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Response functions of proteins

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Abstract

The thermodynamics of protein folding can be studied by a variety of different techniques such as differential scanning calorimetry, differential scanning densimetry and sound velocity measurements. These three methods monitor the different response functions heat capacity, expansion coefficient and compressibility that characterise various aspects of protein dynamics such as equilibrium energy and volume fluctuations and energy–volume correlations. For the development of a comprehensive thermodynamic description of protein behaviour information on these response functions should be combined. As a starting point we provide in the present paper analytical solutions for the determination of the response functions and demonstrate on several examples how to extract a maximum of thermodynamic information on proteins from the measurements of C_p, α_p and κ_T.

Keywords: Fluctuation; Heat capacity; Volume; Compressibility; Expansion coefficient; Protein

1. Introduction

The quantitative investigation of the energetic and mechanical properties of proteins requires thermodynamic approaches. A significant thermodynamic characterisation of proteins involves for example the measurement of the change in the extensive properties volume V and enthalpy H in response to the variation of the conjugate intensive properties pressure p and temperature T. Volume and enthalpy can be related to each other by the partition function $Y(p,T)$ which in turn defines the Gibbs energy $G = -k_B T \ln Y(p,T)$.

In statistical thermodynamics mean values are calculated from derivatives of the partition function with respect to its variables. For example the derivative of the partition function $Y(p,T)$ with respect to the pressure variable $\gamma = p\beta$, with $\beta = 1/k_B T$, gives the volume

$$V = -\left(\frac{\partial \ln Y}{\partial \gamma}\right)_T, \tag{1}$$

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and the derivative with respect to the reciprocal temperature, $\beta = 1/k_B T$, provides the enthalpy [1,2]

$$H = -\left(\frac{\partial \ln Y}{\partial \beta}\right)_p. \tag{2}$$

When employing the relative partition function $Q = Y/Y_N$, which is defined relative to the partition function of the native state Y_N [3,4], these equations become

$$V - V_N = -\left(\frac{\partial \ln Q}{\partial \gamma}\right)_T \tag{3}$$

$$H - H_N = -\left(\frac{\partial \ln Q}{\partial \beta}\right)_p. \tag{4}$$

The changes in the relative partition functions reflect directly the experimentally accessible volume and enthalpy changes relative to the native state. The second derivatives of ln Q provide a measure of the corresponding fluctuations in volume relative to the native state

$$\sigma_V^2 - \sigma_{V_N}^2 = \left(\frac{\partial^2 \ln Q}{\partial \gamma^2}\right)_T = -\left(\frac{\partial (V - V_N)}{\partial \gamma}\right)_T \tag{5}$$

or, respectively, in enthalpy

$$\sigma_H^2 - \sigma_{H_N}^2 = \left(\frac{\partial^2 \ln Q}{\partial \beta^2}\right)_p = -\left(\frac{\partial (H - H_N)}{\partial \beta}\right)_p \tag{6}$$

The mixed derivative yields an expression for the correlation of enthalpy and volume

$$\begin{aligned}\sigma_{V,H} - \sigma_{V_N,H_N} &= \left(\frac{\partial}{\partial \beta}\left(\frac{\partial \ln Q}{\partial \gamma}\right)_T\right)_p \\ &= -\left(\frac{\partial (V - V_N)}{\partial \beta}\right)_p \\ &= \left(\frac{\partial}{\partial \gamma}\left(\frac{\partial \ln Q}{\partial \beta}\right)_p\right)_T \\ &= -\left(\frac{\partial (H - H_N)}{\partial \gamma}\right)_T\end{aligned} \tag{7}$$

These theoretical quantities are related to experimentally accessible properties such as the isothermal compressibility, κ_T

$$\kappa_T^* = V\kappa_T = \frac{\sigma_V^2}{k_B T}, \tag{8}$$

the isobaric heat capacity, C_p

$$C_p = \frac{\sigma_H^2}{k_B T^2} \tag{9}$$

and the isobaric expansion coefficient, α_p

$$\alpha_p^* = V\alpha_p = \frac{\sigma_{V,H}}{k_B T} \tag{10}$$

V is the appropriate volume of the system in study. For example, it can be the partial specific or partial molar volume of the protein. The usage of the Boltzmann constant k_B in these equations indicates that the relations refer to single proteins. Equivalently all quantities can be formulated per mole of protein. In that case k_B has to be replaced by the gas constant R [3].

