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THERMODYNAMIC CHARACTERISATION OF RNAase A IN THE PRESENCE OF UREA AND GuHCl

G. Barone, P. Del Vecchio, D. Fessas, C. Giancola, G. Graziano and A. Riccio

Department of Chemistry-University 'Federico II' Via Mezzocannone 4, 80134 Naples, Italy

Abstract

It is presented a study concerning the influence of guanidinium chloride (GuHCl) and urea on thermal stability of Bovine Pancreatic Ribonuclease A (RNAase A) at different *pH* values. As expected, at increasing the denaturant concentration, the protein thermostability decreases. This is shown by a decrease of both the thermodynamic parameters, temperature and heat effect, characterising the denaturation process. In order to analyse the calorimetric curves we adopt a statistical thermodynamic approach. The individual one-dimensional DSC profiles have been expanded into another dimension by varying the GuHCl concentration, so that a heat capacity surface is defined for each *pH*. By means of the ICARUS program, developed in our laboratory, we accomplish a two dimensional deconvolution of the experimental data linking the binding equilibrium to the denaturation process. This analysis provides a well founded and complete statistical thermodynamic characterisation of denaturation process of RNAase A in the presence of GuHCl and allows to calculate the thermodynamic parameters associated to the binding of denaturant molecule.

Keywords: denaturation models, guanidinium chloride, micro-DSC, ribonuclease A, urea

Introduction

Bovine Pancreatic Ribonuclease A is one of the best studied enzymes. Its crystallographic structure has been exhaustively determined by many laboratories [1–3]. The wealth of structural information and the small size have made it one of the mostly used enzymes in studies concerning the thermodynamic stability of proteins [4, 5]. Sela and Anfinsen [6] showed that the native form of RNAase A represents the minimum of the Gibbs energy function for the system and the validity of 'thermodynamic hypothesis' for the study of proteins. Presently, it is firmly established that the thermal denaturation process of RNAase A well conforms to a reversible one-step transition mechanism, with two macroscopic states, the native and denatured one, significantly populated [4, 5, 7–9]. Differential scanning calorimetry can usefully used as a tool to improve the knowledge of the interaction of this enzyme with denaturants. Re-

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cently a paper has appeared [10], showing as calorimetry can shed light on the problem of protein interaction with denaturants. Urea and guanidinium hydrochloride are widely used as protein denaturants, but notwithstanding the extensive literature on protein denaturation by these agents, the mechanism of their action is still not completely clear. Indeed it is matter of discussion whether the action of these agents is direct, concerning the binding of denaturant molecules on the surface of proteins, or indirect, involving a change in the properties of the solvent water in the presence of urea and GuHCl [11–14].

By means of DSC we obtained a complete set of data at different *pH* values concerning thermal denaturation process of RNAase A in the presence of GuHCl or urea. At higher denaturant concentration (for urea starting from 1.0 *M* at least) the ratio $\Delta_d H/\Delta_d H_{v.H.}$ is less than unity, so in this case the denaturation process cannot be regarded as a one step process and probably a more complex mechanism takes place [4, 7–9]. The effect of these denaturants has been interpreted in the framework of the 'denaturant binding model', proposed by Aune and Tanford in 1969 [15, 16]. This model considers that the influence on protein conformational equilibrium is mainly due to the binding of urea and GuHCl on unspecific, identical and independent sites of the native and denatured state of proteins. It is widely used for analysing denaturant-induced protein denaturation experiments at constant temperature. However using this model, it is possible to link the denaturant binding equilibrium to the thermal denaturation process and to analyse, along these lines, the single DSC curves. For a better estimation of the thermodynamic properties of the system, we followed the formalism proposed by Freire [17–19], giving the heat capacity surface as a function of the temperature and a second independent variable (the denaturant concentration). A two dimensional deconvolution of the experimental heat capacity surface ($<\Delta C_p>$ *vs.* T *vs.* [GuHCl]) has been performed by means of the ICARUS program, developed in our laboratory. The global, non linear least squares analysis of the whole surface allows to estimate simultaneously the intrinsic denaturation parameters and the thermodynamic parameters characterising the binding of denaturant molecule. The so determined best unique set of parameters, describes sufficiently well all data and is in agreement with literature values.

