Thermodynamics of the Helix-Coil Transition: Binding of S15 and a Hybrid Sequence, Disulfide Stabilized Peptide to the S-Protein

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ABSTRACT **Pancreatic ribonuclease A may be cleaved to produce two fragments: the S-peptide (residues 1–20) and the S-protein (residues 21–124). The S-peptide, or a truncated version designated as the S15 peptide (residues 1–15), combines with the S-protein to produce catalytically active complexes. The conformation of these peptides and many of their analogues is predominantly random coil at room temperature; however, they populate a significant fraction of helical form at low temperature under certain solution conditions. Moreover, they adopt a helical conformation when bound to the S-protein. A hybrid sequence, disulfide-stabilized peptide (ApaS-25), designed to stabilize the helical structure of the S-peptide in solution, also combines with the S-protein to yield a catalytically active complex. We have performed high-precision titration microcalorimetric measurements to determine the free energy, enthalpy, entropy, and heat capacity changes for the binding of ApaS-25 to S-protein within the temperature range 5–25°C. The thermodynamic parameters for both the complex formation reactions and the helix-to-coil transition also were calculated, using a structure-based approach, by calculating changes in accessible surface area and using published empirical parameters. A simple thermodynamic model is presented in an attempt to account for the differences between the binding of ApaS-25 and the S-peptide. From this model, the thermodynamic parameters of the helix-to-coil transition of S15 can be calculated. Proteins 2001;42:523–530.**

Key words: isothermal titration calorimetry; ligand binding; RNase-S; S-peptide; structure-based calculations; helix-coil transition

INTRODUCTION

Understanding the energetics of formation of secondary structural elements in proteins has long been recognized as an important step in determining overall protein stability. Direct measurements of the thermodynamic parameters of forming α-helix and β-sheet structures in aqueous solution would require one to monitor the unfolding of isolated secondary structural elements within proteins. However, proteins generally unfold in a cooperative manner, so that it is not possible to observe the unfolding of their secondary structural elements independently.

There are several examples of short peptides corresponding to regions in native proteins that autonomously fold into native-like structures (autonomous folding units), which have proved useful for investigating helical stabilities.[1–4] For example, two fragments of bovine ribonuclease A (RNase-*A*), S15 (residues 1–5) and the S-protein (residues 21–124) combine in a reversible reaction to form the complex ribonuclease S (RNase-S), which has a structure very similar to that of native RNase–A.[5,6] Residues 3–13 of the S15 assume a helical conformation in the RNase–S complex.[7,8] Moreover, the free S15 peptide and other similar peptides have a significant propensity to form an α-helix in aqueous solution, under certain conditions. Other short synthetic peptides reported by Marqusee and Baldwin[3] have as much as 80% helix content. However, attempts to measure the energetics accompanying the thermal unfolding of short helices in aqueous solution have proved difficult, particularly measurements of the heat capacities and enthalpies, since the transition are broad.[9] Very recently, Taylor et al.[10] measured the energetics of the helix-coil transition of a short peptide with covalently closed N- and C-terminal loops, by circular dichroism (CD) and differential scanning calorimetry (DSC).

Some years ago, Pease et al.[11] reported the synthesis and solution structure of a hybrid sequence peptide (ApaS-25) in which stabilization of the S-peptide helical structure was achieved by constraining it within the fold defined by the disulfide bridges of the protein, apamin. The hybrid sequence peptide was found to bind to S-protein, restoring enzymatic activity, suggesting that the helical conformation adopted by the S-peptide region is recognized by the

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S-protein. Furthermore, ApaS-25 induced the production of antibodies in rabbits that cross-reacted with native RNase-A, suggesting that the antibodies recognize the helical conformation of the hybrid peptide. In addition, modeling studies of the complex of the hybrid peptide with S-protein, based on the distance geometry structure of the hybrid peptide and the crystal structure of RNase-S, indicated that the contact face is appropriately exposed. Thus, from the perspective of binding to the S-protein, ApaS-25 represents a conformationally constrained S-peptide analogue.