Several of these relations were applied previously to proteins [5–7] and also to lipids [8]. However, the new aspect of the present study is that we provide analytical solutions, which permit a fit of the experimental data. The equations developed here apply to the response functions of transitions of monomeric proteins. These formulae permit a powerful combination of data derived from complementary thermodynamic methods.

2. Derivation of analytical solutions for the response functions

In the simplest case protein folding can be described by a two-state equilibrium with N and D being the native and the denatured state of the protein and K the equilibrium constant:

$$N \rightleftharpoons D \qquad K = \frac{[D]}{[N]} \tag{11}$$

This system can be described statistically by the relative partition function Q [3]

$$Q = \frac{[N] + [D]}{[N]} = 1 + K \tag{12}$$

For the calculation of the response functions the temperature and pressure dependence of the equilibrium constant is required. The relations are given by the following equations:

$$\left(\frac{\partial}{\partial T}\left(-\frac{\Delta G^0}{RT}\right)\right)_p = \left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H^0}{RT^2} \tag{13}$$

$$\left(\frac{\partial}{\partial p}\left(-\frac{\Delta G^0}{RT}\right)\right)_T = \left(\frac{\partial \ln K}{\partial p}\right)_T = -\frac{\Delta V^0}{RT} \tag{14}$$

The volume relative to the native state is then given by

$$V - V_N = -\left(\frac{\partial \ln Q}{\partial \gamma}\right)_T = -\frac{RT}{Q}\left(\frac{\partial K}{\partial p}\right)_T = \frac{K}{Q}\Delta V^0 = f_D \Delta V^0 \tag{15}$$

where the fractional population of the denatured state, f_D, has been defined by the relation

$$f_D = \frac{K}{Q} = \frac{[D]}{[N] + [D]} \tag{16}$$

The molar volume fluctuations relative to those of the native state can becalculated using Eq. (5):

$$\sigma_V^2 - \sigma_{V,N}^2 = RT\left[f_D\left(\frac{\partial \Delta V^0}{\partial p}\right)_T + \frac{(\Delta V^0)^2}{RT}\frac{K}{Q^2}\right] = RT f_D \Delta \kappa_T^* + (\Delta V^0)^2 f_D(1 - f_D). \tag{17}$$

With these relations and Eq. (8) the change in isothermal compressibility relative to the native state can be expressed as a function of the degree of unfolding, f_D:

$$\kappa_T - \frac{\kappa_{T,N}^*}{V} = f_D \frac{\Delta \kappa_T^*}{V} + \frac{(\Delta V^0)^2}{VRT} f_D(1 - f_D) \tag{18}$$

The difference in compressibility between the native and denatured state is defined by

$$\Delta \kappa_T^* = \kappa_{T,D}^* - \kappa_{T,N}^* = \kappa_{T,D} V_D - \kappa_{T,N} V_N. \tag{19}$$

This relation for the change in compressibility is closely analogous to that for the change in heat capacity as the following equations demonstrate. Starting with the relative partition function Q the proportionality between the degree of transition f_D and the experimental enthalpy, $H - H_N$, is expressed by the relation

$$H - H_N = f_D \Delta H^0 \tag{20}$$

which holds, however, only for monomeric proteins as shown previously by Rösgen and Hinz [3]. The temperature course of the heat capacity is then given by

$$C_p - C_{p,N} = f_D \Delta C_p + \frac{(\Delta H^0)^2}{RT^2} f_D(1 - f_D) \tag{21}$$

The change in heat capacity on unfolding is defined by

$$\Delta C_p = C_{p,D} - C_{p,N} \tag{22}$$

Finally, an important relation that describes the correlation between volume and enthalpy change can be obtained from Eq. (7):

$$\sigma_{V,H} - \sigma_{V,H,N} = RT^2\left[f_D\left(\frac{\partial \Delta V^0}{\partial T}\right)_p + \Delta V^0 \frac{\Delta H^0}{RT^2}\frac{K}{Q^2}\right] = RT^2 f_D \Delta \alpha_p^* + \Delta V^0 \Delta H^0 f_D(1 - f_D). \tag{23}$$

Using Eq. (10) the difference in isobaric expansion between the native and denatured state is defined in the following manner:

$$\Delta \alpha_p^* = \alpha_{p,D}^* - \alpha_{p,N}^* = \alpha_{p,D} V_D - \alpha_{p,N} V_N \tag{24}$$

