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Water-Dependent Domain Motion and Flexibility in Ribonuclease A and the Invariant Features in its Hydration Shell. An X-ray Study of Two Low-Humidity Crystal Forms of the Enzyme

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Abstract

The crystal structures of 88 and 79% relative humidity forms of ribonuclease A, resulting from water-mediated transformations, have been refined employing the restrained least-squares method using X-ray data collected on an area detector to $R = 0.173$ for 15 326 observed reflections in the 10–1.5 Å resolution shell and $R = 0.176$ for 8534 observed reflections in the 10–1.8 Å shell, respectively. The comparison of these structures with those of the native, the phosphate-bound and the sulfate-bound forms demonstrates that the mobility of the ribonuclease A molecule involves hinge-bending movement of the two domains and local flexibility within them, particularly at the termini of regular secondary structures and in loops. The comparison also leads to the identification of 31 invariant water molecules in the hydration shell of the enzyme, many of which are involved in holding different parts of the molecule together and in stabilizing local structure. The conformational changes that accompany the partial removal of the surrounding water, particularly those observed in the 79% form, could be similar to those that occur during enzyme action.

Introduction

Protein hydration and the flexibility of protein molecules are topics which have received considerable attention in recent years (Artymiuk *et al.*, 1979; Poole & Finney, 1983; Ringe & Petsko, 1985; Saenger, 1987; Huber, 1988; Brooks & Karplus, 1989; Caspar & Badger, 1991; Rupley & Careri, 1991). It is recognised that the two are related to each other and that the activity of a protein critically depends upon them. High-resolution X-ray studies of a number of proteins have provided a wealth of information on protein hydration (Blake, Pulford & Artymiuk, 1983; Blundell, Barlow, Borkakoti & Thornton, 1983; Baker & Hubbard, 1984; Teeter, 1984; Wlodawer, Deisenhofer & Huber, 1987; Baker *et al.*, 1988; Kondandapani, Suresh & Vijayan, 1990; Madhusudan & Vijayan, 1991; Malin, Zielenkiewicz & Saenger, 1991; Thanki, Umrania, Thornton & Goodfellow, 1991; Madhusudan, Kodandapani & Vijayan, 1993). These studies indicate that the water molecules present in protein crystals can be broadly classified into two types: those which directly interact with the protein molecule and thus constitute the hydration shell, and those constituting the bulk water which fill the interstices among protein molecules. We have been pursuing an approach involving water-mediated transformations in protein crystals resulting from changes in solvent content when the relative humidity of the environment is systematically varied, to study the variability in protein hydration and the structural consequences of this variability (Salunke, Veerapandian, Kodandapani & Vijayan, 1985; Kodandapani *et al.*, 1990; Madhusudan & Vijayan, 1991; Madhusudan *et al.*, 1993). The X-ray analysis of the structure of low-humidity tetragonal lysozyme and the comparison of this structure with that of the native crystals showed that the removal of a few molecules of bulk water surrounding protein molecules causes substantial disturbances in the protein-bound water molecules constituting the hydration shell (Kodandapani *et al.*, 1990). These disturbances in turn cause structural perturbations in the enzyme molecule. Interestingly, in the case of lysozyme, these perturbations are most pronounced in regions which move during substrate binding. This observation was confirmed through the analysis of the low-humidity form of monoclinic lysozyme (Madhusudan *et al.*, 1993). Low-humidity monoclinic lysozyme, which has the lowest solvent content (22% by volume) in any protein crystal observed so far, also provided useful information on protein–water interactions and water structure. A detailed comparison of the two low-humidity forms and the other available refined crystal structures of lyzozyme also led to the delineation of the rigid and flexible regions of the enzyme molecule and the identification of the invariant water molecules in its hydration shell (Madhusudan & Vijayan, 1991).

