MOLECULAR PRESENTATIONS

Ribonuclease A - Cubic Hydration-Directed Assembly and Catalytic Function

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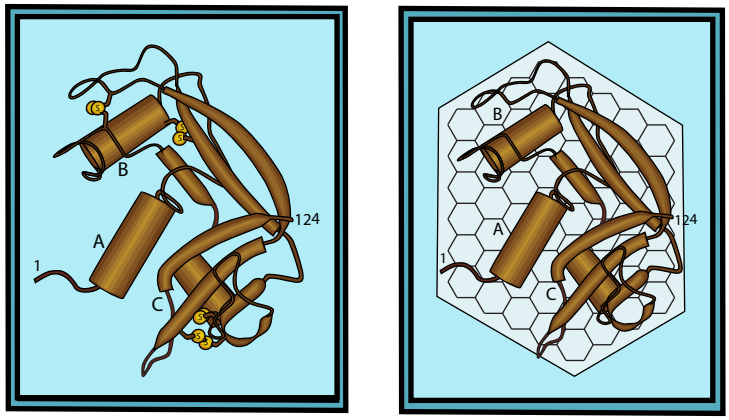
Dedicated to the late Professors Carl Djerassi and William S. Johnson of Stanford University.

Ribonuclese A is a digestive enzyme which is released from the pancreas and cleaves ribonucleic acids at uracil and cytosine nucleotides. It is considered a " killer enzyme" because it functions so rapidly and efficiently.[1] Since its surface is covered with cationic peptides, like lysine and arginine, it is extremely stable to acid - easy to isolate and purify.[2] Literally thousands of studies have been performed on this small 124-peptide protein, including the classical Anfinsen study on polypeptide folding.[3]



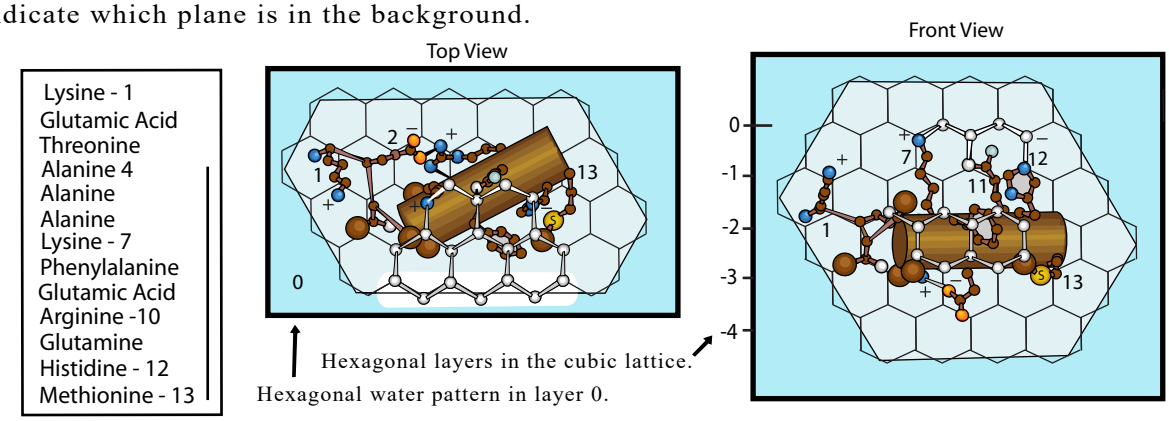
As shown on the left, four disulfide linkages tie coils and linear segments into an extremely stable structure.[4] In order to study folding, Professor Anfinsen, had to reduce the disulfides to sulfides by the addition of mercaptoethanol and then urea to disrupt order in the protein. As the protein unfolded, the long filament of polypeptide increased viscosity of the solution and enzymatic activity was lost. However, when urea and mercaptoethanol were removed from the solution by dialysis and it was permitted to stand in air to reform the disulfides, viscosity decreased and enzymatic activity increased to full potential. When mercaptoethanol was removed from the solution, with urea still present, standing in air decreased viscosity, but only 1% of enzymatic activity was recovered.