Materials and methods

RNAase A was a SIGMA product (type XII A), containing less then 0.14% phosphates (in moles), used without further purifications. Urea was a C. Erba (Milan) product, crystallised twice from ethanol-water mixtures and dried under vacuo on P_2O_5. Guanidinium chloride was a BDH product, at the highest purity grade (minimum assay by NAT 99.8%) and it was used without further

purification. The buffer used were 0.1 *M* acetate for $pH = 5.0$, 0.1 *M* 2-[Morpholino]-ethansulfonate (MES) for $pH = 6.0$ and 0.1 *M* 3-[N-Morpholino]propanesulfonate (MOPS) for $pH = 7.0$ (all are mixture of acids and sodium salts).

Deionised water, twice distilled and filtered on Millipore membrane, was used for all the solutions. The protein concentration was determined spectrophotometrically, using an extinction coefficient of 9 800 $cm^{-1} \cdot M^{-1}$ at 278 nm. The concentration of urea and GuHCl solutions were calculated by weighing the dried substances. The solutions for DSC measurements were prepared by mixing known volumes of protein and denaturant solutions, assuming additivity of volumes.

A second-generation Setaram MICRO-DSC microcalorimeter was used. It was connected to a PC for the automatic acquisition of data utilising the MIDAS informatic system, developed in our laboratory. The instrument signal was corrected for the calibration curve and for the buffer-buffer reference baseline by means of THESEUS program [20]. Finally a consistent baseline was determined assuming the native state as a reference so that the apparent molar heat capacity was converted in the excess molar heat capacity $<\Delta C_p>$ with minimum artefacts as possible. A scanning rate of 0.5 $deg \cdot min^{-1}$ was chosen for all the experiments.

The calorimetric enthalpy $\Delta_d H$ is determined by direct integration of the area under the curve and the van't Hoff enthalpy $\Delta_d H_{v.H.}$ is obtained using the standard formula:

$$\Delta_d H_{v.H.} = 4RT^2_{max} < \Delta C_p >_{max} / \Delta_d H \quad (1)$$

where $<\Delta C_p>_{max}$ is the maximum of the excess molar heat capacity function, T_{max} is the temperature at which occurs $<\Delta C_p>_{max}$ and R is the gas constant. The unitary value of the ratio $\Delta_d H / \Delta_d H_{v.H.}$ is a necessary condition for two-state transition [4, 7–9].

Thermodynamic model and treatment of the data

The effect of a ligand on thermal stability of protein can be considered explicitly by linking the binding equilibrium to the thermal denaturation process [21]. A similar analysis can be extended even to the case of denaturants such as urea or GuHCl, according to the 'denaturant binding model', proposed by Aune and Tanford in 1969 [15, 16]. The model considers that the influence on thermal stability of proteins is mainly due to the binding of urea or GuHCl on multiple and independent sites of the native and denatured states of proteins. Following this line, it is possible to develop an analysis, according to the ca-

nonical partition function formalism, of the DSC curves obtained in the presence of these denaturants. For this purpose we adopted some suggestions of Freire treatment [17–19].

The denaturation process, in the absence of ligand, of a protein that unfolds according to a one-step mechanism can be represented as:

$$N \rightleftarrows D \quad K^{\circ} = [D] / [N] \tag{2}$$

where K° is the associated equilibrium constant. We assume that the native and denatured states bear, respectively, n_N and n_D independent and identical binding sites for a ligand, characterised by the association constants $K_{b,N}$ and $K_{b,D}$. Then in the presence of a ligand L the overall conformational equilibrium constant becomes:

$$K = K^{\circ}[1 + (K_{b,D} \cdot a_L)^{n_D}] / [1 + (K_{b,N} \cdot a_L)^{n_N}] \tag{3}$$

where K° is the equilibrium constant in the absence of ligand and a_L is the activity of the ligand. It has been experimentally demonstrated that the binding constant of urea-like ligands for some proteins is small and in the range of 0.1–1.2 M^{-1} [22], for both the native and denatured states. Then, assuming that the intrinsic binding constant for each site is the same for both of the states [23], the overall denaturation constant becomes:

$$K = K^{\circ}(1 + K_b \cdot a_L)^{\Delta n} \tag{4}$$

where K_b is the mean association constant per site for GuH^+ ions or other non ionic denaturants and Δn is the difference between the number of the denatured and native binding sites; a_L is the activity of the ligand or, in the case of guanidinium chloride, the mean ionic activity.

The obtained equation of K indicates that the destabilization of native conformation induced by the GuHCl is due to the greater number of binding sites on the denatured than on the native protein.

