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Prediction of the heat capacity change on thermal denaturation of globular proteins

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Abstract

The large positive heat capacity change is a common feature of both the transfer of nonpolar compounds to water and the temperature-induced denaturation of globular proteins. In this paper we present a model for the calculation of the denaturation heat capacity change, that is a key parameter of protein thermodynamics, by means of a simple group additivity scheme and some common structural properties of globular proteins. The specific polar and nonpolar contributions to the heat capacity change are determined by analyzing the transfer of several different series of organic compounds to water. Additionally, we derive a general relationship for the evaluation of the fraction of accessible surface area buried in the protein interior, whose knowledge is necessary because only the groups that contact water on unfolding contribute to the heat capacity change. The model, despite its simplicity, works well, as the agreement between the calculated and experimental values of the denaturation heat capacity change is satisfactory for a large set of globular proteins. © 1998 Elsevier Science B.V.

Keywords: Group additivity principle; Water reorganization; Accessible surface area; Denaturation heat capacity change

1. Introduction

The tendency of nonpolar groups to associate in aqueous solution in order to avoid the contact with water, hydrophobic interaction, plays a fundamental role in the stabilization of micelles, biomembranes and native globular protein structures [1]. Hydrophobic interaction is a consequence of the high Gibbs energy cost for the solvation of nonpolar moieties in water: hydrophobic hydration [2]. Other thermodynamic features of this process are the large negative entropy changes and the large positive heat capacity changes. Actually, some authors [3,4] considered the large positive heat capacity change as the hallmark effect of hydrophobic hydration. Therefore, the clarification at the molecular level of such heat capacity change would be useful to reach a better understanding of hydrophobic hydration, whose origin remains a controversial subject [5].

DSC investigations have proved that a large positive increment of the heat capacity is associated also with the temperature-induced denaturation of small globular proteins [6]. This finding suggests that hydrophobic hydration is involved in protein denaturation, as buried nonpolar side-chains contact water on unfolding. However, this is only half of the story. It is now well established [7,8] that polar groups, when contact water, also make a contribution to the heat capacity change, but of negative sign (i.e., they tend to

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counterbalance the effect of nonpolar groups). Additionally, detailed analyses of X-ray structures [9–11], pointed out that proteins bury a large fraction of their polar accessible surface area, mainly formed by backbone peptide groups, as an unavoidable consequence of the chain connectivity in order to achieve a globular shape.

The denaturation heat capacity change, $\Delta_d C_p^\circ$, is a key parameter for the evaluation of the thermodynamic stability of globular proteins as it determines the curvature of the denaturation Gibbs energy as a function of temperature [6], and it is fundamental for the existence of cold denaturation [12]. The development of a theoretical model for the prediction of $\Delta_d C_p^\circ$ from the knowledge of the amino acid sequence of the protein is of general interest in the field of protein stability. In this paper, we devise a simple model for the calculation of $\Delta_d C_p^\circ$, by assuming that the group additivity principle is valid for the thermal denaturation of globular proteins. The specific polar and nonpolar contributions to $\Delta_d C_p^\circ$ are obtained from an analysis of thermodynamic data for the transfer of several different series of organic compounds to water. These specific values have to be multiplied by the fraction of accessible surface area buried in the protein interior on folding, f_b. Exploiting general results on the accessible surface area of native and denatured proteins, we derive a relationship between f_b and the number of residues in the protein, N_{res}. The model is tested against a large set of globular proteins: the predicted values of $\Delta_d C_p^\circ$ agree with experimental ones within the experimental uncertainties. Finally, the model allows the calculation of 'master curves', $\Delta_d C_p^\circ$ vs N_{res}, which well reproduce the experimental finding that $\Delta_d C_p^\circ$, normalized per residue, is limited in the range 30–90 J K^{-1} mol^{-1}.