The well characterized bovine pancreatic ribonuclease A, which is 124 residues long, hydrolyzes single-stranded RNA by cleaving the phosphodiester linkage at the 3′ side of pyrimidine nucleotides (Richards & Wyckoff, 1971). Over the years, ribonuclease A and its complexes have been the subject of several detailed X-

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ray investigations (Borkakoti, 1983; Wlodawer, Miller & Sjolin, 1983; Wlodawer *et al.*, 1987; Wlodawer, Svensson, Sjolin & Gilliland, 1988; Weber, Sheriff, Ohlendorf, Finzel & Salemme, 1985; Campbell & Petsko, 1987; Howlin, Moss & Harris, 1989; Nachman *et al.*, 1990; Aguilar, Thomas, Mills, Moss & Palmer, 1992; Rasmussen, Stock, Ringe & Petsko, 1992; Tilton, Dewan & Petsko, 1992). Encouraged by the results of the studies on lysozyme outlined earlier, we explored the structure of ribonuclease A using the approach involving water-mediated transformations. Crystals of the enzyme have been shown to undergo two water-mediated transformations (Salunke, Veerapandian, Kodandapani & Vijayan, 1985). X-ray analysis of the two low-humidity forms has been carried out at relative humidities of 88 and 79%. The results of this analysis, presented here, provide the first clear crystallographic demonstration of the environment-dependent motion of the two domains in the enzyme about the hinge that connects them. They also help in the identification of the rigid and the flexible regions of the molecule and the conserved water molecules in its hydration shell. Also presented, is evidence to suggest that the atomic movements in the active-site region, in response to the reduction of the amount of water around the protein molecule, are similar to those that occur during catalysis.

Materials and methods

Bovine pancreatic ribonuclease A, type XII-A, was purchased from Sigma. The monoclinic crystals grew from a mixture containing equal volumes of a 3% aqueous solution of the enzyme and ethanol. The relative humidity around the crystal was maintained at the desired level by replacing the mother liquor in the capillary by the saturated solution of the appropriate salt (Rockland, 1960; *CRC Handbook of Chemistry and Physics*, 1980–1981). Potassium chromate and ammonium chloride were used for maintaining the relative humidity at 88 and 79%, respectively. The crystals transformed typically within a day.

Data collection

Data from the 88 and 79% forms were collected to resolution limits of 1.5 and 1.8 Å, respectively, on a Siemens area-detector system mounted on a Marconi-Avionics rotating-anode X-ray generator ($\lambda = 1.5418$ Å). The detector was kept at a distance of 10 cm from the crystal in both cases, and at an angle of 35.5° from the direct beam for the 88% form and 24.5° for the 79% form. Data were processed using the *XENGEN* suite of programs (Howard *et al.*, 1987).

Structure analysis

The 88% form could be refined using the native phosphate-free enzyme structure (Wlodawer, *et al.*, 1988) as the initial model but the 79% form could not. The structure of the latter was solved by the molecular-replacement method using *MERLOT* (Fitzgerald, 1988). Both the structures were refined by use of the Hendrickson–Konnert restrained least-squares program (Hendrickson & Konnert, 1980) incorporated in the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Temperature factors and protein–solvent contacts were not restrained. All five main-chain atoms were restrained for peptide-group geometry. Occupancies were held constant at unity. $2F_o - F_c$, $F_o - F_c$ and 'omit' type maps (Vijayan, 1980; Bhat & Cohen, 1984) were extensively used during the course of the refinement. The details pertaining to the refinement, including R factors and resolution ranges, are given in Table 1 along with the cell parameters and information on intensity data.

Table 1. *Crystal data and details pertaining to data collection and structure refinement*

	88% form*	79% form*
Space group	$P2_1$	$P2_1$
a (Å)	29.74	30.34
b (Å)	38.25	33.27
c (Å)	52.40	52.61
β (°)	112.7	113.3
Solvent volume (%)	38.8	30.9
Total no. of reflections	35813	17852
Total no. of unique reflections	15874	8667
Merging R for all reflections	0.047	0.027
Resolution limits (Å) of reflections used in structure refinement	10–1.5	10–1.8
No. of reflections with $I > 2\sigma(I)$ used in refinement	15326	8534
Crystallographic R factor	0.173	0.176
R.m.s. deviation from ideal		
Bond lengths (Å)	0.012	0.012
Angle distances (Å)	0.034	0.040
Planar angle distances (Å)	0.038	0.048
Planarities (Å)	0.012	0.012
Non-bonded contacts involving		
Single torsions (Å)	0.164	0.190
Multiple torsions (Å)	0.288	0.287
Hydrogen bonds (Å)	0.178	0.202

* The unit-cell dimensions of the native form (Wlodawer *et al.*, 1988) are $a = 30.2$, $b = 38.4$, $c = 53.3$ Å and $\beta = 105.8°$ with a solvent volume of 43.4%.