Based on the results, Anfinsen and the scientific community concluded (and many are still of the opinion) that urea, in some way, bound to the polypeptide and prevented spontaneous folding into the protein. The problem with that conclusion was: 1) eight sulfides had to be held in precisely the proper positions in space to form the correct disulfides and 2) in moving from the open polypeptide to the precisely-folded protein, the polypeptide moved spontaneously from randomness toward order - the opposite direction from that ordained by the Second Law of Thermodynamics.[5] Furthermore, it has since been shown in many studies, that urea, which hydrogen-bonds strongly with water not hydrocarbons, disrupts the structuring properties of liquid water.[6] Thus, it was the structuring property of surface water which held the sulfides in precisely the proper positions to form the correct disulfides and it was that same structuring in surface water which, as we now know forms ice-like linear elements of hydration in cubic forms on lipid and poly-ionic surfaces.[7]

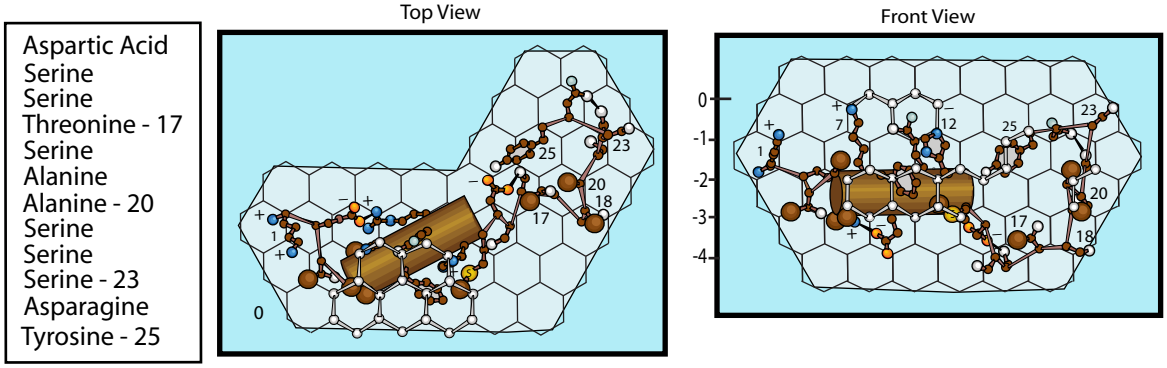
If we view the tertiary structure of the protein over an hexagonal plane of the cubic ice lattice, as shown above (with 2.76 Angstroms between water molecules), it can be seen that the coils and molecule tend to follow linear elements in cubic patterning. Although crystallographic coordinates for the ribonuclease A have been reported in many different molecular orientations, the one chosen for the figure above and the analysis below provided the best statistical fit with the cubic ice lattice. In the spatial anaysis below, atoms were maintained in precisely the same spatial positions as reported.for the crysalline form[4] although many on the surface may be mobile and change position in aqueous solution.

Ribonuclease A Assembly

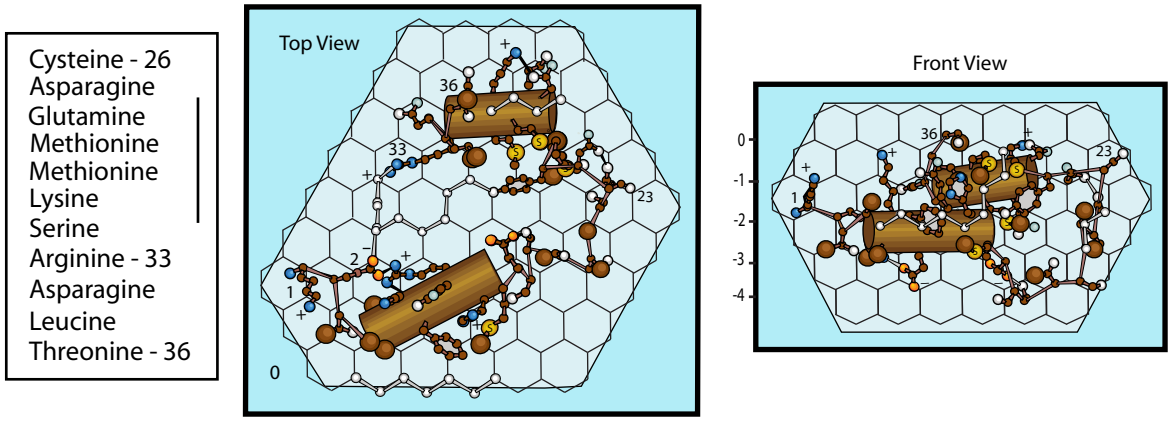
Since hexagonal units in planes of the cubic lattice are not above each other, the planes are numbered in the Front View of the molecule (parallel with the planes), with a number in the Top View, to indicate which plane is in the background.



