MOLECULAR PRESENTATIONS

Carboxypeptidase A - Assembly and Catalytic Action

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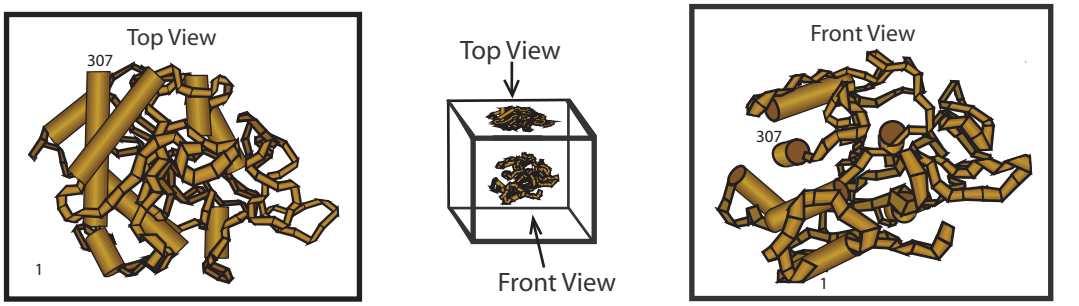
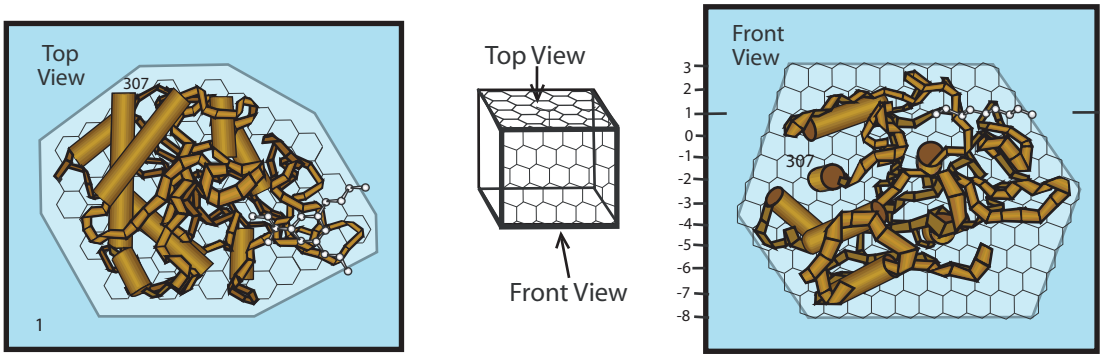
Dedicated to the late Professors Carl Djerassi and William S. Johnson of Stanford University.

Although structures of many proteins are known, the role water may have played in their assembly is unknown. The Transient Linear Hydration hypothesis described below provides an answer.

Carboxypeptidase A is a digestive enzyme which hydrolyzes terminal aromatic amino-acids from the ends of polypeptide chains. It is produced in the pancreas as a single strand of 370 amino-acids which wrap and assemble spontaneously to produce the protein structure shown below. Since the enzyme crystallizes in an orientation that positions most of the coils parallel to linear elements of hydration in the cubic lattice, hydration analysis was performed using the coordinates reported by Professor Lipscome in 1951! The 2.76-Angstrom cubic lattice is displayed behind both the Top and Front Views below. Since positions of hexagonal water molecules differ in the layers, the layers are numbered in the Front View and identified by number in the corner of the Top View.[2]



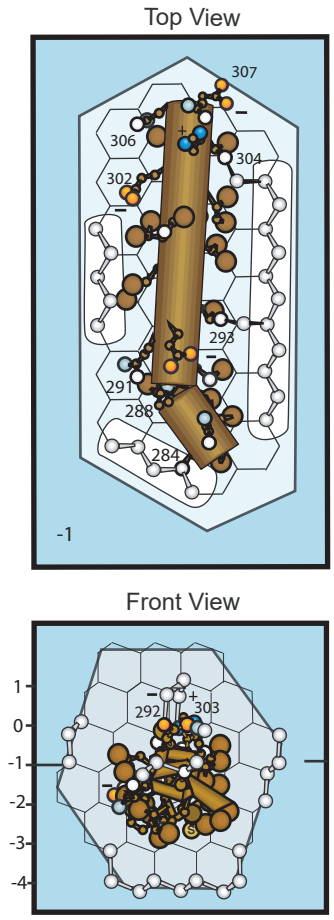


The coil ending in peptide 307, which is surrounded by coils and linear segments in the upper left corner, was selected as the initiating core and used to define the quantized probability orientation and location of covalent linear elements of hydration.[2]