The refined model of the 88% form contains 947 protein atoms (four terminal side-chain atoms of Gln101 were not included because of poor density) and 160 water O atoms. The corresponding numbers in that of the 79% form are 951 and 145, respectively. There was no compelling evidence for multiple conformations of side chains in either model. Luzzati plots (Luzzati, 1952) indicated a coordinate error of about 0.12 Å in both the structures. The criteria employed by Baker & Hubbard (1984) as modified by Kodandapani *et al.*, (1990) were used for delineating hydrogen bonds. The Ramachandran plot (Ramachandran, Ramakrishnan & Sasisekharan, 1963) and the distribution of the side-chain conformation angles follow the expected behaviour (Weaver, Tronrud, Nicholson & Matthews, 1990; Bhat, Sasisekharan & Vijayan, 1979; McGregor, Islam & Sternberg, 1987).

Results and discussion

Structural mobility

Removal of a few molecules of water from the medium that surrounds protein molecules, which is what causes water mediated transformations, is perhaps the gentlest way of causing a structural transformation. The changes in the molecular structure that occur in such a transformation are thus likely to reflect the nature of the inherent flexibility of the protein molecule. A detailed structural comparison of the low-humidity forms with the native forms is, therefore, of considerable interest. The structures of phosphate- (and sulfate-) free ribonuclease A (Wlodawer *et al.*, 1988), the phosphate-bound enzyme (Wlodawer, Borkakoti, Moss & Howlin, 1986) and the sulfate-bound enzyme (Howlin *et al.*, 1989) were used for such a comparison. The three structures will hereafter be referred to as the native, the phosphate-bound and the sulfate-bound forms. The r.m.s. deviations in Cα positions between pairs of them (Table 2), computed using *Insight*II (Biosym Technologies, 1992), clearly show that the 79% form deviates more from the rest of the structures than the rest do among themselves. As illustrated in Fig. 1, many Cα atoms in the 79% form deviate from those in the native form by more than 1 Å. No such deviation is observed among the four remaining structures; in fact, deviations greater than 0.5 Å among them are confined to the amino-terminal residue and a couple of residues near the scissile peptide bond between residues 20 and 21.

A careful examination suggested that the large deviations in the 79% form could be as a result of domain motion. The ribonuclease A molecule consists of two lobes or domains. A detailed computer graphics study indicated that one of them, domain *A*, is made up of residues 1–13, 49–79 and 105–124, while the other, domain *B*, of residues 16–46 and 82–101. Residues 14, 15, 47, 48, 80, 81 and 102–104 constitute the hinge between them. The 'hinge angle', defined as the angle subtended by the centres of mass of the two domains at the centre of mass of the hinge, in each of the five structures is listed in Table 3. Cα atoms with unit weights were used to estimate the centres of mass. The results presented in Table 3 clearly show that the removal of a substantial amount of water from the surroundings of the protein molecule, as in the 79% form, leads to a small, though definite, reduction in the hinge angle. The differences in the hinge angle among the other forms are small. The closing in of the two domains in the 79% form is illustrated in Fig. 2. Indeed, the domain-wise superposition of the structures reduces the overall r.m.s. deviations of Cα-atom positions in the 79% form from those in the others (Table 2). However, the deviations are still larger than those among the four structures other than the 79% form. The reason for this becomes clear when the r.m.s. deviations of Cα atoms in the 79% form from those in other forms in each domain are examined after domain-wise superposition (Table 4). These deviations clearly indicate differences within domains, especially in domain *B*. Thus, the changes in the molecular structure involve movements of domains as a whole and also perturbations within domains. These were examined using difference distance matrices.

Table 2. *R.m.s. deviations* (Å) *in Cα-atom positions after pair-wise superposition of the molecule*

Deviations of Cα atoms in each domain were calculated separately after domain-wise superposition. The r.m.s. values of these deviations for the whole molecule are given in parentheses.

	Phosphate bound	Sulfate bound	88%	79%
Native	0.15	0.13	0.20	0.55
	(0.13)	(0.12)	(0.16)	(0.47)
Phosphate bound	—	0.19	0.27	0.54
		(0.14)	(0.18)	(0.49)
Sulfate bound	—	—	0.23	0.53
			(0.19)	(0.45)
88%	—	—	—	0.54
				(0.48)

Table 3. *Hinge-angle values in the different structures*

Compound	Angle (°)	Reduction with respect to the native form (°)
Native	94.76	—
Phosphate bound	94.34	0.42
Sulfate bound	94.64	0.12
88%	94.71	0.05
79%	93.15	1.61