At changing the temperature, the thermodynamic processes can be described starting from the canonical partition function $Q(T)$, taking the native state of protein as a reference:

$$Q(T) = 1 + K(T) = 1 + K^{\circ}(1 + K_b \cdot a_L)^{\Delta n} \tag{5}$$

where:

$$K^{\circ}(T) = \exp - (\Delta_d H/R)[1/T - 1/T_d] - (\Delta_d C_p/R)[1 - (T_d/T) + \ln (T_d/T)] \tag{6}$$

represents the conformational equilibrium constant in the absence of denaturant, expressed as a function of the temperature and parameters characterising the denaturation process: enthalpy change $\Delta_d H$, heat capacity change $\Delta_d C_p$ and maximum temperature T_d (at which it is $K^o = 1$).

The association constant per site, K_b, is given by:

$$K_b = \exp - [(\Delta_b H/RT) - (\Delta_b S/R)] \quad (7)$$

where $\Delta_b H$ and $\Delta_b S$ represent the enthalpy and entropy changes when one site is occupied by the ligand. They are assumed to be temperature independent.

From the general statistical thermodynamic relationship:

$$<\Delta H> = RT^2(\partial \ln Q/\partial T) \quad (8)$$

it is possible to obtain the excess enthalpy with respect to the native state, chosen as reference:

$$<\Delta H> = [\Delta_d H + \Delta_d C_p(T - T_d) + \Delta n \cdot \Delta H_b(K_b \cdot a/(1 + K_b \cdot a))] \cdot f_D \quad (9)$$

where $f_D = K/Q$ corresponds to the fraction of denatured molecules. Finally, we obtain, from another general relationship, the excess heat capacity with respect to the native state $<\Delta C_p(T)>$:

$$<\Delta C_p(T)> = \frac{<\Delta H^2> - <\Delta H>^2}{RT^2} + \Delta_d C_p \cdot f_D \quad (10)$$

So we obtain an analytical expression for the excess heat capacity function which allows: i) to simulate the DSC curves at different denaturant concentrations; ii) to perform a non linear regression over a whole set of curves, obtained by varying the denaturant concentration at a fixed *pH*. In Fig. 1 are shown the simulated calorimetric curves, obtained, at increasing the guanidinium chloride concentration, by using Eq. (10) for a two-state transition. It is clear that this model represents a good tool to describe the effect of a denaturing agent on protein DSC curves. So it becomes useful and possible to build an excess heat capacity surface as function of temperature and of ligand concentration (i.e. $<\Delta C_p>$ *vs.* T *vs.* $[L]$).

There are six parameters to optimise: $\Delta_d C_p$, $\Delta_d H$, T_d, $\Delta_b H$, $\Delta_b S$, Δn. They would assume a unique value for the whole set of DSC curves (i.e. the entire surface can be generated starting from the unique set of values with Eq. (10). However the optimisation problem can be simplified considering that $\Delta_d C_p$, $\Delta_d H$, and T_d correspond to the values determined from the denaturation of RNAase A in the absence of denaturing agent and must be kept fixed. The pa-

rameters characterising the binding of ligand, the binding enthalpy and entropy changes per site, $\Delta_b H$ and $\Delta_b S$, and the difference in the number of binding sites between denatured and native state, Δn, remain unknown and to be determined by the non linear regression procedure.

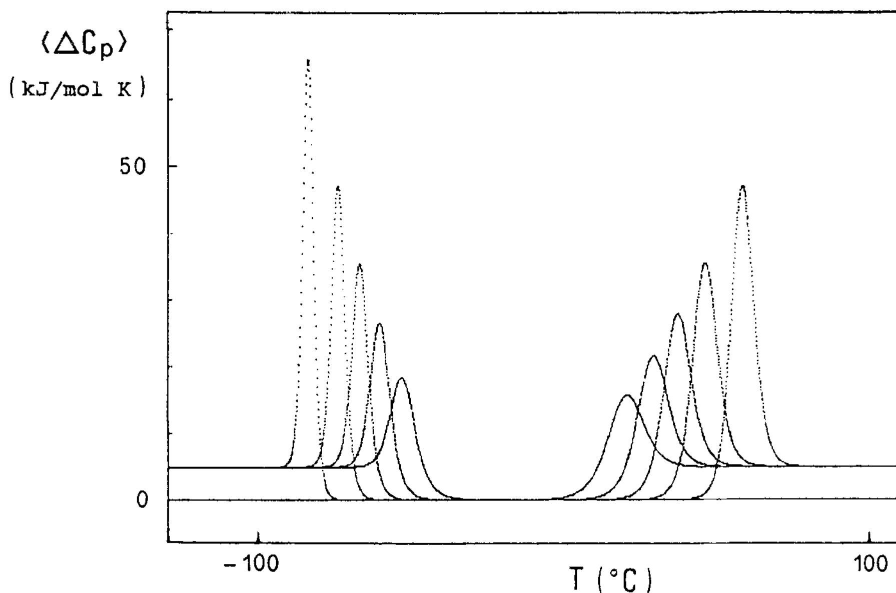

Fig. 1 Simulated excess heat capacity function profiles *vs.* temperature at increasing GuHCl molar concentrations. For illustration purposes, the predicted cold denaturation curves have been included in the figure, even though they are beyond our experimental limits