It is important to realize that the cubic patterning displayed behind the protein is not a ridged lattice, it simply represents most likely positions for short covalent linear elements of hydration which form on non hydrogen-bonding lipid surfaces as the polypeptide wraps and assembles.[3] The elements are stable in ice but last only about 10^{-10} seconds above 0°C.[4] As the linear elements decay and water molecules move back into the liquid state, they absorb energy from the lipid surfaces and move peptides in those surfaces into lower energy coils, beta sheets and lipid/lipid assemblies. On the other hand, small polar peptides like glycine and serine, by hydrogen-bonding directly with surface water, gain energy for polypeptide chain bends and turns. Thus, as assembly proceeds, lipid peptides, by losing unstable water, end up in the anhydrous cores of proteins. Only a few hydration-ordering peptides are left in the upper right-hand corner of the Front View of the enzyme shown above holding water in a linear element to guide substrates into the reaction site for cleavge.[2]

Carboxypeptidase A (307-283)

As the carboxypeptidase A polypeptide emerges from the ribosome, covalent linear elements of hydration form on both sides of the the 307-288 end and withdraw so much energy that it rapidly forms a coil.[5] All of the peptides, except for glycine 295, have alpha CH_2 or CH groups which shield the main chain from bonding with water. This forces surface water to form low-energy bonding which, in moving back into the liquid state, moves the polypeptide into the lower-energy coil.[2]



Asparagine 307
Asparagine
Valine
Threonine 304
Histadine 303
Glutamic Acid 302
Methionine
Isoleucine
Threonine
Leucine
Valine
Glycine 295
Leucine
Tryptophane
Threonine 293
Glutamic Acid 292
Glutamine 291
Alanine
Threonine 289
Proline 288
Isoleucine
Isoleucine
Glutamine
Serine - 284
Alanine - 283

At proline 288, the coil turns because proline cannot form an internal hydrogen bond with isoleucine 287.

In the illustration on the left, a single linear element is pictured as covering the entire right side of the coil. This might be how it would look if we could take a time-lapse picture but, at any instant, only small, three-to five water molecule-units, would be present.[2] Most of the time, water would be in random positions. Although each covalent element lasts only about 10^{-10} seconds, repetitive formation not not only generates the linear elements, but a cubic hydration pattern of water molecules, like the positioning of atoms by electrons around the nucleus of a central atom. Like electrons, water molecules move so rapidly relative to the movements of molecules, that they effectively occupy space but, like the blades of fan, they are not there of the time.

Thus, polypeptides are free to move but, once anhydrous cores are established, relative positions in space are fixed.

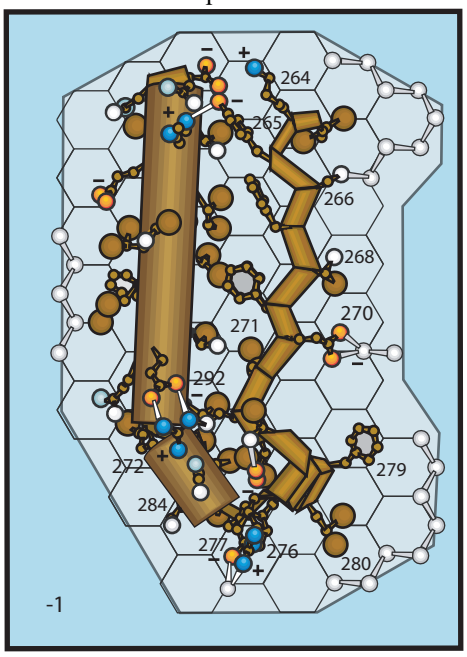
For example, positions and orientations of hydroxyl groups on serines and threonines in polypeptides can either support or disrupt cubic patterning. In the Top View, the hydroxyl groups of threonines 304 and 293 on the right side of the coil are in proper positions and at proper angles to support the formation of ice-like linear elements of hydration adjacent to the coil. Methyl groups on the alpha carbons of these two threonines shield the polypeptide chain from bonding with water. On the other hand, serine 284, although in a proper position to support cubic patterning, does not protect the chain from bonding with surface water - it permits energy input into the chain and initiates a turn.

The Front View illustrates how the hydroxyl group of serine 284 in the center may bond with surface water and how the anionic oxygens of glutamate 292 and the cationic nitrogens of histadine 303 above the coil may be stabilized by the tunneling of protons through covalent linear elements of hydration which continually form between them above the coil.[6] By hydrogen-bonding with surface water at multiple angles, the oxygen and nitrogen atoms of acids and amides usually disrupt linear hydration and the cationic amines of lysine and arginine are so highly hydrated that they usually disrupt cubic hydration patterning.