Table 4. *R.m.s. deviations* (Å) *of Cα-atom positions in each domain in the 79% form on superposition on the corresponding domain in the other forms*

	Native	Phosphate bound	Sulfate bound	88%
Domain *A*	0.22	0.23	0.23	0.26
Domain *B*	0.68	0.71	0.65	0.68

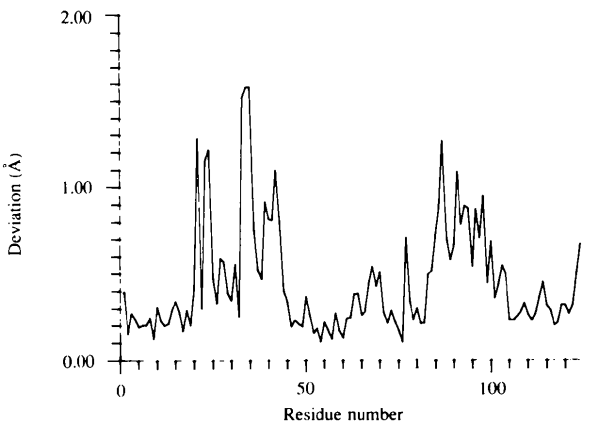

Fig. 1. Differences in Cα-atom positions in the native and the 79% forms.

Difference distance matrices (Nishikawa, Ooi, Isogai & Saito, 1972; Kundrot & Richards, 1987; Madhusudan & Vijayan, 1991) between pairs of structures provide information about the movement of particular residues or segments in relation to the other parts in a molecule. If the distance between the Cα atom of the ith residue and that of the jth residue in one structure is R_{ij} and the same distance in another structure is R'_{ij}, then the element d_{ij} in the difference distance matrix between the two has a value $|R_{ij} - R'_{ij}|$. As in an earlier study on lysozyme (Madhusudan & Vijayan, 1991), a set of residues may be considered relatively rigid if all the elements d_{ij} among them are less than 0.5 Å in all possible difference distance matrices. A larger set of residues results if the threshold value is raised to 1 Å. The subset that results when the set of relatively rigid residues is removed from the larger set may be considered to be made of moderately flexible residues. The residues other than those belonging to the larger set may be considered as constituting the highly flexible regions of the molecule.

The difference distance matrices showed the structures of the enzyme in the native, the sulfate-bound and the phosphate-bound forms to the remarkably similar. No element in the difference distance matrices between them is greater than 1 Å. The same is true even of their difference distance matrices with the 88% form. Only residues 1 and 21 give rise to elements greater than 0.5 Å in the matrices involving the normal humidity forms. There are nine such residues (1–2, 21–23, 52, 68–69 and 101) when the 88% form is also included in the calculations. The number of such residues becomes 62, nearly a third of them giving rise to elements greater than 1 Å, with the inclusion of the 79% form also in the calculations.

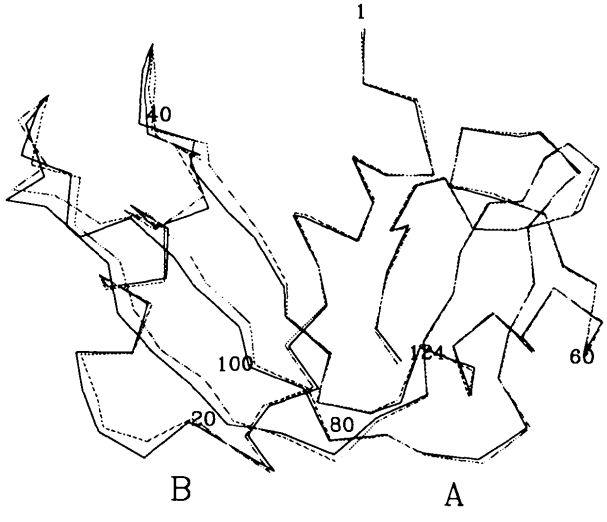

Fig. 2. Cα representations of the molecule in the native (unbroken line) and the 79% (broken line) forms. The A domains in the two structures have been superposed.