As part of a research effort in the laboratories of Drs. Frederick Richards and Julian Sturtevant at Yale University, together with Dr. Rhagavan Varadarajan, one of us (P.R.C.) has reported free energies, enthalpies, entropies, and heat capacities for the binding of a series of S-peptide analogues to the S-protein.[6,12]

The overall binding energetics of these analogues reflect contributions from the changes in conformation of the peptides from coil to helical form, as well as the change in conformation of the S-protein and the association of the peptides with S-protein. In order to obtain a quantitative comparison of S-peptide and ApaS-25 binding to S-protein, we have investigated the thermodynamics of binding of the conformationally constrained hybrid peptide to S-protein. The thermodynamic parameters for the binding of an analogue of S-peptide containing residues 1–5 (S15), and those for the binding of the ApaS-25 peptide to the S-protein are compared in view of the energetics of the helix-to-coil transition of the S-peptide. We have used the S15 analogue instead of the S-peptide, since it was shown that residues 16–20 were not important to binding, and that the two complexes (S20 and S15) with the S-protein were structurally identical.[6] Thus, the presence of these six extra residues in ApaS-25 will not affect the binding process.

MATERIALS AND METHODS

Materials

RNase-S was obtained from Sigma Chemical Company. S-protein and S15 were obtained and purified as described previously.[6] Hybrid II peptide was synthesized as described by Pease et al.,[11] and concentrations of stock peptide solutions were determined by quantitative amino acid analysis as described by Connelly et al.[6]

Titration Calorimetry

Titrations were performed with an OMEGA isothermal titration calorimeter from MicroCal (Northhampton, MA) as described earlier.[6] Briefly, a series of 4–5-μl injections of peptide into 1.3215 ml of S-protein solution were performed, and the heat of injection measured at each step. The reaction heat thus observed at the ith step, q_i, for a simple 1:1 one association reaction, $M + X \rightarrow MX$, is equal to the product of the molar enthalpy and the number of moles of MX formed upon passing from the step $I - 1$ to step i:

$$q_i = \Delta H[MX_i - MX_{i-1}] \quad (1)$$

The number of moles of product present at step i is a function of the total concentration of peptide in the cell $[X_T]_i$, the total concentration of protein $[M_T]_i$, and the association constant, K. The values of the total concentrations of the peptide and protein at each step are determined by their initial concentrations as well as the dilution factors calculated from the volume of injection and the cell volume. Estimates of ΔH and K are determined by a nonlinear least-squares fit to the titration data as described by Connelly et al.[6] Enthalpies at lower temperatures were determined by multiple single injections performed under tight binding conditions.[12]

Structure-Based Calculations

Structure-based calculations have been performed using published empirical parameters obtained from protein unfolding and model compound data[13–15] that relate changes in polar and apolar accessible surface area (ASA) to changes in thermodynamics. We used this approach to calculate the thermodynamic parameters both for the binding reactions with S15 and ApaS-25 and for the helix-to-coil transition. The structure for RNase-S was taken from the Protein Data Bank[16] as reported by Kim et al.[8] As there are no reported X-ray or nuclear magnetic resonance (NMR) structures for the component parts, S15 (helix) and S-protein, the S-protein and the helical form of the S-peptide were taken as the respective part present in the structure of the complex. For the ApaS-25 complex, we used the Sybyl program to model the complex. Starting with the structure of RNase-S, the necessary changes of S15 to transform it into ApaS-25 were made, while maintaining it in the same position in the complex as S15.

The coil structure for S15 was generated using either the Sybyl program or the Ala-X-Ala model. When using the Sybyl program, the helix taken from the crystal structure was transformed into a random coil; an energy minimization was then performed (Force Field TRIPOS, length 1,000, step 1, snap 5, temperature 300 K). The values for this transition are added to the total change in accessible surface area due to complex formation, as indicated in the binding scheme below.

The ASA were calculated using the algorithm of Lee and Richards,[16] as implemented in the ACCESS program (Scott R. Presnell), using a probe radius of 1.4 Å and a slice width of 0.25 Å. The calculation of thermodynamic parameters is described in Appendix A.