2. Analysis of small of molecule data

In a previous work [13], we analyzed the heat capacity changes associated with the dissolution process from pure solid, liquid or gaseous phase into water of several organic compounds. These heat capacity changes depend on temperature, but experimental data over a sufficiently large temperature range are lacking. Thus we restricted ourselves to analyze the values of $\Delta_{tr} C_p^\circ$ at a single temperature, namely 298.15 K. For each homologous series (i.e., liquid alcohols, liquid amides, etc.), the heat capacity change is given by the sum of a constant contribution, due to the functional group common to all compounds, and a variable contribution due to the number of nonpolar hydrogen atoms CH (i.e., hydrogen atoms bonded to a carbon atom, regardless of whether it is aliphatic or aromatic, and assuming, for instance, that a CH_3 group corresponds to three nonpolar hydrogens), present in each molecule. At a fixed temperature, the heat capacity change is described by the following equation:

$$\Delta_{tr} C_p^\circ = \text{constant} + N_{CH} \cdot \Delta C_p^\circ CH \quad (1)$$

where $\Delta C_p^\circ{}_{CH}$ is the heat capacity change due to a single nonpolar hydrogen atom. We analyzed the following series: (a) gaseous hydrocarbons; (b) liquid hydrocarbons; (c) gaseous alcohols; (d) liquid alcohols; (e) liquid ethers; (f) liquid carboxylic acids; (g) liquid amines; (h) liquid amides; (i) solid α-amino acids; (j) solid cyclic dipeptides; (k) solid linear dipeptides; (l) sodium carboxylates; (m) tetralkylammonium bromides; and (n) sodium alkylsulfates. The validity of group additivity was confirmed by the fact that the value of the liner correlation coefficient was always greater than 0.97. More importantly, the value of $\Delta C_p^\circ{}_{CH}$ was practically constant for all the considered series (i.e., the mean value is $\Delta C_p^\circ{}_{CH}$=30.0± 2.0 J K^{-1} mol^{-1}), regardless of the starting phase (i.e., solid, liquid or gaseous).

In this paper we enlarge the set of organic compounds investigated with the following series: (a) liquid monoesters [14]; (b) liquid α,ω-diols [15]; (c) liquid α,ω-dicarboxylic acids [15]; and (d) amines relaxed to water from α-cyclodextrin adducts [16]. Tables 1–3 report the various compounds with the corresponding values of N_{CH}, $\Delta_{tr} H^\circ$ (298.15 K) in kJ mol^{-1}, according to the mole fraction standard state, and $\Delta_{tr} C_p^\circ$ (298.15 K) in J K^{-1} mol^{-1} units. The results of the unweighted least squares regressions, performed according to Eq. (1), are reported at the bottom of each table. By excluding the first entry for the bifunctional compounds, the value of $\Delta C_p^\circ{}_{CH}$ proved to be: 28.7±1.0 J K^{-1} mol^{-1} for monoesters, 28.3±0.2 J K^{-1} mol^{-1} for diols, and 29.8±0.5 J K^{-1} mol^{-1} for diacids. These estimates agree with the average value obtained previously. For the first compound of the α,ω-diols and dicarboxylic acids the general additivity relationship does not work,

Table 1
Number of nonpolar hydrogen atoms (N_{CH}), enthalpy ($\Delta_{tr}H^{\circ}$) and heat capacity ($\Delta_{tr}C_p^{\circ}$) changes associated with the transfer process of monoesters from pure liquid phase into water at 298.15 K. The data are from [14]; *n* is the number of compounds considered in the linear regression

Substance	N_{CH}	$\Delta_{tr}H^{\circ}$ (kJ mol^{-1})	$\Delta_{tr}C_p^{\circ}$ (J K^{-1} mol^{-1})
CH_3OCOCH_3	6	−7.60±0.03	148±3
$C_2H_5OCOCH_3$	8	−9.69±0.04	215±3
$CH_3OCO(CH_2)_2CH_3$	10	−8.33±0.05	261±4
$C_2H_5OCOCH_2CH_3$	10	−10.25±0.04	274±3
$C_2H_5OCO(CH_2)_2CH_3$	12	−9.89±0.04	327±6
$CH_3OCO(CH_2)_4CH_3$	14	−6.70±0.03	373±8
$C_2H_5OCO(CH_2)_3CH_3$	14	−9.51±0.09	388±10

n=7, $\Delta_{tr}C_p^{\circ}$=(−19.6±10.6) + (28.7±1.0) N_{CH}, r=0.9971.