In view of the hinge-bending motion of the two domains, ten difference distance matrices each were subsequently constructed separately for domains A, B and the hinge region. Elements with comparatively large values were again confined mainly to matrices involving the 79% form. Composite difference distance matrices (Madhusudan & Vijayan, 1991), illustrated in Fig. 3, were then constructed for the three regions of the molecule. In the composite matrix, an element was left blank if that element had a value less than 0.5 Å in all the individual matrices. All the elements in the matrix for the hinge region were blank. The residues which contributed to non-blank elements were 1–2, 52, 68–69, 77, 113–114 and 124 in domain A; and 21–23, 32–43, 88-95 and 101 in domain B. Thus, nine residues in domain A and 24 residues in B constitute the flexible regions of the molecule. Of these, residues 1 and 77 in domain A and residues 21, 33–35, 39, 41–42 and 88–94 in domain B constitute the highly flexible regions, as they give rise to elements greater than 1 Å in at least one difference distance matrix.

The relatively rigid and the flexible regions in the molecule, delineated on the basis of the above analysis, are illustrated in Fig. 4. Clearly, the flexible regions primarily occur in loops and the termini of regular secondary structures. The delineation broadly corresponds to that based on displacement parameters. Thus, the average B values of the main-chain atoms in the two regions are 10.9 and 12.6 Å^2, respectively, in the native crystals. The corresponding values in the 88 and 79% forms are 8.4 and 12.0 Å^2, and 7.6 and 15.3 Å^2,

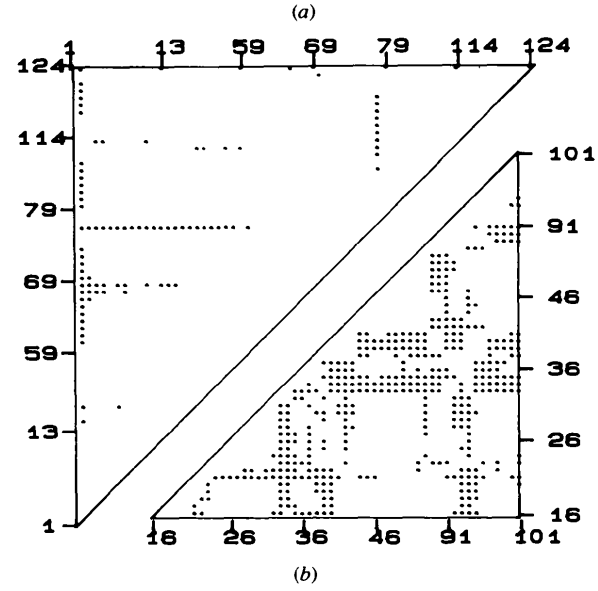

Fig. 3. The composite difference distance matrix for (a) domain A (residues 1–13, 49–79, 105–124) and (b) domain B (residues 16–46, 82–101). An element in it is left blank if the corresponding element in all the ten matrices is less than 0.5 Å.

respectively. Furthermore, on average the flexibility increases with the distance from the centre of the domain and also from the centre of the hinge. Because these are average effects the distribution of rigid and flexible regions cannot be explained exclusively on the basis of distance from domain centres or the hinge.

Invariant water molecules

The availability of several crystal structures, representing different environmental conditions, including the amount of water surrounding the same protein, permits the identification of the invariant water molecules in the hydration shell (Kodandapani *et al.*, 1990; Madhusudan & Vijayan, 1991). The positions of more than a hundred water molecules have been determined and refined in each of the five uncomplexed ribonuclease A crystal structures referred to earlier. In the present analysis, a water molecule was assumed to interact with a protein O or N atom if its distance from the protein atom is less than 3.6 Å. The same distance criterion was also used to identify water–water interactions (Kodandapani, Suresh & Vijayan, 1990; Madhusudan, Kodandapani & Vijauyan, 1993). In each structure, the water sites that surround and interact with the protein molecule were identified using appropriate symmetry elements. These sites, representing water molecules in direct contact with the O and the N atoms in the protein, were considered to constitute the hydration shell in the relevant structure. The protein molecules along with their hydration shells in the phosphate, the sulfate, the 88% and the 79% forms were superposed on the protein molecule and the hyration shell in the native structure, using the method of Rossman & Argos (1975). As in a similar analysis of lysozyme (Kodandapani *et al.*, 1990; Madhusudan & Vijayan, 1991) a water molecule in the hydration shell was considered invariant if it interacts with at least one common protein atom in all five structures and if, in addition, the distance between the corresponding water molecules in every pair of structures is less than 1.8 Å. The invariant water molecules thus identified, with the numbering used in the native structure, are listed in Table 5.