RESULTS

Titration Calorimetry

Figure 1 shows a titration of the ApaS-25 peptide into S-protein. The solid line was calculated from the best-fit parameters given in Table I. In all experiments, the standard error of a point was within the range of 4–16 μJ, which compares favorably with the reported performance of the instrument. Titrations were carried out under two sets of experimental conditions for both the hybrid peptide and S15. The differences in the thermodynamic parameters, calculated relative to the S15 parameters, are also

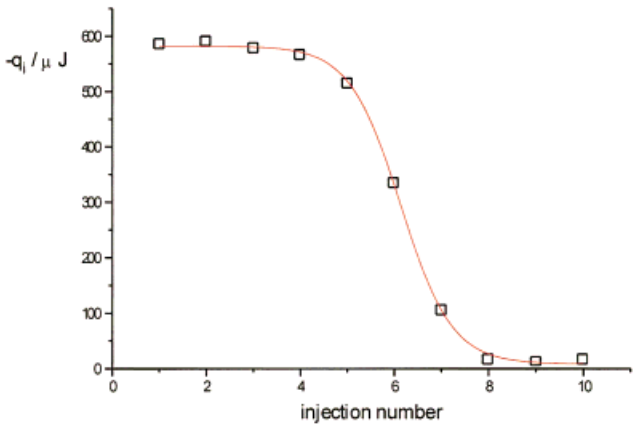

Fig. 1. Calorimetric titration of ApaS-25 peptide into S-protein at pH 6.0, 50 mM sodium acetate, 100 mM sodium chloride, 26.2°C; 5-μl injections of ApaS-25 peptide at 0.9 mM were made into 19.6 μM S-protein. The solid line represents the best fit with $\Delta H = -132.6 \pm 1.7$ kJ mol^{-1} and $K = 6.72(\pm 1.1) \times 10^6$. Errors on the parameters were determined from the asymptotic covariance matrix. The standard error of a point was 11 μJ. [Color figure can be viewed in the online issue, which is available at www.interscience.com.]

given in Table I for both sets of conditions (e.g., $\Delta\Delta H_{\text{obs}} = \Delta H_{\text{ApaS}} - \Delta H_{\text{S15}}$).

The data collected at pH 6.0 correspond to conditions used previously for the determination of binding parameters of S15. Data were also collected in the presence of phosphate. Phosphate is known to stabilize the complex of the S-peptide and S-protein[18]; indeed, we have found that binding of both peptides is significantly tighter in the presence of phosphate. Therefore, in order to obtain an accurate determination of a binding constant in the presence of phosphate, we used a slightly higher temperature (30°C) and a pH value of 5.0.

Figure 2 shows the enthalpies of ApaS-25 binding to S-protein over a temperature range of 5–25°C. The data are well described by a linear function, with the slope indicating a large negative heat capacity change upon binding.

Structure-Based Calculations

The results for the calculated changes in accessible surface areas upon complex formation are shown in Table II. The change in accessible surface area for the coil-to-helix formation are also given. The model finally chosen for the coil was the random coil generated by the Sybyl program. The calculated and experimental results for the binding reactions are given in Table III, and those for the coil-to-helix transition in Table IV.

DISCUSSION

The thermodynamics of binding of S15 to S-protein may be considered to be the result of three processes, consisting of two conformational events and one association event (Fig. 3): (1) the coil (C)-to-helix (H) transition of S-peptide; (2) the change in conformation of the S-protein from the state it assumes when no peptide is bound (state A) to the conformation it assumes in RNase-S (state B); and (3) the association of the helix with conformation B of the S-protein. The observed thermodynamic parameters, ΔJ, for this reaction are given by the sum of the three component processes:

$$\Delta J_{\text{obs,S15}} = \Delta J_{CH} + \Delta J_{AB} + \Delta J_{HB} \quad (2)$$

Specifically, J may be free energy (G), enthalpy (H), entropy (S), or heat capacity (C_p). Similarly, for the binding of ApaS-25 to S-protein, one may outline three events: (1) the change in conformation of the hybrid peptide from the conformation it assumes when free (H') to that which it assumes when bound to the S-protein (H''); (2) the change in conformation of the S-protein from the state it assumes when no peptide is bound (state A) to the conformation it assumes in the complex with ApaS-25 (B'); and (3) the association of the hybrid peptide in state H'' to conformation B' of the S-protein. Again, the sum of the three component processes gives the observed thermodynamic parameters.

$$\Delta J_{\text{obs,ApaS}} = \Delta J_{H'H''} + \Delta J_{AB'} + \Delta J_{H''B'} \quad (3)$$