Peptides 4 to 13, all with alpha and beta methylenes, rapidly induce the formation of the coil,[8] while arginine 10 holds glutamic acid 2 out of the coil. Linear elements of hydration form on the front side as well as above lysine 7 to permit charge-transfer tunneling to histadine 12.[9] Once spatial patterning is established around the coil, it is maintained throughout assembly and function.



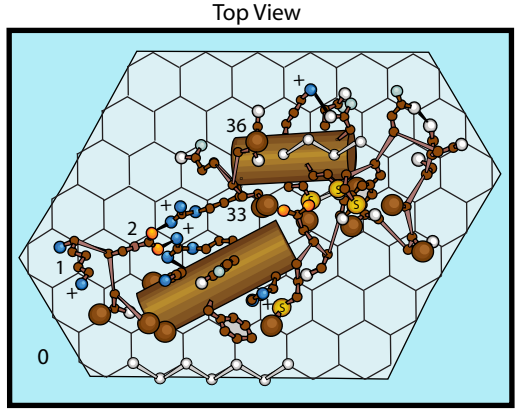
The continuing segment of twelve peptides, which contains eight highly-hydrated small peptides, would be expected to have a great deal of mobility and conformational freedom. Alanines at 19 and 20 and tyrosine 25 would be the only units to influenced by order in adjacent hydration. In fact, the conformation shown above, which is in the finished protein, may not be there until coil 2 is in place. Since coils form rapidly,[8] ordered hydration around them most likely guides them into assembly.



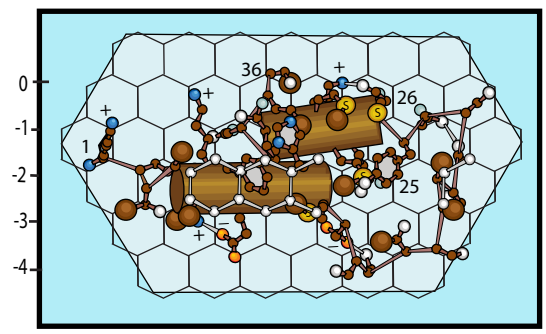
To demonstrate how ionic attraction may possibly direct assembly, a hypothetical intermediate is shown above with the coils separated and the cationic charge of arginine 33 coupled by linear proton tunnelling with anionic glutamate 2.[9]

In the Views shown below, coil 2 is shown behind coil 1 with arginine 33 coupled directly with glutamate 2. Side-chains of methionines fill space previously occupied by ordered water between the coils. Notice in the Front View below, that the upper surface of the assembly is positively-charged, while the lower surface is negative. This assists in directing further assembly and function of the enzyme.