Table 2
Number of nonpolar hydrogen atoms (N_{CH}), enthalpy ($\Delta_{tr}H^{\circ}$) and heat capacity ($\Delta_{tr}C_p^{\circ}$) changes associated with the transfer process of α,ω-diols and dicarboxylic acids from pure liquid phase into water at 298.15 K. The data are from [15]; *n* is the number of compounds considered in the linear regression

Substance	N_{CH}	$\Delta_{tr}H^{\circ}$ (kJ mol^{-1})	$\Delta_{tr}C_p^{\circ}$ (J K^{-1} mol^{-1})
$HO(CH_2)_2OH$	4	−6.87±0.02	43±2
$HO(CH_2)_3OH$	6	−8.67±0.02	93±4
$HO(CH_2)_4OH$	8	−10.46±0.01	150±3
$HO(CH_2)_5OH$	10	−10.59±0.01	206±6
$HOOC(CH_2)_2COOH$	4	−28.71±0.02	96±6
$HOOC(CH_2)_3COOH$	6	−25.09±0.01	103±6
$HOOC(CH_2)_4COOH$	8	–	164±8
$HOOC(CH_2)_5COOH$	10	–	222±10

diols: n=4, $\Delta_{tr}C_p^{\circ}$=(−68.1±4.1)+(27.3±0.6)N_{CH}, r=0.9996.
diols: n=3, ΔtrC_p°=(−76.3±1.2)+(28.3±0.2)N_{CH}, r=0.9999.
diacids: n=4, $\Delta_{tr}C_p^{\circ}$=(−7.4±33.1)=(22.0±4.5)N_{CH}, r=0.9603.
diacids: n=3, $\Delta_{tr}C_p^{\circ}$=(−75.0±3.5)+(29.8±0.5)N_{CH}, r=0.9999.

probably because the two polar groups are very close to each other.

Additionally, for the transfer of amines from α-cyclodextrin adducts to water, $\Delta C_p^{\circ}{}_{CH}$=+29.9± 1.0 J K^{-1} mol^{-1}, which is in exact agreement with the value obtained for the transfer of small molecules from pure phase to water. It has to be noted that for *N*-methylhexylamine and 2-aminoheptane, the values of $\Delta_{tr}C_p^{\circ}$ are very similar to that of hexylamine, even though they have a greater number of CH groups [16]. This finding is readily explained by considering that, at increasing the chain in proximity to the amino group, which is thought to remain in the solution

Table 3
Number of nonpolar hydrogen atoms (N_{CH}), enthalpy ($\Delta_{tr}H^{\circ}$) and heat capacity ($\Delta_{tr}C_p^{\circ}$) changes associated with the transfer of amines from α-cyclodextrin adducts to water at 298.15 K. The data are from [16]. The linear regression was performed only on the first four compounds. See the text for more details

Substance	N_{CH}	$\Delta_{tr}H^{\circ}$ (kJ mol^{-1})	$\Delta_{tr}C_p^{\circ}$ (J K^{-1} mol^{-1})
n-pentylamine	11	13.6±0.2	272±21
n-hexylamine	13	17.6±0.1	327±17
n-heptylamine	15	19.9±0.2	385±17
n-octylamine	17	22.1±0.5	452±25
2-aminoheptane	15	17.9±0.1	318±18
N-methylhexylamine	16	17.6±0.1	302±16

$\Delta_{tr}C_p^{\circ}$=(−59.6±13.8)+(29.9±1.0) N_{CH}, r=0.9989.

before and after association [16], the added nonpolar hydrogens remain in water. Therefore, the nonpolar groups that are not buried into the cavity of α-cyclodextrin do not contribute to $\Delta_{tr}C_p^{\circ}$. A word of caution is necessary: even though the contribution to $\Delta_{tr}C_p^{\circ}$ for the removal of a CH group from the α-cyclodextrin cavity and its solubilization in water is equal to that for the transfer from the pure phase to water, the two processes involve different kinds of interactions. In any case, the effect of the expulsion of water molecules from the inner cavity of α-cyclodextrin involves a constant number of solvent molecules and a similar conformational rearrangement of the macrocycle, regardless of the guest dimension. Thus, the closeness of the $\Delta C_p^{\circ}{}_{CH}$ values suggests that, for the transfer of amines from α-cyclodextrin adducts to water, there is a constant contribution that does not affect the slope of the plot $\Delta_{tr}C_p^{\circ}$ versus N_{CH}.

All these results strongly indicate that the value of $\Delta C_p^{\circ}{}_{CH}$ is entirely determined by the unique physicochemical properties of water. We pointed out that the insensitivity of the $\Delta C_p^{\circ}{}_{CH}$ value to the choice of nonaqueous phase demonstrates that the cause of this insensitivity does not reside in the interaction between the nonpolar solute and water molecule, but rather in the water reorganization process itself [13]. Actually, the water reorganization around nonpolar moieties can be described by a simple model in which each water molecule has two accessible states separated by a small amount of energy [17]. The enthalpy fluctuations associated with the Boltzmann distribution of water over the two states give rise to the excess heat

capacity and account satisfactorily for the experimental values [13,18].