As a first approximation in comparing the binding of the two peptides, we can assume that (1) the conformation of ApaS-25 in the complex contains a helical binding region identical to that of S15 in RNase-S; (2) the conformation of S-protein within the S-protein/ApaS-25 complex is identical to that of the S-protein in RNase-S; and (3) the conformation of free ApaS-25 is identical to the conformation it adopts when bound in the complex. Energetically, the first two assumptions imply that $\Delta J_{AB'} + \Delta J_{H''B'} \approx \Delta J_{AB} + \Delta J_{HB}$, and the third assumption implies that $\Delta J_{H'H''} \approx 0$. Together, these relations imply that the difference in thermodynamic properties of the binding of the two peptides is determined entirely by the changes in the coil-to-helix conformational change of S15,

$$\Delta\Delta J_{\text{obs}} = \Delta J_{\text{obs,ApaS}} - \Delta J_{\text{obs,S15}} \approx -\Delta J_{CH} \quad (4)$$

The qualitative structural picture embodied by the assumptions 1–3 of the simple model given above is supported by computer modeling studies in which the S-peptide in the crystallographically determined RNase–S structure[8,19] is replaced by the distance geometry structure of ApaS-25.[11] These studies demonstrated that the contact face is appropriately exposed in ApaS-25, indicating that the two peptides bind similarly, and that the extra residues protrude out from the surface of the enzyme. Figure 4 shows the structure of ribonuclease S,[8] showing that one face of the peptide is in contact with the S-protein, and the other is exposed to solvent. Further, X-ray data for complexes of S-protein with S peptides analogues (with one mutation at the Met13 residue) shows that they all belong to the same space groups with very similar unit cell parameters.[6]

The observed parallel changes in the thermodynamic properties here determined for the binding of S15 and ApaS-25 to the S-protein in the presence and absence of phosphate, at different pH values (Table I), can be taken as

TABLE I. Thermodynamic Parameters for Binding Peptides to S-Protein

Ligand	t/°C	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}
S15[a]	29.5	−42.2 ± 0.4	−220.5 ± 2.9	−178.2 ± 2.9
ApaS-25[a]	29.6	−40.6 ± 0.4	−183.7 ± 1.7	−143.1 ± 1.7
		$\Delta\Delta G°$ = +1.6 ± 0.4	$\Delta\Delta H°$ = +36.8 ± 3.4	$T\Delta\Delta S°$ = +35.1 ± 3.4
S15[b,c]	26.2	−38.9 ± 0.4	−173.2 ± 5.0	−134.3 ± 5.0
ApaS-25[b]	26.2	−38.9 ± 0.4	−132.6 ± 1.7	−93.7 ± 1.7
		$\Delta\Delta G°$ = 0.0 ± 0.4	$\Delta\Delta H°$ = +40.6 ± 5.3	$T\Delta\Delta S°$ = +40.6 ± 5.3

[a]Experiments carried out at pH 5.0, 50 mM sodium acetate, 50 mM potassium phosphate, and 50 mM sodium chloride.
[b]Experiments carried out at pH 6.0, 50 mM sodium acetate, 100 mM sodium chloride.
[c]Data calculated from the results of Varadarajan et al.[12]

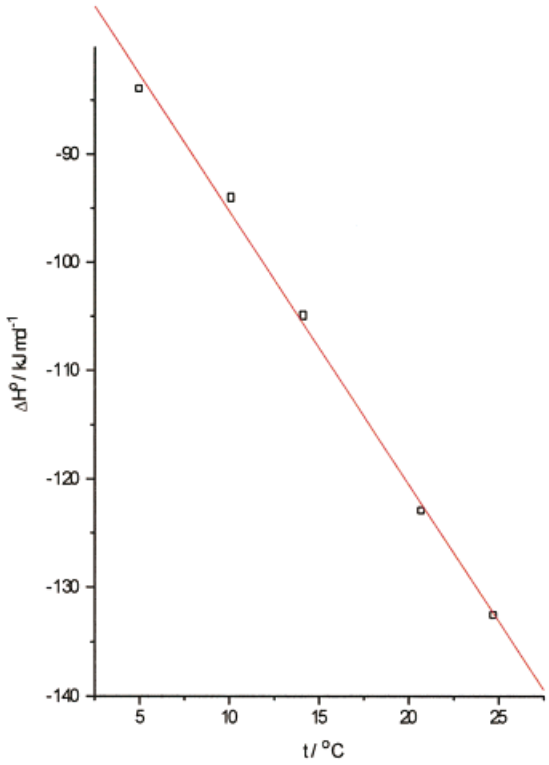

Fig. 2. Enthalpies of ApaS-25 binding to S-protein at pH 6.0, 50 mM sodium acetate, 100 mM sodium chloride, determined from titration calorimetric measurements. The data points, given as temperature (°C), enthalpy (kJ mol^{-1}) pairs, are as follows: (5.0, −84.1), (10.1, −94.1), (14.1, −105.0), (20.7, −123.0), (24.7, −132.6). [Color figure can be viewed in the online issue, which is available at www.interscience.com.]