Top View

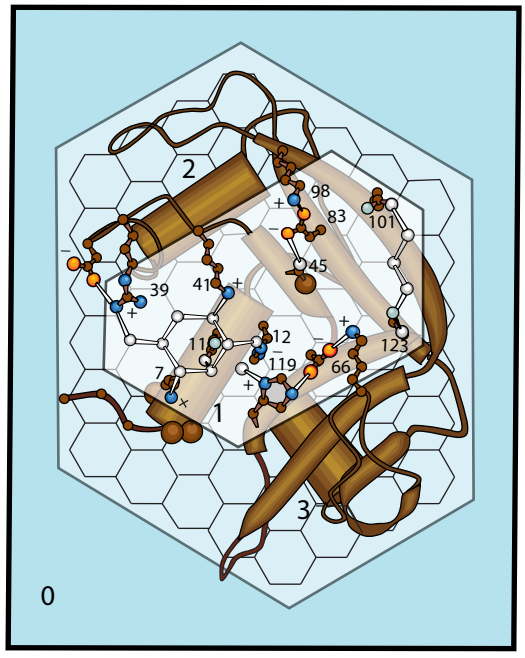


Front View

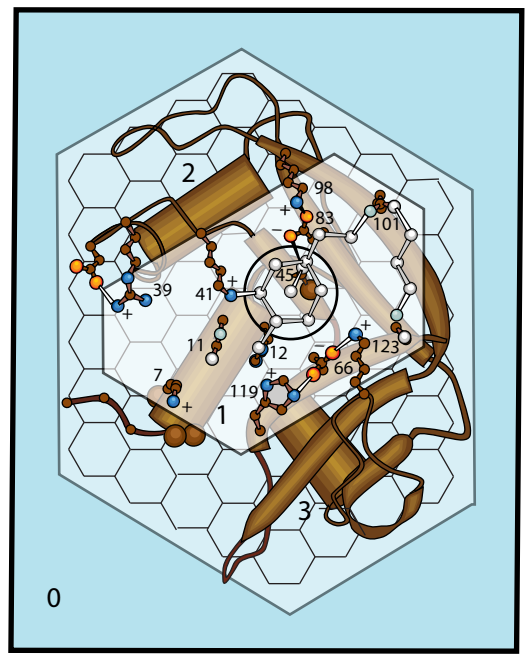


As assembly continues, flexible segments fit hydration-ordering surfaces of beta sheets and coils together to release unstable ordered water. The result is an extremely stable protein with an amazingly flat planar cationic surface between elevated coil 2 and the linear segment above coil 3 on the other side.

Top View A



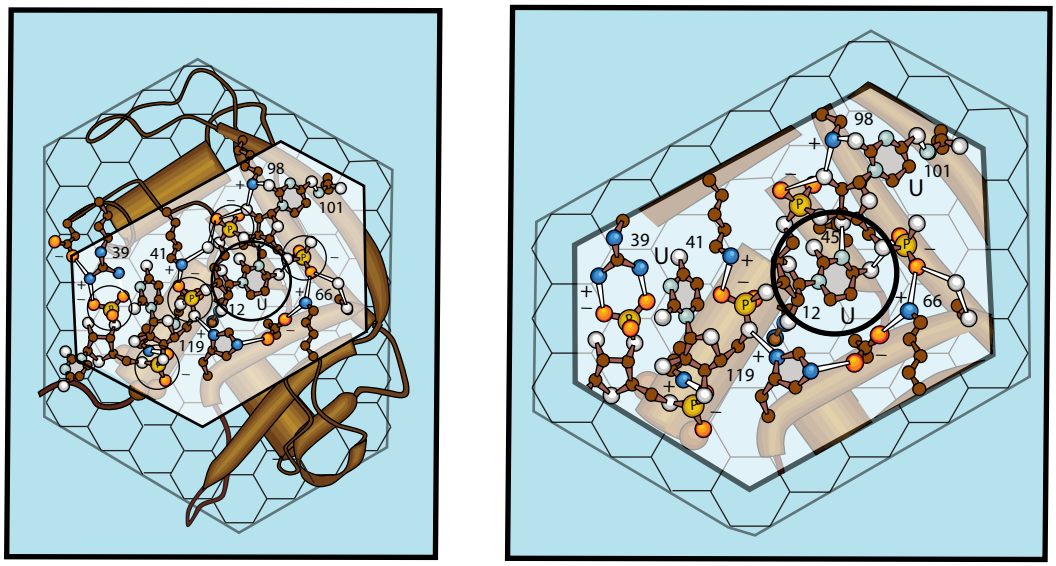
Top View B



Top View A illustrates how hexagonally-ordered water above the ionic groups on coil 1 support cationic charge between arginine 39, lysine 7 and lysine 41. The flat plane continues all the way over to the linear element between glutamines 101 and 123.

