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Heat capacity of folding of proteins corrected for disulfide cross-links

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Abstract

The heat capacities ($\Delta C_{p,f}$) for the temperature-induced folding of proteins: barnase, lysozyme T4, papain, trypsin, ribonuclease T1, chymotrypsin, lysozyme and ribonuclease A have been calculated from the change in solvent accessible surface area between the native state and extended polypeptide chain. To visualize the effect of disulfide cross-links on molar heat capacity, loops of varying number of alanine residues and extended alanine chains with terminal cystein are modeled. The difference in ΔC_p values between the extended state and the loop conformation of proteins is linearly related to the number of residues in the loop. Corrections to the heat capacity of folding ($\Delta C_{p,f}$) are applied for proteins with cross-links based on this observation. There is good correlation between corrected values of $\Delta C_{p,f}$ and experimental values.

Keywords: Protein folding; Protein stability; Heat capacity of folding; Solvent-accessible surface area; Disulfide cross-links

1. Introduction

Since each amino acid influences the free energy of both the folded and unfolded states, insight into the denatured state is crucial for an understanding of protein stability [1]. Lack of information about the denatured state has constrained the proposition of models for the early events of the folding process and has also led to the underestimation of the contributions of hydrophobic interactions to protein stability [2,3]. The exposure of internal non-polar groups of proteins to water would result in heat capacity increment, since the transfer of non-polar compounds to water is associated with a significant increase of the heat capacity [4]. Thus, heat capacity change on denaturation of proteins is a sensitive index of the completeness of the protein unfolding. The comparison of the experimental values of heat capacity of folding with the theoretical values (based on complete unfolding of proteins) obtained by accessible surface area calculation should give an idea about the extent of the completeness of the unfolding process. Myers et al. [5] observed that the correlation between heat capacity of folding and the change in accessible surface area are good, except in some

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cases where there is disulfide cross-links in a native protein. This is expected, because the presence of cross-links in the unfolded state will result in a more compact unfolded state, thus reducing the accessibility of the unfolded polypeptide chain to solvent. To compensate for the effects of cross-links, Myers et al. [5] employed three different ways to estimate the magnitude of the reduction of accessible surface area (ΔA) per disulfide bond. The correction of ΔA per disulfide bond was estimated to be at 900 Å^2. However, using a single value for all cross-links is an oversimplification. The reduction in the accessibility of a protein in the unfolded state due to a particular disulfide bond depends on several factors: the size of the loop connected by the cross-links, the position relative to other cross-links and the overall size of the protein.

In the present work, we have calculated the change in solvent accessible surface area (ΔA) and the heat capacity of folding ($\Delta C_{p,f}$) of various globular proteins: barnase, lysozyme T4, papain, tryspin, ribonuclease T1, chymotrypsin, lysozyme and ribonucease A, with and without disulfide cross-links. Calculated ΔA and $\Delta C_{p,f}$ for proteins with disulfide cross-links were corrected, based on the assumption that the disulfide cross-links remains intact upon unfolding.

2. Materials and methods

Accessible surface areas of the native and denatured conformation of globular proteins were calculated using Lee and Richards analytical molecular surface algorithm 'ACCESS' [6], with a probe radius of 1.4 Å, a slice width of 0.1 Å, and atomic radii listed in Juffer et al. [7].

The changes in heat capacity of folding of proteins ($\Delta C_{p,f}$) were calculated using equations developed by Spolar [8] and Myers et al. [5].

The Spolar equation [8] for the calculation of $\Delta C_{p,f}$ is given as:

$$\Delta C_{p,f} = -0.33\ (\Delta A_{np}) + 0.16\ (\Delta A_p)$$

A similar dependence of heat capacity changes on accessible surface area change is proposed by Myers et al. [5].

$$\Delta C_{p,f} = -(0.28 \pm 0.12)\ (\Delta A_{np}) + (0.09 \pm 0.3)\ (\Delta A_p)$$

Table 1
Change in accessible surface area (ASA) of proteins upon folding

Protein	Change in accessible surface area $A^{\circ 2}$			
	ΔA_{ali}	ΔA_{ar}	ΔA_p	ΔA_{total}
Barnase	−5321	−1432	−3989	−10450
Lysozyme T4	−8949	−1574	−5795	−16319
Papain	−11686	−2471	−7670	−21827
Trypsin	−12302	−1695	−7580	−21576
Ribonuclease T1	−4078	−1550	−3292	−8926
Chymotrypsin	−13422	−2022	−7515	−22959
Lysozyme	−6263	−1253	−4765	−12281
Ribonuclease A	−5662	−1058	−4683	−11403

3. Results and discussions

3.1. Change in solvent accessible surface area upon folding

The changes in non-polar accessible surface area (ΔA_{ali}), polar accessible surface area (ΔA_p) and aromatic accessible surface area (ΔA_{ar}) upon unfolding of proteins: barnase, lysozyme T4, papain, tryspin, ribonuclease T1, chymotrypsin, lysozyme, and ribonuclease A are calculated using different crystal forms of the proteins and are listed in Table 1. The errors in calculation of protein accessible surface area values are within 2–3%.