TABLE II. Changes in ASA for Complex Binding of S-Protein With S15 and ApaS-25, as Well as for the Coil-to-Helix Transition of the S-Peptide

	ΔASA_{ap}/Å^2	ΔASA_{pol}/Å^2
S-protein and S15		
S-protein	−400.65	−257.23
S15 (helix)	−452.68	−292.85
S15 (coil-to-helix)	−243.94	−271.67
Total	−1097.27	−821.75
S-protein and ApaS-25		
S-protein	−362.31	−226.13
ApaS-25	−471.69	−274.33
Total	−834.00	−500.46

ASA, accessible surface area.

a further indication of a similar binding pattern for both peptides.

However, in order to assess the quantitative validity of the model, it is important to compare the various observed thermodynamic differences between S15 and ApaS-25 binding to S-protein to estimates of the individual thermodynamic properties of the coil-to-helix transition of S15.

Heat capacity changes for the unfolding of proteins and for the dissociation of ligands from proteins recently have been discussed.[20–26] An early analysis by Sturtevant[20] indicated that heat capacity changes for protein unfolding are determined largely by the exposure of nonpolar groups to water, but that one must also consider contributions attributable to the changes in the number of easily excitable vibrational modes upon unfolding. Several recent reports have focused on the relationship between the thermodynamic properties for unfolding and binding reactions and changes in polar and apolar ASA.[25,27–31] These studies indicate that one can account quantitatively for ΔC_p based solely on changes in ASA.

From the structure-based calculations, we see in Table III that the calculations for the complex formation with S15 and with ApaS-25 fail to predict the observed values, and in some cases even the sign of the parameter. This is to be expected, as we used the structure for the native S-protein in the complex in the calculations, but it is known that the S-protein is partially unfolded in solution and that it refolds upon binding.[12,26,32] By contrast, this should not affect the possibility of comparing the differences in the thermodynamic properties for the two binding reactions, nor of using them to predict the coil-to-helix transition parameters, given the assumptions above.

Regarding the heat capacity changes for the two binding reactions, the calculated values in both cases are smaller (less negative) than the measured values. Our value for the S15 binding reaction, −1.2 kJ K^{-1} mol^{-1}, is similar to that obtained earlier by Spolar and Record,[27] (−0.8 ± 0.2 kJ K^{-1} mol^{-1}), under the same assumptions (i.e., S-protein structure as in the crystal). We know, as stated above, that part of the measured heat capacity change comes from the refolding of the S-protein upon binding.

TABLE III. Comparison Between Experimental and Calculated Thermodynamic Parameters for Binding S-Protein to S15 and ApaS-25 at 26.2°C

	S15		ApaS-25	
	Calculated	Experimental	Calculated	Experimental
ΔC_p/kJ K^{-1} mol^{-1}	−1.17	−3.8 ± 0.4	−1.02	−2.6 ± 0.4
$\Delta H°$/kJ mol^{-1}	−29.4	−173.2 ± 5.0	−1.64	−132.6 ± 1.7
$\Delta S°$/J K^{-1} mol^{-1}	0	−449 ± 17	+133	−313 ± 6
$\Delta G°$/kJ mol^{-1}	−29.4	−38.9 ± 0.4	−41.4	−38.9 ± 0.4

TABLE IV. Comparison Between Experimental and Calculated Thermodynamic Parameters for the Coil-to-Helix Transition of S15, at 26.2°C

	Calculated		Experimental
	Coil model[a]	− [$\Delta J°$(ApaS-25) − $\Delta J°$(S15)][b]	− [$\Delta J°$(ApaS-25) − $\Delta J°$(S15)]
ΔC_p/kJ K^{-1} mol^{-1}	−0.16	−0.15	−1.2 ± 0.4
$\Delta H°$/kJ mol^{-1}	−21.6	−27.8	−40.6 ± 5.3
$\Delta S°$/J K^{-1} mol^{-1}	−164	−133	−136 ± 18
$\Delta G°$/kJ mol^{-1}	+28	+12	0 ± 0.4
$T\Delta S°$/kJ mol^{-1}	−49	−40	−40.7 ± 5.3

[a]Values for the coil-to-helix transition, calculated as described in Materials and Methods, Structure-Based Calculations.
[b]Values are the differences in the calculated thermodynamic parameters reported in Table III for binding with S15 and ApaS25.

