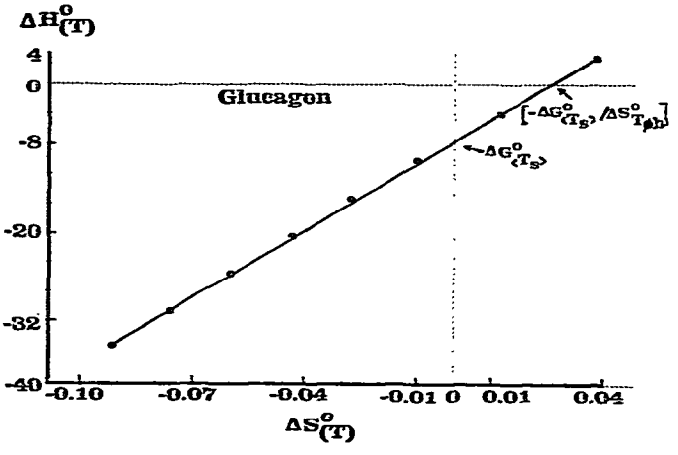
Fig. 4. Second virial coefficient of Cm apo A-II protein as a function of temperature in 0.01 M phosphate buffer, pH 7.4. These values were then used to recalculate the thermodynamic parameters listed in table 4.

$(l/2\gamma)T_{\phi h}^2 + \Delta H_0^\circ/2\gamma R = 0$, where $d(\Delta G_{(T)}^\circ/T)/d(1/T) = 0$, at a temperature we designate as $T_{\phi h}$, the enthalpy of the solution becomes zero. Theoretically, then, $T_{\phi h} \neq T_H$.

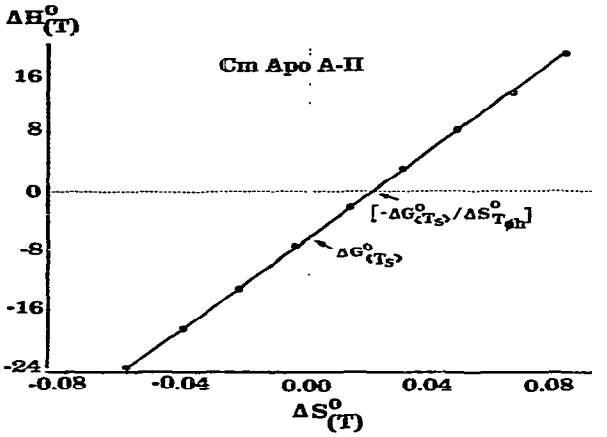
In order to accurately determine the temperature of compensation, $\langle T_c \rangle$, where $\langle T_c \rangle = d\Delta H_T^\circ/d\Delta S_T^\circ$, we plotted values of $\Delta H_{(T)}^\circ$ versus $\Delta S_{(T)}^\circ$. In the resulting linear relationship, shown in fig. 5, we observed that, within the allowances of statistical error, the temperatures of compensation for glucagon, apo A-II and bovine liver L-glutamate dehydrogenase are equivalent at 295 ± 2 K, as seen from table 3. When the compensatory temperatures of these three protein systems were evaluated from a plot of T_{exp} versus



(a)



(b)



(c)

Fig. 5. A plot of $\Delta H_{(T)\text{gross}}^\circ$ versus $\Delta S_{(T)\text{gross}}^\circ$ for bovine liver L-glutamate dehydrogenase (GDH), glucagon and Cm apo A-II protein, with data points taken from tables 2, 4 and 5. The slope of these lines represents $\langle T_c \rangle$, the compensatory temperature for each associating protein system, as listed in table 3.