ΔH^0 and ΔV^0 are the respective volume and enthalpy changes at temperature T. If these val-

ues are known the isobaric expansion coefficient can be calculated in the following manner:

$$\alpha_p - \frac{\alpha^*_{p,N}}{V} = f_D \frac{\Delta\alpha^*_p}{V} + \frac{\Delta V^0}{V}\frac{\Delta H^0}{RT^2} f_D(1-f_D) \tag{25}$$

V is the partial molar volume at temperature T of the protein according to Eq. (15), which is experimentally accessible by densimetry [9]

3. Usage of the response functions for data analysis

3.1. Constant (atmospheric) pressure

For the analysis of numerical data it is useful to formulate the response functions in polynomial form. In most cases first or second order Taylor expansions are sufficient. The corresponding expressions are summarised in the following.

The heat capacity of the native state can usually be approximated well by a linear function of temperature

$$C_{p,N} = C_{p,N,(T_{1/2})} + (T - T_{1/2})(\partial_T C_{p,N})_{(T_{1/2})}, \tag{26}$$

while the heat capacity of the denatured state requires at least a second order polynomial [10]

$$C_{p,D} = C_{p,D,(T_{1/2})} + (T - T_{1/2})(\partial_T C_{p,D})_{(T_{1/2})} + \frac{1}{2}(T - T_{1/2})^2\left(\partial^2_T C_{p,D}\right)_{(T_{1/2})}, \tag{27}$$

The enthalpy difference between the two states is then given by the equation:

$$\Delta H^0 = \Delta H^0_{(T_{1/2})} + (T - T_{1/2})\Delta C_{p,(T_{1/2})} + \frac{1}{2}(T - T_{1/2})^2(\partial_T \Delta C_p)_{(T_{1/2})} + \frac{1}{6}(T - T_{1/2})^3\left(\partial^2_T C_{p,D}\right)_{(T_{1/2})}. \tag{28}$$

The isothermal compressibilities of the native and denatured state are assumed to be linear functions of temperature

$$\kappa_{T,N} = \kappa_{T,N,(T_{1/2})} + (T - T_{1/2})(\partial_T \kappa_{T,N})_{(T_{1/2})} \tag{29}$$

$$\kappa_{T,D} = \kappa_{T,D,(T_{1/2})} + (T - T_{1/2})(\partial_T \kappa_{T,D})_{(T_{1/2})}. \tag{30}$$

The same assumption is made for the volumes of the native and the denatured state:

$$V_N = V_{N,(T_{1/2})} + (T - T_{1/2})\alpha^*_{p,N,(T_{1/2})} \tag{31}$$

$$V_D = V_{D,(T_{1/2})} + (T - T_{1/2})\alpha^*_{p,D,(T_{1/2})} \tag{32}$$

$C_{p,N}(T_{1/2})$, $C_{p,D}(T_{1/2})$, $\Delta H^0(T_{1/2})$, $\kappa_{p,N}(T_{1/2})$, $\kappa_{p,D}(T_{1/2})$, $V_N(T_{1/2})$ and $V_D(T_{1/2})$ are the values of the respective parameters at the transition temperature $T_{1/2}$ chosen as reference temperature. The standard Gibbs energy difference between the D and N state at temperature T is [3,11]:

$$\begin{aligned}\Delta G^0(T) = &-\Delta H^0_{(T_{1/2})}\frac{T - T_{1/2}}{T_{1/2}} \\ &+ \Delta C_{p,(T_{1/2})}\cdot\left(T - T_{1/2} - T\ln\frac{T}{T_{1/2}}\right) \\ &+ (\partial_T \Delta C_p)_{(T_{1/2})} \\ &\times\left(\frac{T^2_{1/2} - T^2}{2} + TT_{1/2}\ln\frac{T}{T_{1/2}}\right) \\ &+ \frac{(\partial_{T,T}\Delta C_p)_{(T_{1/2})}}{2} \\ &\times\left(\frac{T^3 - T^3_{1/2}}{3} + \left(\frac{(T - T_{1/2})^2 T}{2}\right)\right. \\ &\left. - T^2_{1/2}T\ln\frac{T}{T_{1/2}}\right).\end{aligned} \tag{33}$$