Additionally, from both the previous and present analysis, it results that polar groups (i.e., COOH, CONH and NH_2) make negative contributions to the heat capacity change, as already pointed out [7,8]. In particular, we are interested in the contribution of the peptide group, CONH, which is a basic group of proteins. We obtained two estimates very close to each other: $\Delta C_p^{\circ}{}_{CONH} = -60 \pm 6.0$ J K^{-1} mol^{-1} from solid cyclic dipeptides [13]; and $\Delta C_p^{\circ}{}_{CONH} = -65.5 \pm 5.1$ J K^{-1} mol^{-1} from liquid *N*-alkyl amides [13]. Additionally, Privalov and Makhatadze [19], performing a group additivity analysis of the heat capacity changes associated with the hydration (i.e., gas to water transfer) of a large variety of compounds, determined $\Delta C_p^{\circ}{}_{CONH} = -63.4$ J K^{-1} mol^{-1} for the gas to water transfer. Thus, it results that the contribution of the CONH group is practically constant, regardless of the originating phase (i.e., solid, liquid or gaseous). This finding suggests that the water reorganization around the peptide group is the dominant term in determining the heat capacity change, as it occurs for nonpolar groups, even though the two processes have to be different because they give rise to heat capacity changes of different sign. In fact, describing the water reorganization with the two-state hydrogen bond model developed by Muller [20] and modified by Lee and Graziano [18,21], it appears that: (a) the hydrogen bonds in the hydration shell of nonpolar solute are enthalpically stronger but more broken than those in the bulk water; and (b) the hydrogen bonds in the hydration shell of a polar solute are less broken than those in the bulk water.

3. Accessible surface area of globular proteins

The accessible surface area, ASA, is defined as the area over which a probe water molecule can be rolled in order to make van der Waals contacts with protein atoms [22]. The accessible surface area of denatured proteins, ASA_D, has been usually calculated by summing up the contributions of the single residues evaluated from the tripeptides Gly–X–Gly or Ala–X–Ala in *trans*-conformation, where X is the requested residue. Calculations showed that ASA_D is simply proportional to the molecular weight M_w, because the fluctuations caused by differences in the amino acid sequence are small [9–11]. In particular, Karshikoff and colleagues [11], performing calculations for a set of 183 nonhomologous proteins, determined $ASA_D = 1.5\ M_w$. Such calculations, however, overestimate the value of ASA_D because they do not consider the screening effect by neighbouring groups in the unfolded chain. Makhatadze and Privalov [23] showed that the ASA of a whole polypeptide chain in an extended conformation amounts to about 80% of the ASA calculated by summing up the values of the single residues from tripeptides. So, the correct relationship is:

$$ASA_D = 1.2 \cdot M_W \tag{2}$$

Native protein structures, which have very different folding patterns and specific shapes, might show no such correlation. Really, Chothia [24], proved that the accessible surface area of native structures, ASA_N, is also a simple function of M_W. From the recent analysis by Karshikoff and colleagues [11], it can be shown that

$$ASA_N = 6.6 \cdot M_W^{0.732} \tag{3}$$

Janin [25], with simple geometrical considerations, gave clear interpretation of this result. If globular proteins were solid spheres, their ASA and volume, $V = (M_W \cdot v / L_A)$, where v is the partial specific volume, about 0.75 cm^3 g^{-1}, and L_A is the Avogadro's number, would be related to the radius R by:

$$ASA = 4\pi R^2 \text{ and } V = (4/3)\pi R^3 = (M_W \cdot v / L_A) \tag{4}$$

which give:

$$ASA = 5.6 M_W^{0.667} \tag{5}$$

Comparison with Eq. (3) shows that ASA_N is approximately twice that of a sphere of the same mass and density. The excess ASA_N of globular proteins is largely due to the roughness of their surface viewed at the atomic level. On the other hand, the globular shape expresses the need to achieve a minimum ASA, compatible with molecular mass, in order to maximize the Gibbs energy gain from hydrophobicity on folding, a quantity proportional to the buried ASA [24].