3.2. Change in heat capacity of folding

The heat capacity of folding of various proteins calculated using Spolar [8] and Myers's equations [5], along with the experimental values of $\Delta C_{p,f}$ are listed in Table 2.

It is clear that for proteins with disulfide bonds or other cross-links, the calculated $\Delta C_{p,f}$ values are greater than the experimental values. This is expected because the presence of cross-links in the unfolded state will result in a more compact

Table 2
Heat capacity of folding of proteins

Proteins	$\Delta C_{p,f}$ (cal/mol per K)			No. of disulfide cross-links
	Spolar	Myers	Experimental	
Lysozyme T4	−2545	−2425	−2610[a]	0
Papain	−3445	−3274	−3014[b]	3
Trypsin	−3406	−3237	−2856[c]	6
Ribonuclease T1	−1331	−1280	−1270[d]	2
Chymotrypsin	−3894	−3648	−3373[e]	5
Lysozyme	−1718	−1676	−1540[f]	4
Ribonuclease A	−1468	−1460	−1230[g]	4
Barnase	−1606	−1541	−1650[h]	0

[a] Makhatdze and Privalov, 1995 [11].
[b] Mendiola et al., 1993 [12].
[c] Privalov, 1979 [13].
[d] Yu et al., 1994 [10].
[e] Privalov and Gill, 1988 [14].
[f] Privalov and Gill, 1988 [14].
[g] Privalov and Gill, 1988 [14].
[h] Griko et al., 1994 [15].

unfolded state, thus reducing the accessibility of the unfolded polypeptide chain to solvent.

3.3. Corrections in ΔASA and $\Delta C_{p,f}$ of proteins

The corrections in ΔASA and $\Delta C_{p,f}$ of proteins for disulfide cross-links were carried out as follows. The loops with varying number of alanine residues with disulfide linkage were modeled on the Insight II program from Biosym (MSI). The energy optimization of the model built structures was performed using the CVFF force field [9]. The minimization protocol employed the steepest descent, followed by the conjugate gradient algorithms, until the RMS deviation between successive structures attained a value of 0.01 Å. The non-polar accessible surface areas and polar surface areas of model built structures are listed in Table 3. Extended alanine chains were also modeled. The calculated values of non-polar accessible surface areas and polar surface areas of extended alanine chain are also listed in Table 3. The changes and percentage changes in non-polar accessible surface areas and polar accessible surface areas from extended to loop conformation, and corresponding changes in the $\Delta C_{p,f}$ calculated using Myers and Spolar's equations are listed in Table 4.

A linear relation exists between heat capacity of folding and the number of residues in the loop. This clearly indicates that the change in heat

Table 3
Accessible surface area of $(ALA)_n$ with and without a disulfide bond

Residues in loop (n)	With disulfide cross-link			Without disulfide cross-link		
	A_{np} ($A^{\circ 2}$)	A_p	A_{total}	A_{np}	A_p	A_{total}
5	355	234	590	365	323	687
10	595	320	915	746	475	1221
20	1179	470	1649	1508	780	2287
30	1751	526	2277	2271	1084	3355
35	2075	645	2720	2653	1236	3889
40	2322	756	3078	3033	1388	4422
45	2547	891	3437	3414	1540	4954

Table 4
Change in accessible surface area and heat capacity due to formation of a disulfide bond

No. of residues in loop	ΔA_{np} ($A^{\circ 2}$)	ΔA_p	% change in A_{np}	% change in A_p	$\Delta C_{p,f}$ (cal/mole per K)	
					Spolar	Myers
5	−9	−88	3	27	11	5
10	−151	−155	20	33	−25	−28
20	−329	−309	22	40	−59	−64
30	−520	−559	23	51	−82	−95
35	−577	−591	22	48	−96	−108
40	−711	−632	23	45	−134	−142
45	−867	−649	25	42	−182	−184

Table 5
Heat capacity of folding of proteins corrected for disulfide cross-links

Protein	$\Delta C_{p,f}$ (cal/mol per K) Corrected for disulfide cross-links						Experimental
	Spolar	Myers	a	b	c	d	
Barnase	−1606	−1541	−1606	−1541	−1606	−1541	−1650
Lysozyme T4	−2545	−2425	−2545	−2425	−2545	−2425	−2610
Chymotrypsin	−3894	−3648	−3169	−2938	−3582	−3306	−3373
Lysozyme	−1718	−1676	−1138	−1108	−1643	−1588	−1540
Ribonuclease A	−1468	−1460	−888	−892	−1235	−1200	−1230
Papain	−3445	−3274	−3010	−2885	−3033	−2874	−3014
Trypsin	−3406	−3237	−2536	−2385	−3093	−2895	−2856
Ribonuclease T1	−1331	−1280	−1041	−996	−1323	−1265	−1270
Correlation Coefficient	0.983	0.983	0.989	0.986	0.994	0.996	
Covariance Factor	763628	704207	690512	636401	684457	624557	