The bidimensional deconvolution is performed by means of the ICARUS program developed in our laboratory. We exploited the idea of creating a DSC super-curve constituted by all the DSC curves of a given set, placed in sequence at increasing $[L]$ values and of analysing it in its totality. In this manner we take in consideration simultaneously the effect of temperature and of denaturant concentration and obtain a better statistical estimation of parameters.

However to accomplish the deconvolution the heat capacity change $\Delta_d C_p$, associated to the denaturation must be put at zero. Looking at Eq. (10) one can see that the excess heat capacity function $<\Delta C_p>$ is given by the sum of two terms: the first corresponds to latent heat associated to the transition and gives rise to the well shaped peak; the second corresponds to the heat capacity difference between denatured and native state and gives rise to the sigmoidal baseline in the experimental profile. The second term must be cancelled to allow the execution of bidimensional deconvolution. This can be readily done by means of an

iterative algorithm based on very reasonable assumptions (i.e. keeping in mind that the sigmoidal baseline is directly proportional to the fraction of molecules in the unfolded state).

It is worthnoting that, because $\Delta_d C_p$ is statistically correlated to the binding parameters, $\Delta_b H$ and $\Delta_b S$, the identification of the global minimum in the space of parameters is feasible only if the initial guess values are sufficiently close to the most probable values. Moreover, for strongly correlated parameters a simple approximate, non linear supporting method must be employed in order to obtain a better estimation of non-linear asymmetric joint confidence intervals for all model parameters [24, 25]. The implementation of such a procedure is under advanced progress in our laboratory.

In conclusion with a bidimensional analysis the entire set of DSC data is used to minimize the error surface and to obtain the best set of parameters that describe the behaviour of the system along the surface function of the two independent variables, temperature and ligand concentration.

Results and discussion

DSC measurements were carried out in order to characterise in a thermodynamic manner the thermal denaturation of RNAase A in the presence of different amount of GuHCl and urea denaturants. In all the investigated conditions the denaturation process was highly reversible. Figure 2 shows a tridimensional plot ($<\Delta C_p>$ *vs.* T *vs.* [GuHCl]) of experimental curves in a 0–2.5 *M* range of GuHCl concentration at $pH = 6.0$. The results in Table 1 clearly show the influence of guanidinium chloride on thermal stability of RNAase A; indeed, for instance, at *pH* 6.0 the values of T_d and $\Delta_d H$ of 62.2°C and 498 kJ/mol in the absence of GuHCl, become 35.9°C and 211 kJ/mol in the presence of GuHCl 2.5 *M*. Similar results are obtained in the presence of urea and reported in Table 2. Figure 3 shows a tridimensional plot ($<\Delta C_p>$ *vs.* T *vs.* [urea]) of experimental curves in a 0–3 *M* range of urea concentration at *pH* 5.0. In the case of urea the denaturing power is lower than GuHCl; indeed, for instance, at *pH* 5.0 the T_d and $\Delta_d H$ values of 60.9°C and 460 kJ/mol in the absence of urea, become 46.9°C and 292 kJ/mol in the presence of urea 3.0 *M*. Then, especially the transition temperature, corresponding to the maximum of DSC peak, remains at higher values in the presence of urea. The determined $\Delta_d C_p$ values do not show any particular trend, in contrast to the suggestion of Freire [17], who supported the idea that in the presence of GuHCl the heat capacity change should be increase. The increase should be attributed to the partially loss of negative contribution of the interaction of water molecules with polar groups of polypeptide chain, due to the binding of GuH^+ ions.

In the last column of both the Tables are reported the values of R, the calorimetric to van't Hoff enthalpy ratio. The *R* value is unitary for RNAase A in the absence of denaturants, as expected for a two-state transition process, and at low GuHCl concentrations. At increasing the GuHCl concentration and starting at least from 1.0 *M* urea, the *R* values become lower than one, indicating that a more complex transition process takes place. The explanation of these results is very difficult because they are unexpected. Urea and GuHCl have been largely used as denaturant agents for studying the protein thermodynamic stability but the analyses have been always performed assuming the validity of the two state model.