The symbols ∂_T and $\partial_{T,T}$ indicate the first and second partial derivatives with respect to temperature at $T_{1/2}$. The parameters that are determined from the experimental curves are the

transition temperature $T_{1/2}$ and the properties of the protein at this temperature: the enthalpy change $\Delta H^0(T_{1/2})$, the heat capacities $C_{p,i}$ and their first or second derivatives with respect to temperature. The same holds for the compressibility and the volume. Knowledge of the standard Gibbs energy change ΔG^0 permits calculation of the equilibrium constant:

$$K(T) = \exp\left(\frac{-\Delta G^0(T)}{RT}\right) \tag{34}$$

To illustrate the application of the equations Figs. 1–4a show representative heat capacity curves of RNase A, lysozyme, cytochrome *c* and α-lactalbumin. The experimental data are taken from Hinz et al. [12]. A fit of the data to Eq. (21) results in the parameters given in Table 1. Figs. 1b–4b exhibit the change in partial specific volume as a function of temperature for the same proteins. These data can evidently be fitted with excellent results to Eq. (15), if the fraction of unfolding, f_D, is taken as a known quantity from the analysis of the heat capacity curves. The fits are shown as grey lines in Figs. 1b–4b. The isobaric expansion coefficient, α_p, can be obtained as a function of temperature from the partial specific volume curves on the basis of Eq. (25). The resulting values have also been included in Figs. 1b–4b.

Figs. 1c–4c show calculated compressibility values for the four proteins. Since there is no information on the isothermal compressibility of the native and the denatured state, $\kappa_{T,N}$ and $\kappa_{T,D}$, over a broad temperature range, we plotted only the transitional contribution, which corresponds according to Eq. (18)

$$\kappa_{T,tr} = \frac{(\Delta V^0)^2}{VRT} f_D(1-f_D). \tag{35}$$

The experimental determination of the adiabatic compressibility is usually achieved by ultrasound velocity measurements, i.e. at frequencies in the order of MHz. The isothermal compressibility, κ_T, can be obtained from these values, if the expansion coefficient α_p, the density ρ and the isobaric heat capacity C_p are known [7,13]. Since protein folding is characterised by relaxation times between milliseconds and weeks, ultrasound cannot be expected to yield much information on the folding process. The only compressibility contributions that will be detected by ultrasound measurements are those, which result

Table 1
Thermodynamic parameters at the transition temperature $T_{1/2}$ obtained from a fit of the data in Figs. 1–4 according to Eqs. (15) and (21)[a]

	RNase A	Lysozyme	Cytochrome *c*	α-Lactalbumin
pH	2.5	1.9	3.2	7
c (DSC) (g/l)	14.4	8.3	16.1	7.17
c (DSD) (g/l)	14.4	32.2	16.1	12.2
MW (kg/mol)	13.7	14.3	12.4	14.2
$T_{1/2}$ [*K* (/°C)]	317.8 (44.7)	328.3 (55.2)	333.1 (60.0)	339.4 (66.3)
ΔH^0 (kJ/mol)	319	374	250	284
$C_{p,N}$ (kJ/mol*K*)	27.6	24.8	26.5	30.8
$C_{p,D}$ (kJ/mol*K*)	31.3	30.9	29.9	33.8
$\partial_T C_{p,N}$ (J/molK^2)	177	142	152	87
$\partial_T C_{p,D}$ (J/molK^2)	23	33	−67	115
V_N (l/mol)	9.379	10.359	8.562	10.636
V_D (l/mol)	9.369	10.356	8.593	10.737
ΔV^0 (ml/mol)	−10	−3	31	101
$\partial_T V_N$ (ml/mol*K*)	4.7	5.2	6.4	6.4
$\partial_T V_D$ (ml/mol*K*)	8.1	8.3	7.2	7.0

[a] The experimental conditions are given in the figure legends and in Hinz et al. [12].