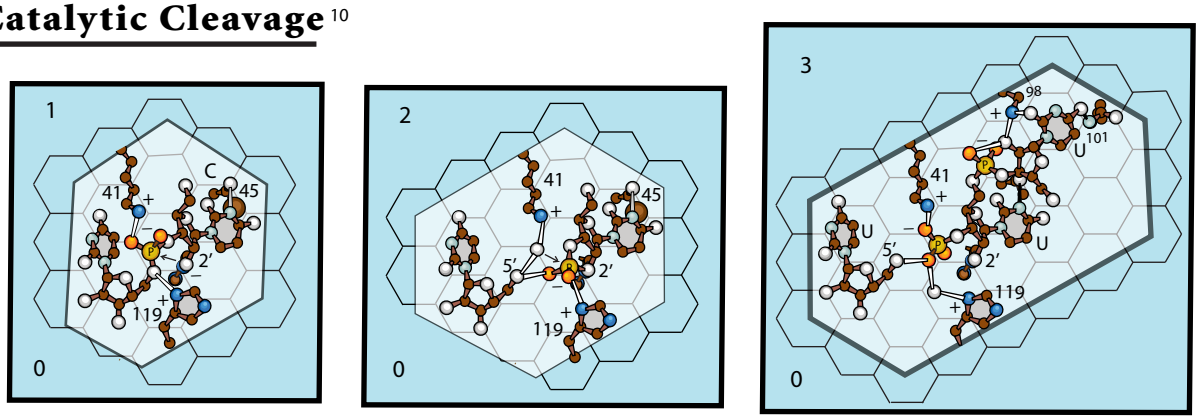
View B illustrates how a depressed pocket in the surface in the center is large enough to support a unit of cubically-ordered water bonded on the surface to lysine 41, histidine 12 and glutamine 101 and below to aspartate 83. The pocket is precisely the size to selective bind the pyrimidine rings of uracil and cytosine but not the larger rings of adenine and guanine. It seems almost impossible that a flat planar surface such as this could form spontaneously from a polypeptide with so many possible modes of freedom.

Ribonucleic Acid Binding



On the left above, the phosphates of five nucleotides are circled and illustrated bonded to cationic amines at positions 7, 19, 39, 41, 66 and 98.[10] As shown in the enlargement on the right, the phosphate of the central uracil is tightly bound between histidine 119 and lysine 41 with the 2' hydroxyl oxygen atom on the ribose ring of that nucleotide hydrogen-bonded to basic ring of histidine 12. The nitrogen atom of its pyrimidine ring is hydrogen-bonded to the hydroxyl group of threonine 45 in the depressed pocket. Once the phosphate bond between the central uracil and the one on the left is cleaved, the one on the right is ready to move into the site.[10] It is a flat ionic machine with a binding site in the middle.

Catalytic Cleavage [10]



In Figure 1, the uracil nucleotide is illustrated bound tightly in the reaction site with cationic amines on both sides of the central phosphate increasing the positive charge on the phosphate atom to the extent that it breaks the bond with the 5' oxygen of the next nucleotide and forms a cyclic phosphate intermediate with the 2' oxygen of the ribose ring of the uracil nucleotide, as shown in Figure 2. The water molecule bound between lysine 41 and the 5' oxygen of the released nucleoside in Figure 2 then reacts with the cyclic phosphate, releasing the free phosphate, as in Figure 3. During this two-step process, the uracil ring remains tightly hydrogen-bonded with threonine 45 in the cavity. However, following the cleavage, dynamic hydration of the free phosphate releases uracil from the pocket and the uracil on the right is pulled into it for cleavage. Whenever adenines or guanines are pulled over the pocket, their basic rings are too large to fit into the cavity and the chain is pulled along between peptides 41 and 119 until another uracil or cytosine is in the binding site.

As illustrated in Figure 3, the binding site is so perfectly "designed" that, if the next nucleotide in the chain is a uracil or cytosine, it is already in a perfect orientation to move into the reaction site for cleavage.

In spite of the fact that the principles of surface hydration presented above may differ substantially from present views, they provide rational explainations for many of the questions which lack viable answers in molecular biology today. Hopefully, this presentation will encourage others to take seriously the possibility that it is the formation of ice-like linear and cubic spatial forms which form on hexagonal-patterning surfaces regulate motions and interactions in living cells.

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