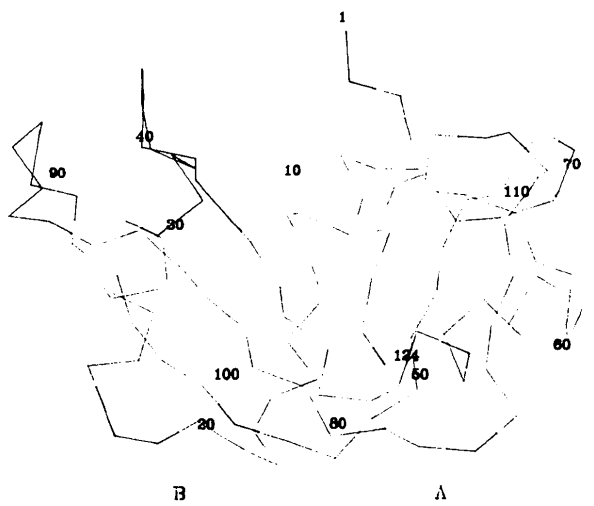

Fig. 4. Flexible regions, shown in red in the ribonulease A molecule.

Table 5. *Invariant water molecules in the hydration shell of ribonuclease A*

The numbering of the water molecules corresponds to that in the native form.

Water	Protein atoms with which the water molecule interacts in all the structures	Additional protein atoms with which the water molecule interacts in at least one structure
201	23 O, 27 N, Asn27 OD1 ND2, 97 O, Thr99 OG1	26N
204	Glu49 OE2, 50 N, Asp53 OD2	Ser50 OG
205	Glu2 OE2	—
206	117 O	5O, Glu55 NE2
208	53 O, Gln60 NE2	—
210	3 N	—
211	83O, 98O, Thr100 OG1	Asp83 OD2, 85 N, Arg85 NH1, 100 N
213	6 O	—
214	67 N, Asn67 OD1, Asp121 OD1	66 N, Lys66 NZ, Asn67 ND2, His119 NE2
215	52 N	51 N
216	Gln60 OE1, 76 N, 77 N	Glu60 NE2
217	13 O, 15 N	Ser15 OG
218	111 O	—
219	27 O, 95 O	—
221	92 N	—
224	35 O, 37 N, 38 N, 39 O, 39 N	—
227	52 O	—
228	114 O	—
229	Gln11 OE1, 118 O	His12 NE2
233	36 O, 93 O	Lys31 NZ, Thr36 OG1
247	Glu49 OE1	Glu49 OE2
250	58 O	—
293	23 O, 99 N	Lys98 NZ
294	4 O, 118 O	Lys7 NZ
295	Asn71 ND2, Glu111 OE1	Gln69 NE2
307	Ser59 OG, Gln60 NE2	56 O
316	9 O	13O, Arg33 NH1
324	Asn62 OD1, 70 O	Thr70 OG1
337	5 N	4 N
348	Asp14 OD2	Asp14 OD1, Ser16 OG
356	Glu86 OE1	Glu86 OE2, 90 O

The majority of the invariant water molecules are involved in multiple interactions with the protein. Many of them appear to contribute to the stability of the three-dimensional structure by bridging different regions of the molecule. For example, as illustrated in Fig. 5(*a*), six invariant water molecules participate in bridging the N-terminal helix with the C-terminal strand and the preceding loop. Incidentally, there are no direct protein–protein hydrogen bonds between these two regions of the molecule and the water bridges are substantially responsible for holding them together. Likewise, three such water molecules connect the 24–33 helix and the 94–100 strand, as shown in Fig. 5(*b*). Some invariant water molecules appear to stabilize secondary structural features. Three examples of such stabilization are given in Fig. 6. Such specific structural roles cannot of course be readily assigned to all the invariant water molecules.

If the 79% form is omitted from consideration, the remaining four structures have 15 water molecules common to their hydration shells, in addition to the 31 invariant water molecules listed in Table 5. Out of these 15, one water (310 in the native structure) is located in the interdomain region and interacts with residues 45, 83 and 123, thus forming a water bridge between the two domains. It is tempting to suggest that the removal of this water molecule is probably responsible for triggering the slight domain closure observed in the 79% form.