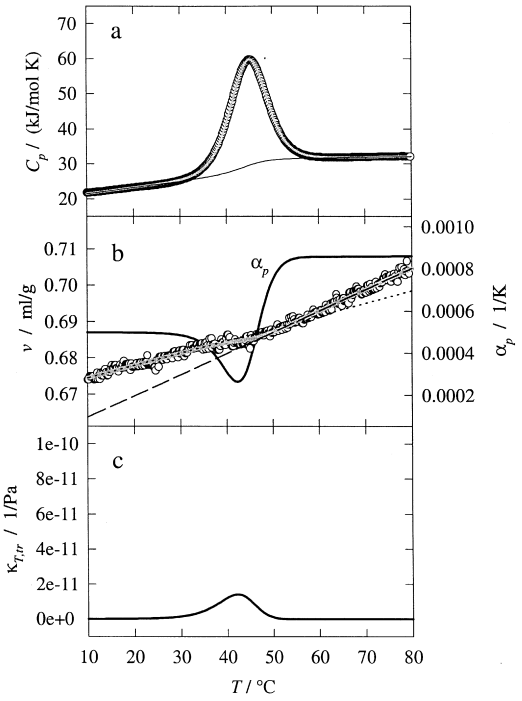

Fig. 1. Variation with temperature of the response functions and the partial specific volume of RNase A at pH 2.5 in 0.1 M sodium-phosphate buffer. The experimental data (circles) are taken from Hinz et al. [12]. The fits to Eqs. (15) and (21) are shown as grey lines. Parameters are given in Table 1: (a) heat capacity and baseline (total heat capacity minus the transitional contribution as a black line); (b) volume of the protein and volumes of the native (dotted) and denatured state (dashed) and expansion coefficient [solid black line, calculated according to Eq. (25)]; (c) transitional compresssibility contribution [calculated according to Eq. (35)].

from microscopic changes faster than microseconds. Such changes are likely to reflect the contributions to the compressibility of the pure states.

We can therefore draw the conclusion that neither ultrasound measurements nor density measurements alone will yield the full compressibility function of a protein. However, a combination of the result of both methods with the heat capacity data will accomplish that. The DSC measurements provide the information on the population sizes of native and denatured states, the density measurements permit the calculation of the contribution to the compressibility of the transition [Eq. (35) or last term in Eq. (18)] and the ultrasound measurements yield the compressibility of the pure states [first term on the r.h.s. of Eq. (18)].

It is worth noting that for the proteins studied the volume difference between the two states decreases with decreasing transition temperature of the proteins. At the transition midpoint the ΔV^0-value becomes even negative for RNase A, which has the lowest $T_{1/2}$ value among these four proteins. Up to now volume measurements on proteins were mainly performed at relatively low temperatures with different results. Negative ΔV^0 values were observed [14–17] but it was also suggested that ΔV^0 could assume both positive or negative values [18]. In that context effects of

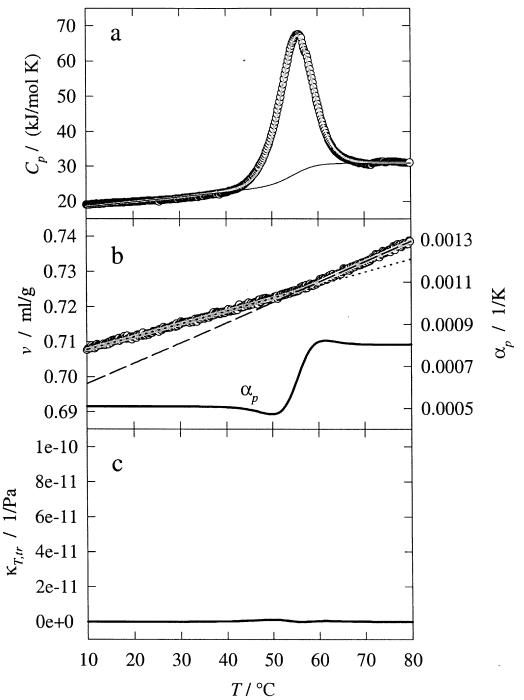

Fig. 2. Variation with temperature of the response functions and the partial specific volume of lysozyme at pH 1.9 in 0.1 M sodium phosphate buffer. The experimental data (circles) are taken from Hinz et al. [12]. The fits to Eqs. (15) and (21) are shown as grey lines. Parameters are given in Table 1: (a) heat capacity and baseline (total heat capacity minus the transitional contribution as a black line); (b) volume of the protein and volumes of the native (dotted) and denatured state (dashed) and expansion coefficient [solid black line, calculated according to Eq. (25)]; (c) transitional compressibility contribution [calculated according to Eq. (35)].