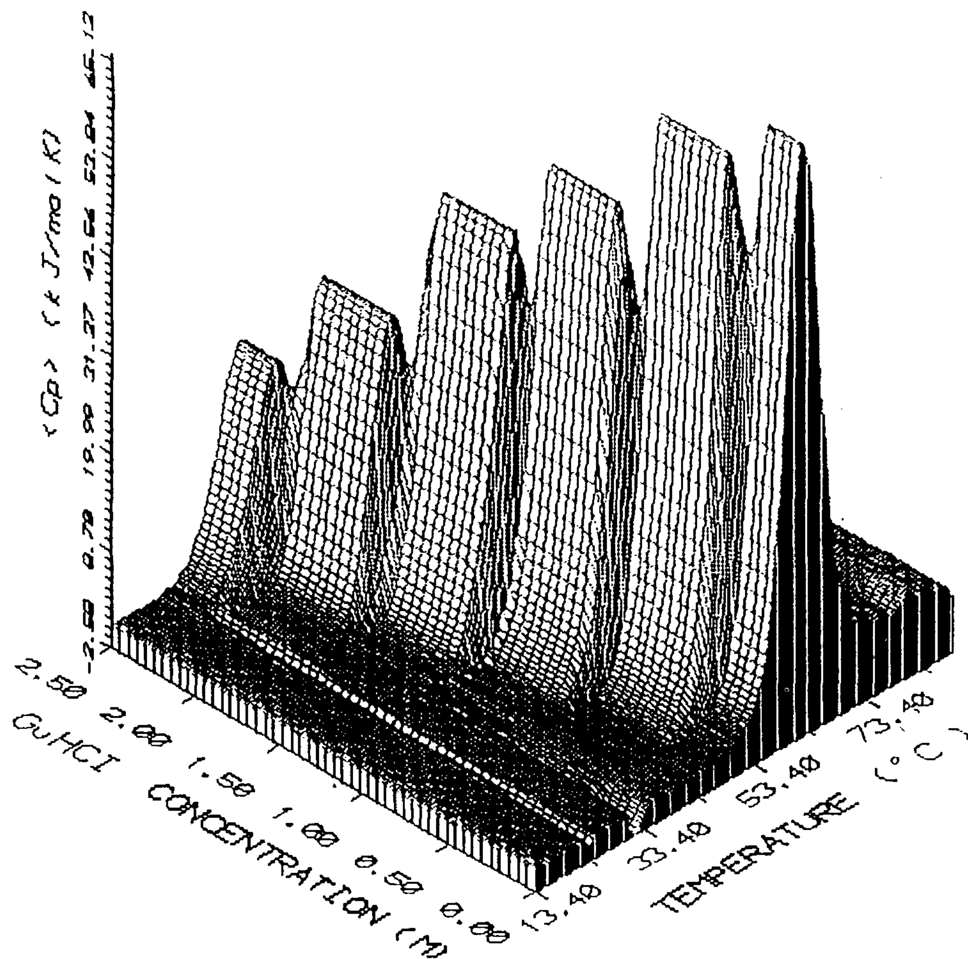

Fig. 2 Experimental DSC curves of RNAase A at different GuHCl molar concentrations and *pH* 6.0, shown in a tridimensional plot as a function of temperature and GuHCl molar concentration

Table 1 Thermodynamic parameters of denaturation of RNAase A in the presence of GuHCl at different *pH* values

	C_{GuHCl} / M	$\Delta_d H$ / kJ·mol^{-1}	T_d/ °C	$\Delta_d C_p$/ kJ·mol^{-1}·K^{-1}	$R(*)$
pH = 5.0					
	0	460	60.9	6.3	1.00
	0.5	445	58.0	5.8	0.84
	1.0	361	52.9	5.0	0.77
	2.0	300	47.7	5.6	0.83
pH = 6.0					
	0	498	62.2	5.6	0.98
	0.5	462	53.1	7.4	0.79
	1.0	407	53.1	7.4	0.97
	1.5	373	48.3	6.5	0.94
	2.0	269	42.3	6.3	0.73
	2.5	211	35.9	4.6	0.71
pH = 7.0					
	0	520	62.8	8.8	1.00
	0.5	466	59.3	5.6	1.00
	1.0	406	53.8	5.2	0.93

(*) = $\Delta_d H/\Delta_d H_{v.H.}$

Note: reported values are the averages on three or four measurements for each set of experimental conditions. The standard deviation in $\Delta_d H$ and $\Delta_d C_p$ are less than 10 per cent of the reported values. Errors on T_{max} are ±0.2°C