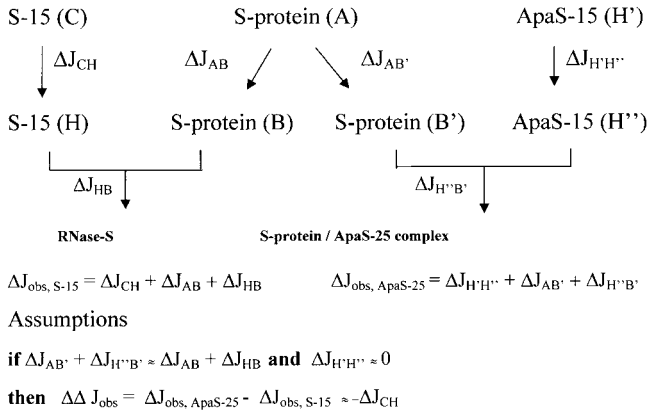

Fig. 3. Reaction schemes for S15 and ApaS-25 peptide binding to S-protein showing three events contributing to the thermodynamic state functions for the binding of each peptide. *J* can be any thermodynamic state function, i.e., heat capacity (*C*), enthalpy (*H*), entropy (*S*), free energy (*G*). Individual terms are defined in the text.

According to the proposed scheme, this contribution should have the same value in both binding reactions (as $\Delta J_{AB'} + \Delta J_{H''B'} \approx \Delta J_{AB} + \Delta J_{HB}$) and will be equal to the excess heat capacity of the S-protein at 25°C, which is given as $\Delta H^2/RT^2\,[K/(1+K)^2] + \Delta C_p\,[K/(1+K)]$. According to the thermodynamics of unfolding of the S-protein reported by Graziano et al.[26] (T_m = 38.6°C; ΔH_m = 182 kJ mol^{-1}; ΔC_p = 1.6 kJ K^{-1} mol^{-1}), the excess heat capacity at 25°C will be 1.63 kJ K^{-1} mol^{-1}. Subtracting this value from the experimental ΔC_p for each binding reaction we would get ΔC_p = −2.2 kJ K^{-1} mol^{-1} in the case of S15 and ΔC_p = −1.0 kJ K^{-1} mol^{-1} for ApaS-25.

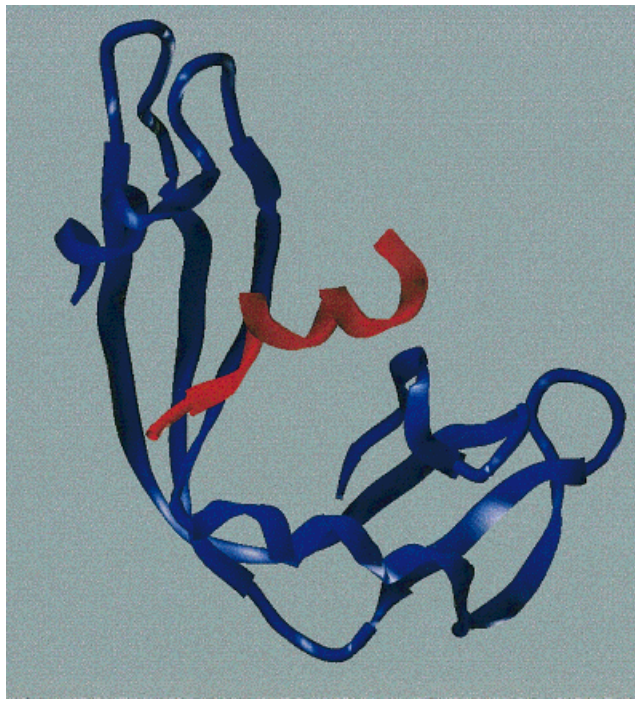

Fig. 4. Perspective of the structure of ribonuclease S.