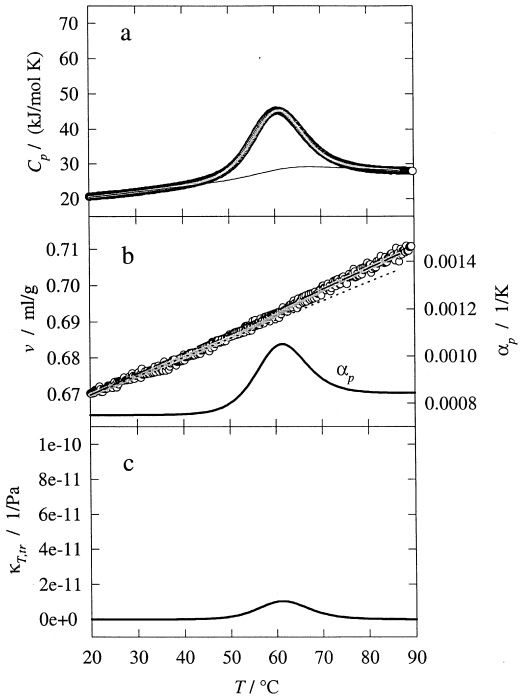

Fig. 3. Variation with temperature of the response functions and the partial specific volume of cytochrome *c* in 0.1 M potassium phosphate buffer at pH 3.2. The experimental data (circles) are taken from Hinz et al. [12]. The fits to Eqs. (15) and (21) are shown as grey lines. Parameters are given in Table 1: (a) heat capacity and baseline (total heat capacity minus the transitional contribution as a black line); (b) volume of the protein and volumes of the native (dotted) and denatured state (dashed) and expansion coefficient [solid black line, calculated according to Eq. (25)]; (c) transitional compressibility contribution [calculated according to Eq. (35)].

protein size on the sign of ΔV^0 were discussed [19]. Small proteins were suggested to exhibit negative volume changes, while larger proteins were associated with positive volume changes. However, a generalisation is clearly not possible, since experimental ΔV^0 data are available that demonstrate that also small proteins can exhibit positive volume changes on denaturation both at room temperature [20,21] and at higher temperatures [12,22]. Therefore, the size of the protein seems to be of lower importance for the volume difference between the native and the denatured state than the experimental conditions at which the measurements are carried out.

The volume data of RNase A that were analysed in the present paper were determined in 0.1 M sodium-phosphate buffer at pH 2.5 [12]. The other volume data on RNase A in the literature are those in Tamura and Gekko [16]. The protein has been studied in water adjusted to low pH values (1.6, 1.9 and 2.08). Due to the different buffer conditions these data obtained at related but lower temperatures are not directly comparable to the data derived from DSD temperature scans [12]. However, if one neglects the larger uncertainty involved in the extrapolation of the volume of the native and the denatured state resulting from the smaller number and the large

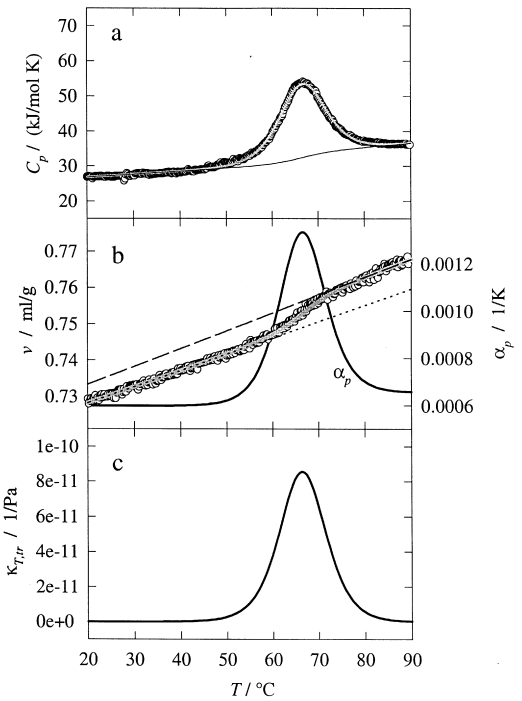

Fig. 4. Variation with temperature of the response functions and the partial specific volume of α-lactalbumin in water at pH 7. The experimental data (circles) are taken from Hinz et al. [12]. The fits to Eqs. (15) and (21) are shown as grey lines. Parameters are given in Table 1: (a) heat capacity and baseline (total heat capacity minus the transitional contribution as a black line); (b) volume of the protein and volumes of the native (dotted) and denatured state (dashed) and expansion coefficient [solid black line, calculated according to Eq. (25)]; (c) transitional compressibility contribution [calculated according to Eq. (35)].

scattering of data points in Tamura and Gekko [16] it turns out that at the pH values studied (pH 1.6, 1.9 and 2.08) each ΔV^0 ($T_{1/2}$) value (−320 ml/mol, −490 ml/mol, −670 ml/mol) is considerably more negative than the value obtained at pH 2.5 (−10 ml/mol). In view of the present results this can be rationalised, if one assumes that the volume difference between the native and the denatured state at a given temperature decreases with decreasing $T_{1/2}$.