However it must be kept in mind that in the calorimetric measurements, the denaturation process is induced either by a physical denaturing agent, the temperature, either by a chemical denaturing agent, the GuHCl or urea. The simultaneous action of these two factors can lead to an unexpected behaviour. Finkelstein and Shakhnovich [26, 27] have shown, with general theoretical arguments, that the denaturation process for small globular proteins can be considered as the sum of two phenomena: a first-order phase transition passing from the native to a compact denatured state, and a second-order phase transition passing from the compact denatured state to random coil. These denatured states, strongly depend on the solvent features, because the solvent and cosolvent molecules can penetrate in the macromolecule interior. In these conditions it is correct to adopt the concepts of conventional theory of macromolecules: when the solvent is poor, the denatured protein is globular; when the solvent is good ('theta conditions'), the protein is a random coil. These authors have also calculated some phase diagrams which show the stability regions of states of a protein at changing both the temperature and the denaturant concentration. The

Table 2 Thermodynamic parameters of denaturation of RNAase A in the presence of urea at different pH values

	C_{urea} / M	$\Delta_d H$ / $kJ \cdot mol^{-1}$	T_d/ °C	$\Delta_d C_p$/ $kJ \cdot mol^{-1} \cdot K^{-1}$	$R(*)$
$pH = 5.0$					
	0	460	60.9	6.3	1.00
	1.0	382	56.7	5.8	0.84
	2.0	320	51.7	5.0	0.77
	3.0	292	46.9	5.6	0.83
$pH = 6.0$					
	0	498	62.2	5.6	0.98
	1.0	415	58.4	7.5	0.79
	2.0	353	54.0	5.7	0.79

(*) = $\Delta_d H/\Delta_d H_{v.H.}$

Note: reported values are the averages on three or four measurements for each set of experimental conditions. The standard deviation in $\Delta_d H$ and $\Delta_d C_p$ are less than 10 per cent of the reported values. Errors on T_{max} are ±0.2°C

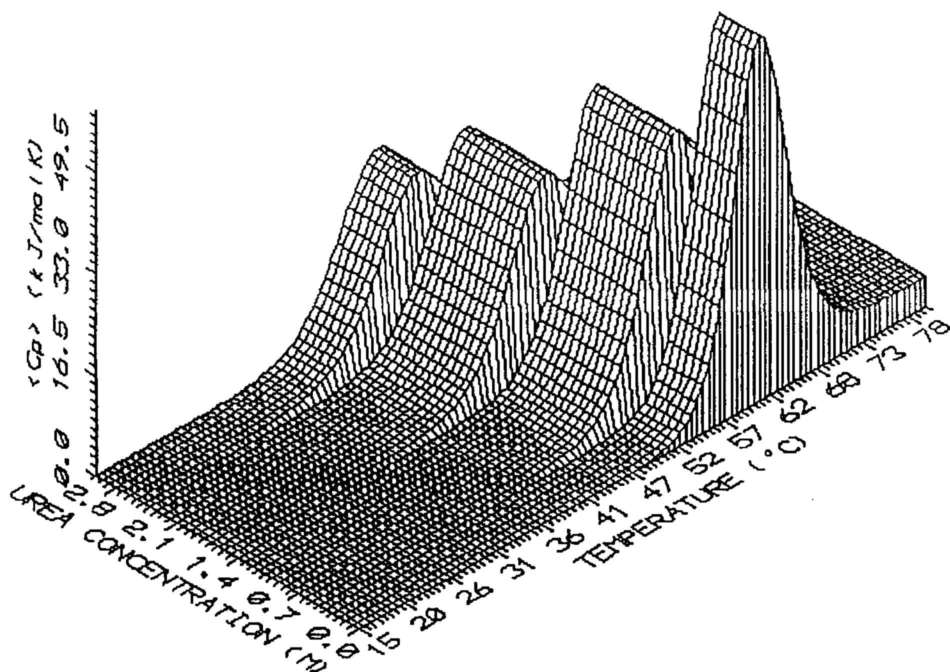

Fig. 3 Experimental DSC curves of RNAase A at different urea molar concentrations and $pH = 5.0$, shown in a tridimensional plot as a function of temperature and urea molar concentration

diagrams emphazise the presence of regions where are stable states such as the wet molten globule and the swollen globule. This theoretical analysis then points out that the assumption of very general validity of two-state model may be incorrect and misleading. However the necessity of treating the experimental data pushes the researchers to use a model, perhaps simplest, but very useful.