We are interested in the fraction of ASA buried on folding, f_b, which, using Eqs. (2) and (3) proves to be

and gives:

$$f_b = [(ASA_D - ASA_N)/ASA_D] = 1.0 - 5.5 \cdot M_W^{-0.268} \quad (6)$$

This relationship, by assuming $M_W = 115 \cdot N_{res}$, where 115 is the average molecular weight of amino acid residues, becomes:

$$f_b = 1.0 - 1.54 \cdot N_{res}^{-0.268} \quad (7)$$

It is evident that the fraction of ASA buried on folding depends on the number of residues, but when N_{res} becomes large, f_b reaches a practically constant value. This behaviour, dictated by the geometrical features of globular proteins above clarified, readily explains the trend observed in the past for limiting values of hydrophobicity and polarity indexes [26,27].

For our purposes it would be necessary to evaluate separately the fraction of ASA polar buried, f_{pol}, and the fraction of ASA nonpolar buried, f_{np}, on folding. Actually, the analyses performed by various authors [9–11] pointed out that f_{pol}, to a good approximation, can be considered constant and equal to about 0.70, after correction for the overestimation of ASA_D. On the other hand, Chothia and co-workers [10] found that nonpolar groups contribute almost as much to the ASA of native proteins as they do to that of unfolded polypeptide chains (i.e., about 58% in a large set of globular proteins). Thus, by applying Eq. (6) to evaluate f_{np}, it results that it is well represented by Eq. (7), and so we fix $f_{np} \equiv f_b$. Additionally, Eq. (7) emphasizes that the amphiphilic nature of amino acid residues and the chain connectivity impose a complex constraint, rendering it impossible to bury all the nonpolar ASA in the protein interior (i.e., otherwise the structure would resemble a micelle). It has to be noted that Eq. (7) is of general validity because it is based on the results of Karshikoff and colleagues [11], who performed calculations on a set of 183 non-homologous proteins with known three-dimensional structure.

4. Calculation of the denaturation heat capacity change

A globular protein, as a first approximation, is essentially composed of polar peptide groups, CONH, and nonpolar hydrogen atoms, CH. The working hypothesis is that the denaturation heat capacity change, normalized per residue and assumed temperature-independent, can be calculated with a group additivity scheme, by adding polar and nonpolar contributions. Actually, Privalov and co-workers [28], with careful DSC measurements, showed that $\Delta_d C_p^\circ$ is slightly temperature-dependent. However, due to enthalpy–entropy compensation, its temperature-dependence little affects the protein stability curve [29], $\Delta_d G^\circ$ vs T. Therefore, we believe it reliable to consider $\Delta_d C_p^\circ$ to be temperature-independent.

It is clear that the polar and nonpolar groups contributing to $\Delta_d C_p^\circ$ are those buried in the protein interior that contact water on unfolding. By considering a single residue, the polar contribution is given by:

$$\Delta_d C_p^\circ pol = f_{pol} \cdot \Delta C_{pCONH}^\circ \quad (8)$$

where f_{pol}, the fraction of ASA polar buried, is considered constant and equal to 0.70; and $\Delta C_p^\circ{}_{CONH} = -65.5\ J\ K^{-1}\ mol^{-1}$, from the group additivity analysis of the transfer to water of liquid *N*-alkyl amides [13]. The polar groups present in side-chains, in majority, are in contact with water in the native structures, and so do not contribute to the heat capacity change. The nonpolar contribution is given by:

$$\Delta_d C_p^\circ np = f_{np} \cdot \Delta C_{pCH}^\circ \cdot < N_{CH} > \quad (9)$$

where f_{np} is the fraction of ASA nonpolar buried determined by Eq. (7), $\Delta C_p^\circ{}_{CH} = 30.0\ J\ K^{-1}\ mol^{-1}$, the general value obtained from the group additivity analysis of small molecule data [13], and $<N_{CH}>$ is the average number of CH per residue, readily obtained from the amino acid sequence of a give protein. By adding together the polar and nonpolar contributions, Eq. (10) results:

$$\Delta_d C_p^\circ = -46.0 + [1.0 - 1.54 \cdot N_{res}^{-0.268}] \cdot 30.0 \cdot < N_{CH} > \quad (10)$$