Implications to enzyme action

One of the interesting results of the earlier studies on the low-humidity forms of lysozyme (Kodandapani *et al.*, 1990; Madhusudan *et al.*, 1993) has been the observation that the perturbations in the molecule during water-mediated transformations were, by and large, in the regions which have been shown to move during inhibitor binding. This appeared to suggest that the small structural changes that result on partial removal of water surrounding the protein could be in some way related to movements during enzyme action. It was, therefore, of interest to examine the low-humidity forms of ribonuclease A, particularly the 79% form, from this point of view. In this context, it was fortunate that the high-resolution crystal structure of ribonuclease A complexed with a substrate analogue, uridine vanadate, has already been determined (Wlodawer *et al.*, 1983).

Appropriate pairwise superposition of the molecules in the native form, the 79% form and the substrate analogue

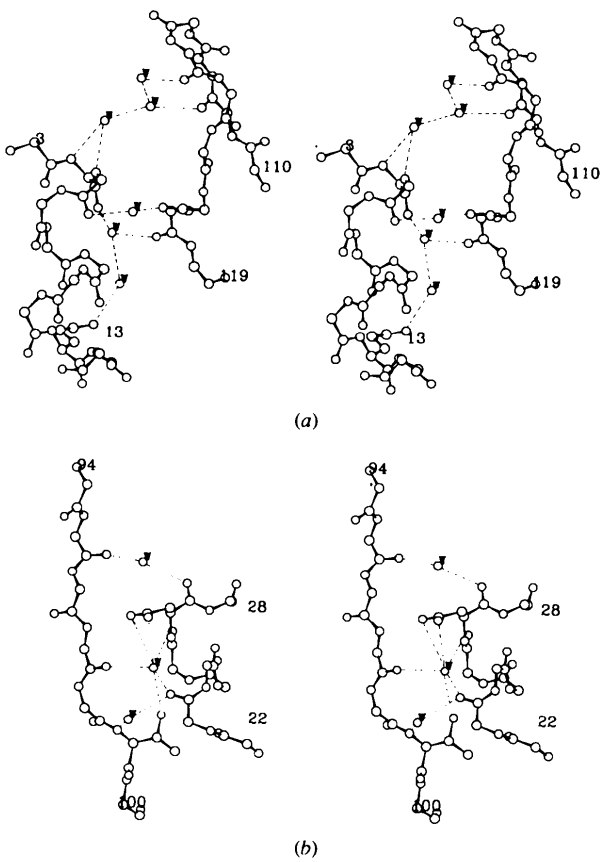

Fig. 5. Invariant water molecules that bridge (*a*) the N-terminal helix and the C-terminal strand and the preceding loop, and (*b*) the 24–33 helix and the 94–100 strand.