The denaturant binding model assumes, however, the validity of the two-state transition, so the bidimensional deconvolution using ICARUS program, was carried out only for DSC experimental curve of RNAase A in the presence of GuHCl for which the R value is close to one. For the mean ionic activity of GuHCl the following polynomial expansion, as function of molarity, is used [28]:

$$a_L = 0.6761 \cdot M - 0.1468 \cdot M^2 + 0.02475 \cdot M^3 - 0.00132 \cdot M^4 \tag{11}$$

In the Figs 4a and 4b are reported the superposition between the experimental super-curve and the super-curve obtained from non-linear regression performed by ICARUS at pH 5.0 and 6.0 respectively. The agreement is satisfactory. The determined values of parameters characterising the binding of GuH^+ ion on a single site at the investigated pH values are collected in Table 3. The enthalpy and entropy changes, $\Delta_b H$ and $\Delta_b S$, averaged over the three pH values, are equal to –9 233.4 J/mol and –39.4 J/mol K, respectively. As expected, there is not a strong dependence of the determined values on the pH of solution and they are within the range of literature data (for GuHCl $\Delta_b H$ = –11 000± 2000 J/mol and $\Delta_b S$ = – 41±8 J/mol K averaged over three proteins, lysozyme, ribonuclease A and cytochrome C [10]). The binding of GuH^+ ion is an exothermic process with a negative entropy change. The exothermicity demonstrates that the energetic interaction of GuH^+ ion is more favourable with polar groups of polypeptide chain than with water molecules. Instead the entropy decrease associated to the binding is surely due to the loss of translational degree of freedom of GuH^+ ion. However it has been also suggested that, if the denaturant binding model is a realistic representation of the GuHCl action, the entropy decrease can be partially ascribed to the stiffening of protein denatured conformation which would be far away from a random coil for the presence of bounded GuH^+ ions [10].

In any case starting from the values of $\Delta_b H$ and $\Delta_b S$, assumed temperature independent, we can calculate the association constant per site, K_b at 25°C. At changing the pH of solution the values of K_b are between 0.295 and 0.393 M^{-1}, perfectly within the range 0.1–1.2 M^{-1}, determined for a certain number of proteins in direct GuHCl denaturation studies [22].

Finally, the average value of Δn, the difference in the number of binding sites between denatured and native state is equal to 34 and is very close to that

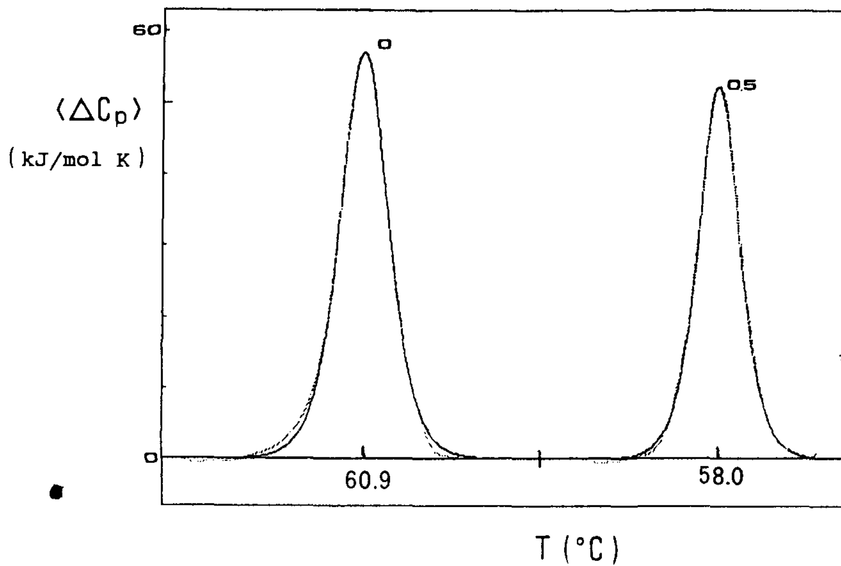

Fig. 4a Superposition of experimental and theoretical DSC super-curve for RNAase A at $pH = 5.0$ and different GuHCl molar concentrations, as determined by the bidimensional deconvolution using ICARUS program