3.2. Effect of pressure variation

A complete set of heat capacity, expansion and compressibility data at atmospheric pressure permits an extrapolation of the transition behaviour to other pressures. The extrapolation can be based on an expression for the Gibbs energy at different temperatures and pressures. Such a relation is obtained by means of a two-dimensional Taylor series expansion about a given temperature T_0 and pressure p_0, for example $T_0 = T_{1/2}$ and $p_0 = 1$ bar. When using terms up to fourth order the expression reads

$$\begin{aligned}\Delta G^0(T,p) = {} & \Delta G^0(T,p_0) \\ & + \left(\partial_p \Delta G^0\right)_{(T_0,p_0)}(p-p_0) \\ & + \left(\partial_{p,T} \Delta G^0\right)_{(T_0,p_0)}(p-p_0)(T-T_0) \\ & + \frac{\left(\partial_{p,p} \Delta G^0\right)_{(T_0,p_0)}}{2}(p-p_0)^2 \\ & + \frac{\left(\partial_{p,T,T} \Delta G^0\right)_{(T_0,p_0)}}{2}(p-p_0) \\ & \times (T-T_0)^2 + \frac{\left(\partial_{p,p,T} \Delta G^0\right)_{(T_0,p_0)}}{2} \\ & \times (p-p_0)^2(T-T_0) \\ & + \frac{\left(\partial_{p,p,p} \Delta G^0\right)_{(T_0,p_0)}}{6}(p-p_0)^3 \\ & + \frac{\left(\partial_{p,T,T,T} \Delta G^0\right)_{(T_0,p_0)}}{6}(p-p_0) \\ & \times (T-T_0)^3 \\ & + \frac{\partial_{p,p,T,T} \Delta G^0_{(T_0,p_0)}}{4}(p-p_0)^2 \\ & \times (T-T_0)^2 \\ & + \frac{\left(\partial_{p,p,p,T} \Delta G^0\right)_{(T_0,p_0)}}{6}(p-p_0)^3 \\ & \times (T-T_0) \\ & + \frac{\left(\partial_{p,p,p,p} \Delta G^0\right)_{(T_0,p_0)}}{24}(p-p_0)^4 \end{aligned} \tag{36}$$

ΔG^0 (T,p_0) is the temperature dependence of the Gibbs energy at constant pressure as given in Eq. (33). The various partial derivatives with respect to pressure and temperature are abbreviated as before by $\partial_{p,p}$ or $\partial_{p,p,T}$, etc. After introduction of the expressions for the partial derivatives of ΔG^0 (T,p) Eq. (36) is transformed as follows:

$$\begin{aligned}\Delta G^0(T,p) = {} & \Delta G^0(T,p_0) \\ & + \Delta V^0(T_0,p_0)(p-p_0) \\ & + \Delta \alpha_p^*(T_0,p_0)(p-p_0) \\ & \times (T-T_0) \\ & - \frac{\Delta \kappa_T^*(T_0,p_0)}{2}(p-p_0)^2 \\ & + \frac{\partial_T \Delta \alpha_p^*(T_0,p_0)}{2}(p-p_0) \\ & \times (T-T_0)^2 \\ & - \frac{\partial_T \Delta \kappa_T^*(T_0,p_0)}{2}(p-p_0)^2 \\ & \times (T-T_0) - \frac{\partial_p \Delta \kappa_T^*(T_0,p_0)}{6} \\ & \times (p-p_0)^3 \\ & + \frac{\partial_{T,T} \Delta \alpha_p^*(T_0,p_0)}{6}(p-p_0) \\ & \times (T-T_0)^3 \\ & - \frac{\partial_{T,T} \Delta \kappa_T^*(T_0,p_0)}{4}(p-p_0)^2 \end{aligned}$$

$$\times(T-T_0)^2$$

$$-\frac{\partial_{p,T}\Delta\kappa_T^*(T_0,p_0)}{6}(p-p_0)^3$$

$$\times(T-T_0)$$

$$-\frac{\partial_{p,p}\Delta\kappa_T^*(T_0,p_0)}{24}(p-p_0)^4 \quad (37)$$

The only constants that can not be measured in isobaric experiments are the pressure derivatives $\partial_p\Delta\kappa_T^*$, $\partial_{p,p}\ \Delta\kappa_T^*$ and $\partial_{p,T}\kappa_T^* = \partial_{p,p}\Delta\alpha_p^*$. However, spectroscopic measurements of protein transitions at different pressures can be used for the evaluation of compressibilities, that are comparable to the values obtained from purely thermodynamic methods, by a determination of the $\Delta G^0(T,p)=0$ curve [7]. This is maybe easiest using NMR [23]. Since spectroscopic techniques appear to involve fewer problems at higher pressures than, e.g. expansion, ultrasound or heat capacity studies, values for the last three undetermined parameters are likely to result from spectroscopic measurements. The strategy for obtaining the highest degree of information is then to perform global fits that comprise the experimental heat capacity, compressibility, volume and spectroscopic pressure denaturation data. The data should permit a reliable extrapolation of the response functions into high-pressure regions.