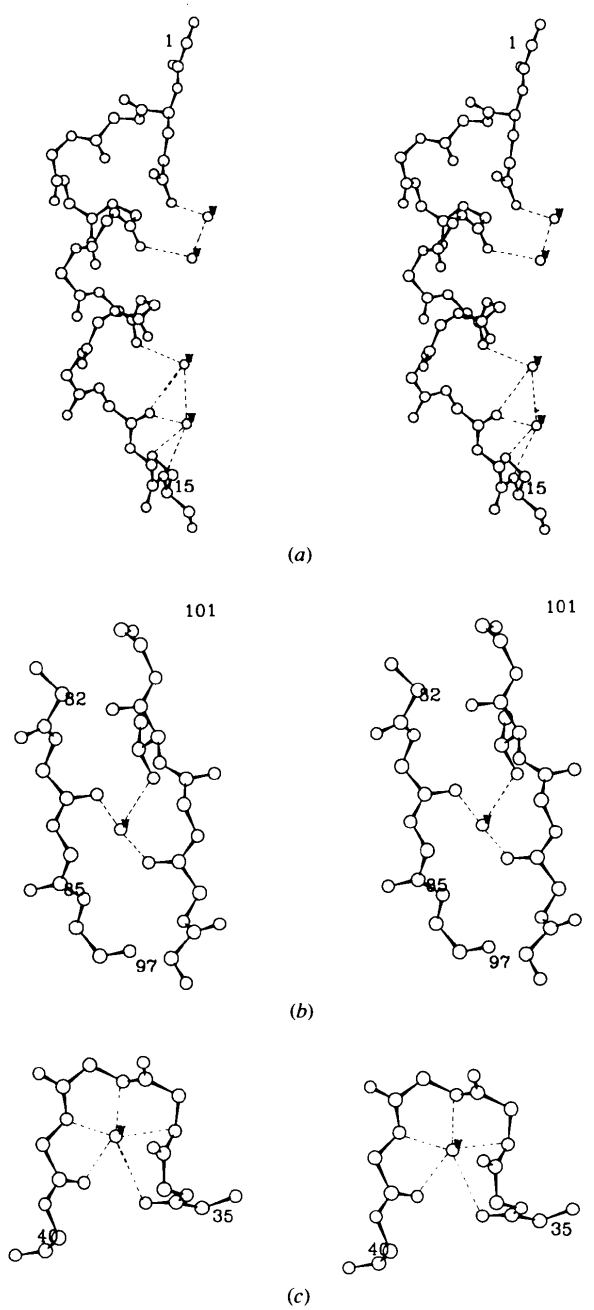

Fig. 6. Invariant water molecules that appear to lend stability to (*a*) 3–13 helix, (*b*) the 82–101 region of the β-structure and (*c*) the 35–40 loop.

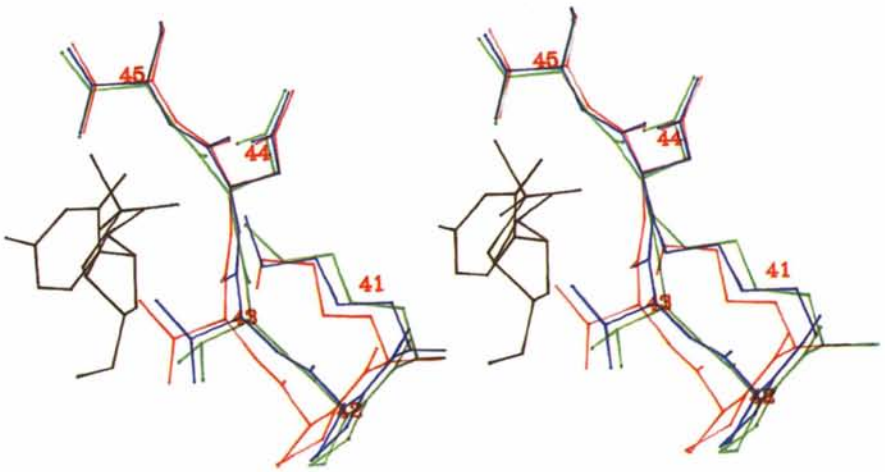

Fig. 7. Residues 41–45 in the native form (shown in green), the uridine vanadate complex (blue) and the 79% form (red) after superposition of all Cα atoms. The location of uridine vanadate is also shown (black).

complex, showed that 90 out of 124 Cα atoms move nearly in the same direction from their positions in the native structure, in the 79% form and the complex. Therefore, the overall changes in the structure during the binding of the substrate analogue resemble those that result from partial dehydration. The magnitude of the movements is higher in the 79% form than in the complex. As shown earlier (Table 3), the hinge angle reduces by 1.6° when going from the native to the 79% form. Slight domain closure occurs in the complex also; the magnitude of the reduction is, however, lower at 0.8°. In addition to the gross motion of the domains, the similarity between the two structures is seen in segmental motion as well. Substantial deviation from the native structure is observed in residues 41–45 in the 79% form and the complex, as illustrated in Fig. 7. These deviations are in the same direction, although their magnitude is larger in the 79% form. Residues 41–45 form part of the substrate-binding site of the enzyme. Thus, it appears that by gently decreasing the water content in the crystals, we have to some degree simulated the conformational changes observed in the transition-state complex, but probably to a higher magnitude. The earlier reports of minor conformational changes in the substrate binding based on NMR and other solution studies (Hahn & Rüterjans, 1985; Markus, Barnard, Castellam & Saunders, 1968; Meadows, Roberts & Jardetzky, 1969; Roberts, Dennis, Meadows, Cohen & Jardetzky, 1969) are consistent with the above observations.

Concluding remarks

The mobility of ribonuclease A molecule presents a complex picture involving hinge-bending movement of the two domains as well as different extents of flexibility within them. The flexible regions, which occur mainly at chain termini of regular secondary structures and in loops, are, on average, more distant from the respective domain centres and the hinge than the relatively rigid regions. The hydration shell of the protein contains 31 invariant water molecules many of which are involved in holding different parts of the molecule together or in stabilizing local structure. A comparison between the 79% humidity form and the enzyme complex with a transition-state analogue indicates that a reduction in the amount of surrounding water might induce conformational changes similar to those that occur in the transition-state complex.*

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* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 79% relative humidity form, 1RHA; 88% relative humidity form, 1RHB). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: SE0153).

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