The value for ApaS-25 is close to that calculated from the change in ASA, but for S15 it is still about twice the calculated value. It seems, then, that in the case of S15 there is an extra heat capacity change that is not accounted for from the changes in ASA upon binding. This effect was already pointed out by one of us[12] in a study of

heat capacity changes in binding reactions of S15 and six other peptide analogues with S-protein, where it is stressed that regardless of the method used to calculate the changes in ASA, the derived values are too small to account for the experimentally derived heat capacity changes.

The same type of reasoning can be used to correct the calculated values for the enthalpy changes upon binding for both reactions, again using the data of Graziano et al.[26] The unfolding enthalpy at 26.2°C is 162.2 kJ mol^{-1}. As the S-protein will be about 6% unfolded at this temperature, the contribution will equal $\Delta H \times [K/(1 + K)]$, that is, −9.8 kJ mol^{-1}. Adding these values to the calculated enthalpy changes shown in Table III, we will get −39.2 kJ mol^{-1} for the S15 binding reaction and −11.4 kJ mol^{-1} for the ApaS25 binding reaction. This result shows that the partial unfolding of the S-protein does not explain the discrepancy between the calculated and observed $\Delta H°$ values.

The results shown in Table IV compare the predicted values for the coil-to-helix transition calculated both in terms of the coil model and as differences in the calculated binding energetics, and the experimentally determined differences ($\Delta\Delta J°$) between the two binding reactions. In each case the sign of the observed change is predicted correctly, and both models provide similar values. However, the predicted value for the enthalpy change is about 20 kJ mol^{-1} smaller (i.e., less negative) than that observed experimentally. It should be noted that the predicted enthalpy change using the Ala-X-Ala model for the coil is about 20 kJ mol^{-1} higher than the experimental value (values not presented). This shows that the predicted values are extremely sensitive to the models used and that the experimental value is between the two obtained from different models of the coil state. The predicted value for the entropy change is in very good agreement with experiment. The difference between the calculated and the observed $\Delta G°$ values reflects the poor enthalpy estimate obtained by this coil model.

The most striking difference is obtained for the predicted value of the heat capacity change, which is almost one order of magnitude smaller than the observed value. This reflects, perhaps, that the heat capacity is the thermodynamic function that is most sensitive to fluctuation behavior. In these calculations, we are considering just one model for the coil, that is, just one possible form for the unfolded peptide, which is an oversimplification. As the heat capacity is proportional to the variance of the enthalpy values, this should increase the observed heat capacity as $\Delta H^2/4RT^2$ if the $\Delta G°$ for helix formation is 0, as suggested by the experimental results. Nevertheless, the assumption of a fully unfolded S15 peptide at 25°C is supported by CD measurements.[6]

The values obtained can also be compared with other estimates and experimental measurements of the coil-to-helix transition. Estimates of enthalpy changes for the helix-to-coil transitions of peptides[33] have been placed at ~4 kJ (mol res)$^{-1}$. Some of the earliest data on enthalpies of helix formation come from the Hermans[1] measurement on the helix-to-coil transitions of poly-L-glutamic acid and poly-L-lyzine (4.4 kJ (mol res)$^{-1}$and 37 kJ (mol res)$^{-1}$, respectively).[34] The determination of the enthalpy of helix-to-coil transition of a 50-residue peptide by DSC gives a value of 5.4 kJ (mol res)$^{-1}$ with a lower bound of 3.8 kJ (mol res)$^{-1}$, although no heat capacity change could be evaluated.[9] A recent NMR study of the thermal transitions of a model helical peptide[35] gives the values of 3.6 ± 0.7 kJ (mol res)$^{-1}$, 12 ± 2.5 J K^{-1} (mol res)$^{-1}$ and 18.7 ± 4.5 J K^{-1} (mol res)$^{-1}$ for the enthalpy, entropy, and heat capacity changes, respectively. In this study, the heat capacity change was determined from fitting the thermal dependence observed for each carbonyl carbon atom, assuming a two-state transition.