We validated our model over a large set of globular proteins, by selecting proteins for which reliable DSC determinations of $\Delta_d C_p^\circ$ exist, even though their denaturation process is not well represented by the two-state transition model. The present set consists of 50 proteins spanning a large range of chain length (i.e., from 43 to 691 residues) [30–40]. As new data appear in the literature, the set can be updated. Some features

of these proteins and the experimental and calculated values of $\Delta_d C_p^\circ$ are collected in Table 4. The overall agreement is good, as the average relative error is equal to 10%, within the range of experimental uncertainties, typically 10–20% [6,28,29]. To clarify the performance of the model we consider three closely homologous proteins: ribonuclease A, RNase A, ribonuclease S, RNase S, and monomeric bovine seminal ribonuclease, mBS-RNase. All these proteins have 124 residues. RNase S is obtained from RNase A by the proteolytic cleavage of the peptide bond between Ala20 and Ser21 by subtilisin, and by the specific noncovalent association of the two parts [36]. mBS-RNase is the monomeric derivative of BS-RNase, obtained by selective reduction and alkylation of the two intersubunit disulfide bridges and possesses more than 80% of sequence homology with RNase A [37]. There is close agreement between the calculated and experimental value of $\Delta_d C_p^\circ$ for RNase A (i.e., the relative error is ~0.2%). For RNase S the experimental value is somewhat lower than the theoretical one (i.e., the relative error is 15.6%), probably because the fraction of ASA exposed to the solvent is larger than in RNase A, as a consequence of the proteolytic cleavage. Finally, for mBS-RNase, the calculated value is significantly larger than the experimental one (i.e., the relative error is 29.8%). The reason is that mBS-RNase is not a natural protein, but a subunit of a dimeric enzyme. As a consequence, a fraction of its ASA nonpolar, that is buried on forming the complex, is exposed to solvent and does not contribute to $\Delta_d C_p^\circ$. The discrepancy is large also for hevein, a small disulfide rich protein; fragment 1–90 of cytochrome b5, a membrane protein; interleukin 1ß that has a nearly all ß structure; and hexokinase that has two domains arranged in a bilobed conformation. The failure of the algorithm in some specific cases is understandable in view of the several approximations embodied in the model. Clearly, an improvement in the estimation of $\Delta_d C_p^\circ$ would be the direct calculation of the ASA polar and nonpolar buried in the native structure of the selected protein. In fact, Freire and coworkers [41] have recently improved the calculation of $\Delta_d C_p^\circ$ through a minimization of the differences between the calculated and experimental values, based on the available structural information in the Protein Data Bank. However, this way has a drawback: the native structure has to be solved by X-ray diffraction or NMR. Thus, the use of empirical relationships, such Eqs. (2) and (3), derived from an analysis of a large set of proteins, may be a convenient choice, particularly for those globular proteins the structure of which is still unknown.

Other models have been developed to predict $\Delta_d C_p^\circ$ and all exploited the additivity of polar and nonpolar contributions. However, they differ for the compounds selected to mimic the amino acid residues. Murphy, Gill and Freire [7,42] used the dissolution into water of solid cyclic dipeptides. Record and co-workers [8,43] used the transfer of liquid hydrocarbons and liquid *N*-alkyl amides to water. Privalov and Makhatadze [19,23] used specific contributions derived by analyzing the gas to water transfer of a large number of organic compounds. Ooi and Oobatake [44] devised a model very similar to that of Privalov and Makhatadze. All these approaches reached a satisfactory agreement between calculated and experimental values of $\Delta_d C_p^\circ$, even though the starting phase and the employed compounds are very different. At first sight, this result is astonishing, but the explanation is simple. The reason is that the contribution of water reorganization around both the polar and nonpolar groups is, by far, the fundamental process in determining the heat capacity change and, as shown above, it provides practically constant contributions, regardless of the molecular species and originating phase (i.e., $\Delta C_p^\circ{}_{CONH} \approx -65.0$ J K^{-1} mol^{-1} and $\Delta C_p^\circ{}_{CH} = 30.0 \pm 2.0$ J K^{-1} mol^{-1}).

An important feature of the experimental values of $\Delta_d C_p^\circ$ normalized per residue, reported in Table 4, is that they are limited to the range 30–90 J K^{-1} mol^{-1}. This experimental finding is well reproduced by our model. Additionally Eq. (10) allows one to calculate 'master curves' of $\Delta_d C_p^\circ$ vs N_{res}, by fixing the value of $\langle N_{CH} \rangle$, which can be considered as an index of protein hydrophobicity. A reliable estimate is obtained from an average over the 50 proteins of Table 4, $\langle N_{CH} \rangle = 5.75 \pm 0.75$. The 'master curves' of $\Delta_d C_p^\circ$ vs N_{res}, for $\langle N_{CH} \rangle$ in the range 5.0–6.5, are reported in Fig. 1. Similar 'master curves' were obtained by us and others using different and less general approaches [32,45]. The combined dependence of $\Delta_d C_p^\circ$ on N_{res} and $\langle N_{CH} \rangle$ seems physically correct, as Fig. 1 points out two different effects. First, the heat capacity change is larger for the more hydrophobic proteins (i.e., those possessing larger values of $\langle N_{CH} \rangle$).