To illustrate the temperature and pressure dependence of a protein the stability of RNase A was calculated as a function of temperature for a variety of pressures using the parameters given in Table 1 and a compressibility difference of 1.397×10^{-7} ml/(Pa mol) between the native and the denatured state [12,24]. Since not all experimental parameters required for the calculation of protein stability according to Eq. (37) are known the accuracy of the stability curves is expected to be best at a low pressure. For a more precise calculation of the Gibbs energy change at pressures in the kilobar region more information is needed both on the compressibility itself but also on the pressure and temperature derivatives of the compressibility (Fig. 5).

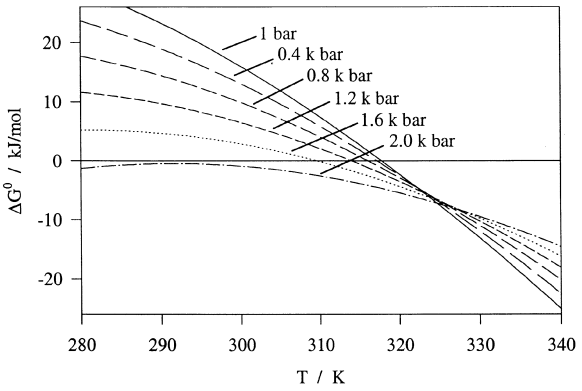

Fig. 5. Stability of RNase A as a function of pressure and temperature. The curves were calculated according to Eq. (37) using the parameters given in Table 1 and a compressibility difference of $\Delta\kappa_T^* = 1.397\times10^{-7}$ ml/(Pa mol) between the native and the denatured state [12,24].

4. Calculation of response functions that are not experimentally accessible

If three response functions are known other response function can be determined [13]. This applies also to properties that are not experimentally accessible. Some interesting response functions that can be calculated from the ones given above are the isochoric tension coefficient β_V

$$\beta_V = \frac{\alpha_p}{p\kappa_T} \quad (38)$$

and the isochoric heat capacity C_V

$$C_V = C_p - \frac{\alpha_p^2}{\kappa_T}TV. \quad (39)$$

p, V and T are respectively pressure, volume and temperature. A comparison of isochoric and isobaric heat capacity for lysozyme is shown in Fig. 6. As noted previously [12] the isochoric heat capacity of the protein could decrease on denaturation while the isobaric heat capacity increases. However, to verify this result further, particularly with respect to compressibility data, sound veloc-

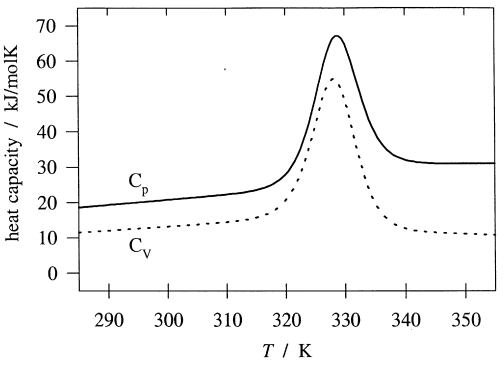

Fig. 6. Isobaric (—) and isochoric (– –) heat capacity of lysozyme calculated according to Eq. (39) using the data given in Table 1 and $\Delta\kappa_T^* = 1.397 \times 10^{-7}$ml/(Pa mol) [12,24].

ity measurements should be performed on both the native and denatured protein over a broad temperature range.

5. Conclusions

In the present study we have shown that a combination of thermodynamic methods can yield significant insight into the mechano-thermal properties of proteins that is superior to the information obtained by the individual method. We derived formulas that permit the extraction of maximal information from the various thermodynamic measurements. These equations link the parameters obtained by different methods and allow e.g. the calculation of the static compressibility of proteins from a combination of calorimetric, volumetric and sound velocity measurements.

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