More recently, Taylor et al.[10] performed a CD and DSC study of the folding-unfolding of an α-helix with covalently closed N- and C-terminal loops (29 residues). These investigators assumed a two-state transition for the full peptide. On the basis of the DSC results, they obtained a value for the heat capacity change of 18.4 J K^{-1} (mol res)$^{-1}$. They also calculated the enthalpy, entropy, and Gibbs functions. The enthalpy and entropy changes reported were (2.8 ± 0.3) kJ (mol res)$^{-1}$ and (9.6 ± 0.3) J K^{-1} (mol res)$^{-1}$, respectively. The $\Delta G°$ value is around 0 at 25°C.

Since there are 10 residues involved in helix formation in S15, we can obtain estimates of $\Delta H_{(h\rightarrow c)}$ of 40, 37, (38–54), 36, and 28 kJ mol^{-1} at 25°C, using the values above, respectively. In comparison, $\Delta\ \Delta H_{\text{obs, ApaS-25-S15}}$ = 40.6 kJ mol^{-1} at 26.2°C. Thus, except for the last value, the agreement is very good. For the entropy change, for a 10-residue helix, the estimated values are 130 and 96 J K^{-1} mol^{-1}, respectively, using the Shalongo[35] and Taylor[10] values. Our value of $\Delta\ \Delta S_{\text{obs, ApaS-25-S15}}$ = 136 J K^{-1} mol^{-1} is in good agreement with the first estimate, but less so with the second estimate.

The heat capacity change that can be estimated for a 10-residue helix is 0.18 kJ K^{-1} mol^{-1} in both cases, much lower than the observed heat capacity change of 1.2 kJ K^{-1} mol^{-1}, but in excellent agreement with the calculated value.

The free energy change of a helix-to-coil transition is estimated by the model to be about 0–1.6 kJ mol^{-1} between 25 and 30°C. Within the confines of the present model, this value should be seen as an order of magnitude. The results presented by Taylor et al.[10] also give a value close to 0 at 25°C. Thus, the free energy of S15 helix formation is small at 25°C, as has also been found previously for helix formation.[34]

The thermodynamic results obtained provide positive evidence in support of the general view that ApaS-25 binds to the S-protein in a similar fashion as the S-peptide. In addition, within the confines of the simple model presented, the data are in accord with values reported for enthalpies of helix-to-coil transitions of other peptides and provide a first-order estimate of the heat capacity of the helix-to-coil transitions of S-peptides. Further, the poor agreement between calculated and experimental ΔC_p values suggest that other heat capacity effects are present

which are not accounted for by the changes in ASA. However, comparison with other experimental values of peptide helix-to-coil transitions does not show the large heat capacity effects observed in the RNaseS system.

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APPENDIX A

To calculate the thermodynamic parameters associated with binding, the following expressions were used[13–15]:

$$\Delta C_p = 1.88\Delta\mathrm{ASA}_{\mathrm{ap}} - 1.09\Delta\mathrm{ASA}_{\mathrm{pol}} \quad (5)$$

$$\Delta H(60^\circ\mathrm{C}) = 131\Delta(\mathrm{ASA})_{\mathrm{pol}} - 35.3\Delta(\mathrm{ASA})_{\mathrm{ap}} \quad (6)$$

$$\Delta H(t) = \Delta H(60^\circ\mathrm{C}) + \Delta C_p(t - 60) \quad (7)$$

$$\Delta S^\circ = \Delta S^\circ_{\mathrm{solv}} + \Delta S^\circ_{\mathrm{conf}} + \Delta S^\circ_{\mathrm{mix}} \quad (8)$$

$$\Delta S^\circ_{\mathrm{solv}} = \Delta C_p \ln(T/385.15) \quad (9)$$

$$\Delta S^\circ_{\mathrm{mix}} = R \ln(1/55.5) \quad (10)$$

$$\Delta S^\circ_{\mathrm{conf,SC}} = \sum_l \frac{\Delta\mathrm{ASA}_{\mathrm{SC},l}}{\mathrm{ASA}_{\mathrm{AXA},l}} S^\circ_{\mathrm{SC}} \quad (11)$$

$$\Delta S^\circ_{\mathrm{conf,backbone}} = \Delta S^\circ_{bb} + \Delta S^\circ(ex \rightarrow bu) \quad (12)$$

In the case of ApsS-25 only expression 11 was used to obtain the configurational entropy component, as it is considered that the helix is already formed from start, so we do not need to consider backbone contributions in this case. For the S15, eqs. 11 and 12 were summed up to give the total configurational entropy contribution. For the coil-to-helix transition, the entropy change was considered as given by eq. 12.

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