Table 4
Number of residues (N_{res}), average number of nonpolar hydrogen atoms per residue (<N_{CH}>), and experimental and calculated denaturation heat capacity changes ($\Delta_d C_p^\circ$) per residue for a set of globular proteins

Protein	N_{res}	<N_{CH}>	$\Delta_d C_p^\circ$(exp) (J K^{-1} mol^{-1})	$\Delta_d C_p^\circ$(calc) (J K^{-1} mol^{-1})	Error %
Hevein	43	6.0	44.4[a]	32.8	24.9
Protein G domain B1	56	6.4	46.4[b]	45.5	1.9
Protein G domain B2	56	6.5	51.8[b]	46.9	9.5
BPTI	58	5.4	28.5[c]	32.0	12.3
BPTI-RCOM	58	5.4	26.7[c]	32.0	19.9
Erabutoxin B	61	5.3	31.2[c]	31.6	1.3
SH3	62	6.4	50.0[d]	48.2	1.9
Sso7d	63	6.4	47.6[e]	48.6	2.1
p53tet	64	4.9	25.8[c]	26.7	4.7
Tendamistat	74	5.4	39.1[c]	37.3	4.6
Ubiquitin	76	6.4	57.1[c]	53.4	6.5
CI-2	83	5.5	39.8[f]	41.3	3.8
K4 fragment of plasminogen	86	5.2	40.7[c]	37.2	8.6
Barstar	89	6.2	46.1[c]	54.0	17.1
Fragment 1–90 of cytochrome b5	90	6.0	66.7[c]	51.0	23.5
Ribonuclease T1	104	4.9	46.6[c]	35.8	23.2
Cytochrome c	104	6.2	64.4[c]	57.5	10.7
Parvalbumin	108	5.4	46.3[c]	44.9	3.0
E.coli thioredoxin	108	5.7	63.9[c]	49.9	21.9
Barnase	110	5.8	54.6[c]	52.0	4.8
apo α lactalbumin	122	6.0	62.3[c]	57.5	7.7
Ribonuclease A	124	5.4	47.6[c]	47.5	0.2
Ribonuclease S	124	5.4	41.1[g]	47.5	15.6
m-ribonuclease BS	124	5.5	37.9[h]	49.2	29.8
ROP protein	126	5.8	63.5[c]	54.7	13.9
Adrenal ferredoxin	128	5.6	58.6[c]	51.5	12.1
Hen lysozyme	129	5.4	51.9[c]	48.2	7.1
Equine lysozyme	129	5.7	57.3[i]	53.4	6.8
Human lysozyme	130	5.5	55.0[c]	50.1	8.9
λ Cro repressor	132	6.1	52.3[c]	60.9	16.4
Staph. nuclease	149	6.2	64.4[c]	65.1	1.1
met-myoglobin	153	6.4	70.6[c]	69.2	2.0
Interleukin 1ß	153	6.2	49.7[c]	65.6	32.0
ß-lactoglobulin	162	6.4	66.1[c]	70.4	6.5
T4 lysozyme	164	6.1	63.4[c]	65.2	2.8
Diphteria toxin Domain A	183	5.8	79.8[c]	61.7	22.7
Serum retinol Binding protein	190	5.3	56.3[c]	53.0	5.9
Papain	212	5.5	65.1[c]	58.5	10.1
SSI	226	5.4	54.0[c]	57.6	6.7
Chymotrypsinogen	245	5.5	64.9[c]	60.8	6.3
Carbonic anhydrase B	256	5.8	63.3[c]	67.4	6.5
Subtilisin BPN″	275	5.7	73.1[c]	66.6	8.9
Pepsinogen	365	5.7	69.9[c]	70.8	1.3
Cytochrome P450cam	414	6.0	80.4[c]	78.9	1.9
PGK	415	6.4	75.7[c]	87.2	15.2
Hexokinase	461	6.0	65.1[j]	80.4	23.5
Amylase A	478	5.8	76.2[c]	76.7	0.7
S-ßgly	541	5.6	87.8[k]	74.1	15.6
apo-ovo transferrin	686	5.8	80.5[c]	81.4	1.1
apo-human transferrin	691	5.8	87.1[c]	81.5	6.4

[a] Ref. [30]; [b] Ref. [31]; [c] Ref. [32]; [d] Ref. [33]; [e] Ref. [34]; [f] Ref. [35]; [g] Ref. [36]; [h] Ref. [37]; [i] Ref. [38]; [j] Ref. [39]; [k] Ref. [40].