$T_{exp}\Delta S_{(T)\text{gross}}^\circ/\Delta C_{P(T)\text{gross}}^\circ$, however, values of 303, 290 and 313 K were obtained for apo A-II, glucagon and bovine liver L-glutamate dehydrogenase, respectively. Theoretically, as $T_{exp} \rightarrow T_s$, $\Delta S_{(T)\text{gross}}^\circ \rightarrow 0$. Therefore, it should be true that $\langle T_c \rangle = \langle T_s \rangle = \langle T_H \rangle$

4. Discussion

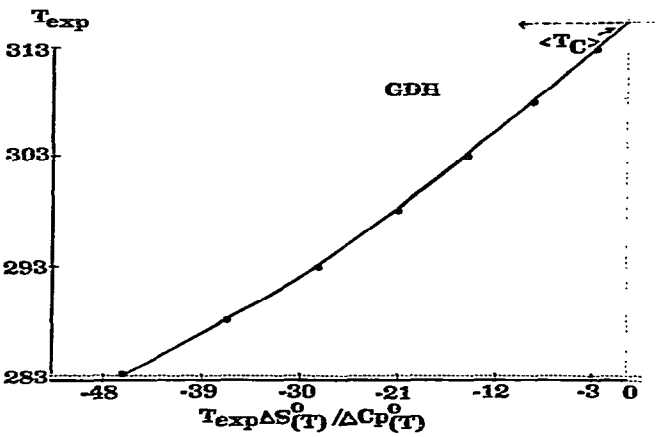
4.1. Thermodynamic analysis of the non-linear Van 't Hoff expression

The three interacting protein systems which we have examined (bovine liver L-glutamate dehydrogenase, glucagon and apo A-II protein) are characterized by temperature-dependent, entropically-driven association and a negative change in heat capacity.

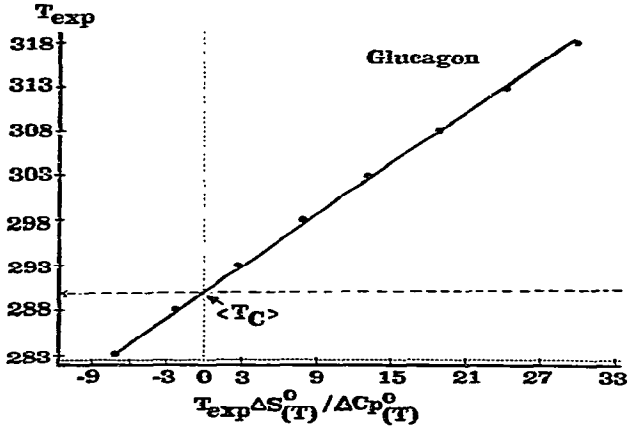
The positive dependence of the enthalpy term, ΔH°, on temperature suggests that the accompanying change in heat capacity, ΔC_P°, is directly related to reorganization of the water molecules surrounding the non-polar groups of the associating species. In such hydrophobic interaction, the change in ΔH° and ΔS° for the solution of hydrocarbons and non-polar groups will be negative, as the water "freezes" to ice [12,45].

Thermodynamic analysis of these apolar groups suggests that the chief contributor to the change in heat capacity is the exposure of hydrogen atoms in hydrocarbon chains (CH_2) to water [18]. Hydrogen bonding appears to be a minimal factor [39,40], since it is favored by non-polar solvents but is extremely weak in water and other polar solvents [41]. Hence, the positive, unfavorable enthalpy term at low temperature is a direct consequence of negative heat capacity changes.

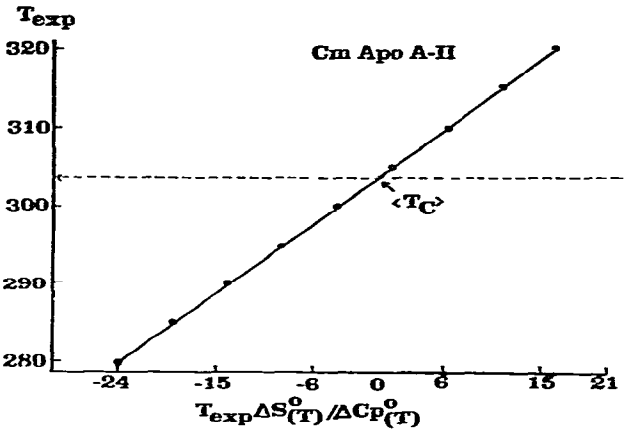
Lumry and Rajender [25] have described the compensating temperature, $\langle T_c \rangle$, as a constant where $\Delta G^{\circ}_{comp} = 0$. We have found in these studies, however, that the compensatory temperature is more accurately defined as $\langle T_c \rangle = T_{exp} + T_{exp}(\Delta S^{\circ}_{(T)gross}/\Delta C^{\circ}_{P(T)gross})$; $\langle T_c \rangle$ is evaluated from the linear plot of $\Delta H^{\circ}_{(T)gross}$ versus $\Delta S^{\circ}_{(T)gross}$ shown in fig. 5.