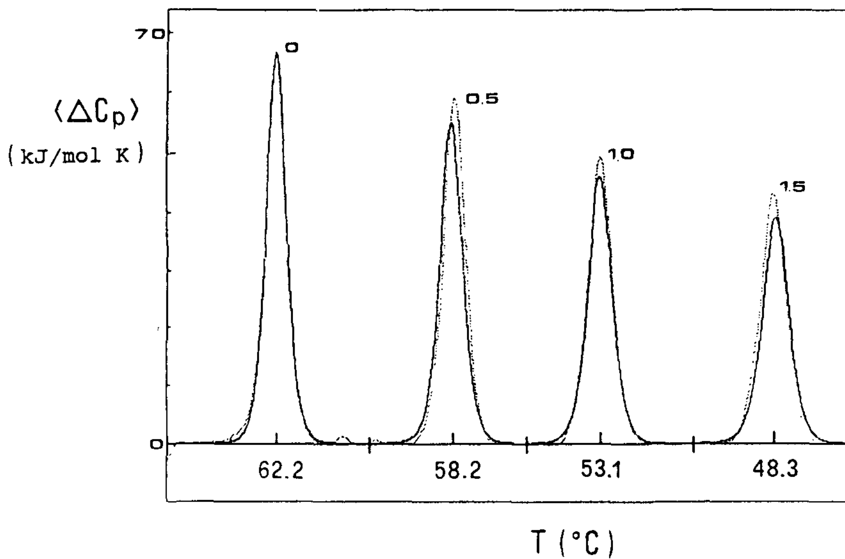

Fig. 4b Superposition of experimental and theoretical DSC super-curve for RNAase A at $pH = 6.0$ and different GuHCl molar concentrations, as determined by the bidimensional deconvolution using ICARUS program

Table 3 Binding parameters of denaturation of RNAase A various *pH* in the presence of GuHCl derived by the deconvolution analysis

pH	$\Delta_b H$ / J·mol^{-1}	$\Delta_b S$ / J·mol^{-1}K^{-1}	Δn	$K_{b(25°C)}$ / M^{-1}	σ / J·mol^{-1}
5.0	–8 500	–37	32	0.295	6 220
6.0	–9 000	–39	35	0.347	5 100
7.0	–10 200	–42	34	0.393	6 130

Note: σ is the standard deviation of the fit calculated by the square root of chi-square to the experimental point number ratio

determined by Makhatadze and Privalov for RNAase A [10] (i.e. $\Delta n = 31$). These authors on the basis of the well known crystal structure of RNAase A were able to characterise the binding sites of GuH^+ ions and to clarify the type of interaction. They determined that the sites are unspecific and correspond to a certain number of neighbour polar groups on which the GuH^+ ions bind through the formation of four or five hydrogen bonds. However it must be pointed out that this parameter can assume very different values starting from initial guess values far away from the best estimates and it has to be considered with caution.

In conclusion our study points out that the denaturation process of RNAase A, induced by both the temperature and GuHCl or urea, cannot be described always within the framework of the usual two-state model. A reasonable explanation of this behaviour has been proposed on the basis of theoretical results. However, when the denaturation process well conforms to a two-state transition, the application of the bidimensional deconvolution allows to characterise thermodynamically the interaction of GuH^+ with RNAase A and to obtain results in agreement with the experiments and literature data. The denaturant binding model is thus able to describe the effect of GuHCl on protein stability, only when the unfolding is a two-state process.

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Zusammenfassung — Es wird eine Untersuchung des Einflusses von Guanidinchlorid (GuHCl) und Harnstoff auf die thermische Stabilität von Rinderbauchspeicheldrüsen-Ribonuklease A (RNAase A) bei verschiedenen *pH*-Werten dargelegt. Wie erwartet sinkt die Wärmebeständigkeit des Proteins mit zunehmender Konzentration des Denaturierungsmittels. Dies wird durch ein Absinken der beiden thermodynamischen Parameter Temperatur und Wärmeeffekt gezeigt, welche den Denaturierungsprozeß charakterisieren. Zur Analyse der Kalorimeterkurven wendeten wir eine statistische thermodynamische Näherungslösung an. Die einzelnen eindimensionalen DSC-Profile wurden durch Variieren der GuHCl-Konzentration in eine andere Dimension erweitert, so daß für jeden *pH*-Wert eine Wärmekapazitäts-Oberfläche definiert wurde. Mit Hilfe des in unserem Labor entwickelten ICARUS-Programmes konnte eine zweidimensionale Dekonvolution der experimentellen Daten erzielt werden, die das Bindungsgleichgewicht mit dem Denaturierngsprozeß verknüpft. Diese Analyse bietet eine fundierte und vollständige statistische thermodynamische Beschreibung des Denaturierungsprozesses von RNAase A in Gegenwart von GuHCl und gestattet die Berechnung der thermodynamischen Parameter bezüglich der Bindung der Moleküle des Denaturierungsmittels.