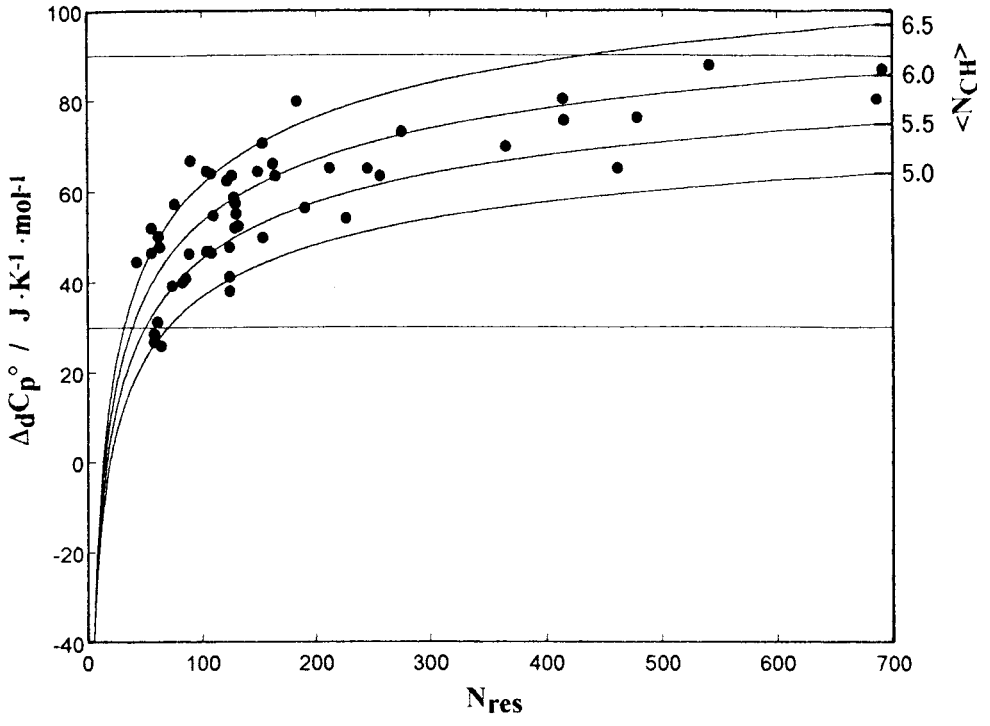

Fig. 1. Theoretical dependence of $\Delta_d C_p^\circ$ on the number of residues in the protein. Each curve is calculated according to Eq. (10) by fixing the $<N_{CH}>$ value as reported on the right margin. Experimental values are shown as filled circles. The horizontal lines represent the limiting values of $\Delta_d C_p^\circ$, 30 and 90 J K^{-1} mol^{-1}.

Second, it increases for proteins of equal hydrophobicity at lengthening the polypeptide chain. The lower and upper bounds $\Delta_d C_p^\circ$, 30 and 90 J K^{-1} mol^{-1}, are determined by physical and geometrical factors: (a) the chemical nature and dimensions of the 20 natural residues that are the building blocks of proteins; and (b) the globular shape of proteins that determines the ratio between buried and exposed surface. Indeed, if $\Delta_d C_p^\circ$ were lower than the limit, this would imply the absence of a close-packed core, inaccessible to water molecules. On the other hand, $\Delta_d C_p^\circ$ cannot rise indefinitely at increasing the chain length because f_b reaches a practically constant value.

5. Conclusion

We have shown that $\Delta_d C_p^\circ$ can be predicted with accuracy by a simple model, based on the group additivity principle. It couples common structural features of globular proteins to specific polar and nonpolar contributions to $\Delta_d C_p^\circ$ derived from an analysis of the transfer of small organic compounds to water. The model is tested against a large set of proteins and readily explains the experimental finding that $\Delta_d C_p^\circ$ is limited in the range 30–90 J K^{-1} mol^{-1}.

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