(a) Correction for disulfide cross-link to the values of heat capacity of folding, calculated using Spolar's equation, based on William Doig's estimation of the changes in ΔA_{np} (640 $A^{\circ 2}$) per disulfide bond. (b) Correction for disulfide cross-link to the values of heat capacity of folding, calculated using Myers's equation, based on William Doig's estimation of the changes in ΔA_{np} (640 $A^{\circ 2}$) per disulfide bond. (c) Correction for disulfide cross-link to the values of heat capacity of folding, obtained by calculating the change in accessible surface area from loop to extended conformation and using Spolar's equation. (d) Correction for disulfide cross-link to the values of heat capacity of folding, obtained by calculating the change in accessible surface area from loop to extended conformation and using Myers's equation.

capacity of folding of proteins due to disulfide cross-links is a function of the size of the loop.

Applying Myers's equation, the following linear relation is obtained.

$$\Delta\Delta C_{p,f} = 23.5 - (4.23) \times n \quad (1)$$

Where n is number of residues in the particular loop.

For more than one disulfide bond, this formula can be extended as

$$\Delta\Delta C_{p,f} = 23.5 \times m - (4.23) \times \sum n \quad (2)$$

where m represents the number of disulfide bonds, while $\sum n$ is the sum of residues in the loops.

Applying Spolar's equation, the following linear relation is obtained.

$$\Delta\Delta C_{p,f} = 28.8 \times m - (4.15) \times \sum n \quad (3)$$

where m represents the number of disulfide bonds while $\sum n$ is the sum of residues in the loops.

Corrections to the calculated values of heat capacity of folding using Spolar and Myers's equations [Eqs. (2) and (3)] were applied for proteins with disulfide cross-links. The bigger loops with more than 80 residues are neglected, since they do not lead to a significant change in accessible surface area from loop to extended conformation. Therefore, the 1–122 loop in chymotrypsin, 6–127 and 30–115 loops in lysozyme, 22–157 and 128–232 loops in trypsin, and the 6–103 loop in ribonuclease T1, are not considered. The calculated values of $\Delta C_{p,f}$, corrected for disulfide cross-links, along with the experimental values are listed in Table 5.

Doig and Williams [2] estimated the change in ΔA_{np} per disulfide bond from the dependence of hydration free energy and heat capacity of folding ($\Delta C_{p,f}$) on cross-links to be 590° A^2 and 690° A^2, respectively. Because the fraction of total polar area buried is 0.55 times the total non-polar area buried, the change in the $\Delta C_{p,f}$ value per disulfide bond would correspond to $0.33 \times 640 - 0.55 \times 640 \times 0.16 \cong 145$ cal mol^{-1} K^{-1} when Spolar's equation is applied and corresponds to 142 cal mol^{-1} K^{-1} when Myers's equation is applied. The $\Delta C_{p,f}$ of various proteins with disulfide cross-links is thus corrected for the effect of disulfide

cross-links and the values are also listed in Table 5.

The correlation coefficients and the covariance (COVAR) factors, the average of the products of deviation for each data point pair in two data sets (experimental and calculated) by various methods, are listed in Table 5.

The best correlation coefficient and lowest value of covariance factor in the values of $\Delta C_{p,f}$ is from this work, which shows that the correction for disulfide cross-links, as outlined in the present work, significantly improves the calculated values of $\Delta C_{p,f}$ and among the various methods, gives values closest to the experimental values.

For ribonuclease T1, the values of heat capacity of folding uncorrected for disulfide cross-links are closer to the experimental values, suggesting that the denatured form of ribonuclease T1 is without disulfide cross-links. However, it is not so. If the size of the loop is not taken into account, we should have expected a lower value of heat capacity of protein folding. Ribonuclease T1 has one big loop (6–103) and one short loop (2–10). Because of their size, both of them do not affect heat capacity significantly. Yu et al. [10] measured the heat capacity of the denatured state of the ribonuclease T1 and compared it with its heat capacity, to both disulfide bonds, reduced and cystein residue, carboxymethylated. When the heat capacity effect of the additional four CH_2COOH groups introduced by modification are taken into account, the values obtained are found to be closer to the heat capacity of the denatured ribonuclease T1. Thus, the denatured state of ribonuclease T1 and reduced ribonuclease T1 has same heat capacity of folding.

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