(a)



(b)



(c)

Fig. 6. A plot of T_{exp} versus $T_{exp}(\Delta S^{\circ}_{(T)gross}/\Delta C^{\circ}_{P(T)gross})$ for bovine liver L-glutamate dehydrogenase (GDH), glucagon and Cm apo A-II protein with data points taken from tables 2, 4 and 5. (a) GDH—residue square = 0.9997, PR > F, 0.0001, sum of square of error = 0.06. Standard deviation of $\langle T_c \rangle$ = 0.114. (b) Glucagon—residue square = 0.9997, PR > F, 0.0001, sum of square of error = 0.29. Standard deviation of $\langle T_c \rangle$ = 0.228. (c) Cm apo A-II—residue square = 0.9996, PR > F, 0.0001, sum of square of error = 0.625. Standard deviation of $\langle T_c \rangle$ = 0.298.

It is clear from our thermodynamic analysis of the non-linear Van 't Hoff plot for these three self-associating protein systems that the variation of the equilibrium constants as a function of temperature depends not only on the concentration, but also upon the value of the second virial coefficient, which is also a function of concentration and temperature, and is indicative of alteration in the molecular asymmetry, accounting for the geometric exclusion volume effect and molecular size [1,5,43,44].

We have shown that the non-ideality term, BM_1, contributes significantly to the molecular volume changes in self-associating protein systems [42]. Our evaluation of thermodynamic parameters from the non-linear Van 't Hoff expression has established the fact that β or BM_1 plays an important role in the change in heat capacity of the sample protein systems as a function of temperature. We have yet to deter-

mine, however, why an abrupt change is observed in the second virial coefficient of glucagon at T_s, where the entropy of the solution approaches zero, suggesting a major structural alteration upon association.

Any such changes in the folding of the tertiary structure which accompany self-association will involve a change in the heat capacity term, $C_P^\circ \approx \sigma_H^2/kT^2$. Here σ_H^2 is the variation of H°, the enthalpy of the system, a heat quantity which describes fluctuations in internal energy, exclusion volume and molecular asymmetry of the interacting species [24].

4.2. Compensatory temperature, $\langle T_c \rangle$, defined

At $\langle T_c \rangle$ in the linear compensation process, as defined by eq. (7) where $d\Delta G^\circ_{(T)\text{gross}}/dT = 0$,

$$T_{\text{exp}} = \langle T_c \rangle - [T_{\text{exp}} \Delta S^\circ_{(T)\text{gross}}/\Delta C^\circ_{P(T)\text{gross}}].$$

Hence we would assume that the values for $\langle T_c \rangle$, T_s and T_H would be equivalent, within the allowance for statistical error, in each of the systems we examined. Our results presented in table 3, however, show that although there is relatively good agreement in the values of $\langle T_c \rangle$, the variance in the other values is much greater than we might expect.

Why is this the case? Are we in fact determining the compensatory temperature, $\langle T_c \rangle$, in plotting $\Delta H^\circ_{\text{gross}}$ versus $\Delta S^\circ_{\text{gross}}$, or does the slope of the resulting line only represent $\langle T_c \rangle_{\text{app}}$? Two possibilities present themselves.

First, we might assume that the variation in T_H, T_s and $\langle T_c \rangle$, which was at most ten degrees Kelvin or within the 3 percent error limit, is indicative of the fact that the value of 295 ± 2.0 K which we have designated $\langle T_c \rangle$ is in fact $\langle T_c \rangle_{\text{app}}$, a temperature close to the compensatory temperature, but still influenced by sensitive changes in entropy and the heat capacity of system, as in eq. (10).

This equation can be modified to consider such slight variations, as follows:

$$\langle T_c \rangle \frac{\Delta C^\circ_{P(T)\text{gross}}}{T_{\text{exp}}} - \Delta S^\circ_{(T)\text{gross}} - T_{\text{exp}} \frac{\Delta C^\circ_{P(T)\text{gross}}}{T_{\text{exp}}} = 0,$$

where $\langle T_c \rangle_{\text{app}} = \langle T_c \rangle + (\langle T_c \rangle_{\text{app}} - T_s)$. Substitution into the following equation,

$$\langle T_c \rangle_{\text{app}} = T_{\text{exp}} \left[\frac{\Delta S^\circ_{(T)\text{gross}} + \Delta C^\circ_{P(T)\text{gross}}}{\Delta C^\circ_{P(T)\text{gross}}} \right]$$

gives

$$T_{\text{exp}} = \langle T_c \rangle + (\langle T_c \rangle_{\text{app}} - T_s) - T_{\text{exp}} \left(\frac{\Delta S^\circ_{(T)\text{gross}}}{\Delta C^\circ_{P(T)\text{gross}}} \right).$$

A second possibility is that our choice of the polynomial function for ΔG° as a function of temperature in evaluating our thermodynamic parameters was not the best possible selection for the precise evaluation of that unique temperature which we have designated $\langle T_c \rangle$ for any interacting system.

If, as is true in our case, $\Delta G^\circ_{(T)}$ is experimentally determined from a plot of K_{eq} as a function of temperature from equilibrium measurements, based on l'Hopital's rule, the resulting polynomials derived from $\Delta G^\circ_{(T)}$, where $d\Delta H^\circ_{(T)}/d\Delta S^\circ_{(T)} = \Delta H^\circ_{(T)}/\Delta S^\circ_{(T)}$, will always result in a linear function of temperature.

If, however, the polynomials for $\Delta H^\circ_{(T)}$ and $\Delta S^\circ_{(T)}$ are independently, experimentally determined as a function of temperature, the resulting plot may be non-linear. Thus, by our procedure, it is essential to precisely evaluate K_{eq} as a function of temperature to give the best possible fit of the data. This, in turn, will affect the accuracy of the polynomial chosen to represent $\Delta G^\circ_{(T)}$, from which a plot of $\Delta H^\circ_{(T)}/\Delta S^\circ_{(T)}$ is derived.

In either case, the argument for the existence of a unique compensatory temperature for interacting protein systems undergoing the thermodynamic compensation process remains a sound one. We have defined $\Delta S^\circ_{(T)}/\Delta C^\circ_{P(T)} = \langle \Delta T_c' \rangle / \langle T_{\text{exp}} \rangle$, where $\langle \Delta T_c' \rangle = (\langle T_c \rangle - T_{\text{exp}})$. It is to be hoped that further refinement of our experimental techniques, which will result in more precise experimental data, will permit the precise determination of $\langle T_c \rangle$ in future examinations of any self-associating protein system.

5. Conclusions

1. Our data demonstrate the existence of a linear thermodynamic compensation process operating in any associating biological system.

2. We have demonstrated the existence of a compensatory temperature at which the enthalpy and entropy of the interacting system are in balance, for maximum effectiveness of biological function.

3. At $\langle T_c \rangle$, the standard Gibbs free energy of association is at a minimum in the thermodynamic sense.

Hence, ΔH° and $T\Delta S^\circ$ are balanced, and the protein structure retains its maximum stability.

Our definition of the compensatory temperature suggests a number of interesting hypotheses which will require further examination. For example, we might speculate that all systems *in vivo* have a similar, unique compensatory temperature — for example, 37 degrees C. in the human body — at which entropy and enthalpy are maintained in balance, permitting all individual body processes to operate at a maximum level of effectiveness.

We would speculate that even if the primary and secondary structural sequences of a protein are altered, its compensatory temperature in vitro will remain unchanged as long as it undergoes self-association (tertiary—tertiary interaction) and retains its biological function.

Our results suggests that this unique compensatory temperature may very well be at or near 295 K in any self-associating system examined *in vitro*.

Ultimately, the questions we must ask ourselves are: Does temperature determine the structure of proteins and their interaction? Or do protein structure and interaction determine the compensatory temperature?

In terms of evolutionary events in biological systems, it would seem that the latter case is more likely.

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