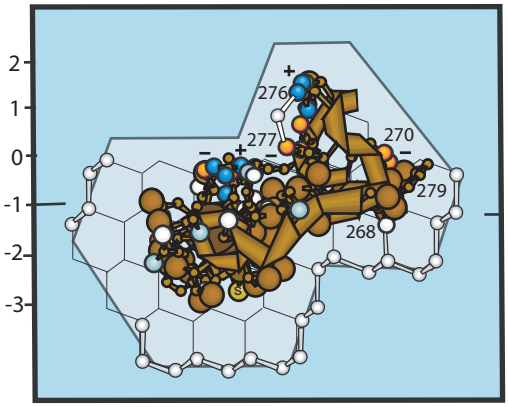
Carboxypeptidase A (307-263)

As expected, the chain moves to the right and, at glycine 278, turns upward in a beta-turn to permit arginine 276 to be bridged by a water molecule to tyrosine 277 to make a turn - then downward in a beta turn at glycine 275 to couple arginine 272 directly to glutamate 292 above the coil.

Top View



Front View



Serine 284
Alanine
Proline
Leucine
Leucine
Phenylalanine 279
Glycine 278
Tyrosine 277
Arginine 276
Glycine 275
Threonine
Aspartate 273
Arginine 272
Leucine
Glutamate 270
Phenylalanine
Threonine 268
Phenylalanine
Serine 266
Tyrosine 265
Lysine 264
Isoleucine

With these diagonal units in place, the the linear segment from leucine 271 to tyrosine 265 is in position to fit its two aromatic rings into the hydrophobic space next to the coil with tyrosine 265 hydrogen bonded to histadine 303 above the coil. Note that the side chains on this linear segment of polypeptide alternate from side to side permitting different hydration properties on each side. In this case, the left side is hydrophobic while the hydroxyl group of serine 266 is precisely in the proper position to support hexagonal patterning while 268 is out of position.

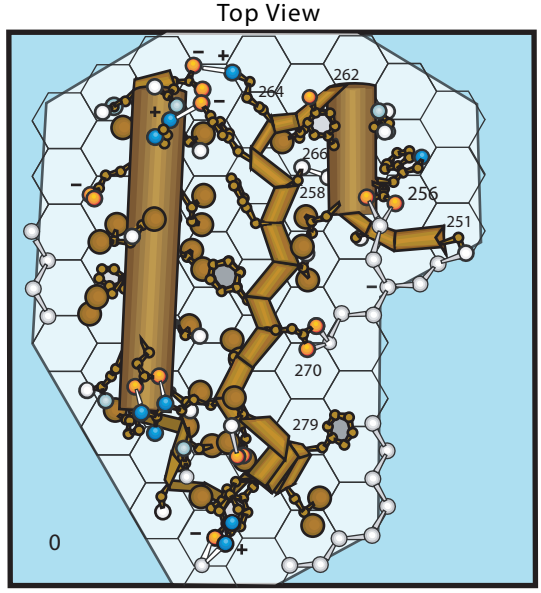
While the hydroxyl group of threonine 268 and glutamate 270 disrupt order in planes 0 and -1, phenylalanine 279 and leucine 280 on the end reinforce cubic order in those two planes. Notice that most of the ionic peptides are on the ends neutralizing each other while glutamate 270 is alone in the center with no close cations to neutralize its charge. Regions of hydration order on the right-hand side at both ends of the assembly are separated by this central disordering acid with water clustered around it to dissipate its charge. As you shall see, this isolated acid will play a critical role, not only in directing further assembly at the upper and lower ends, but in being a critical part of the reaction site.

It is important to mention that arrangements of linear segments of polypeptide next to coils are extremely common in proteins. It is easy to visualize how various combinations of surface groups on the sides of coils might complex with complimentary groups on the sides of linear segments, like those shown above.

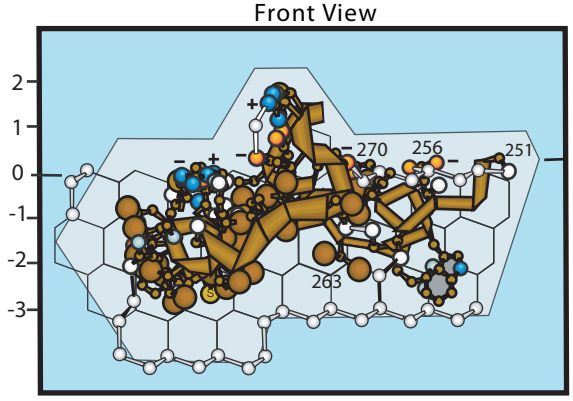
Carboxypeptidase A (307-251)

After placing the lipid group of isoleucine 263 in the ordered void below the complex, as shown in the Front View, glycine 262 permits the chain to change direction and, with three order-forming peptides, initiate a short new coil parallel to the initial one. This positions the carboxyl group of aspartate 256 in the plane of glutamate 270 so that a single negative charge can be stabilized by transient linear elements between them.

Top View



Front View



Lysine 264
Isoleucine
Glycine 262
Glutamine
Asparagine
Tyrosine
Serine 258
Tryptophane
Aspartate 256
Isoleucine
Serine 254
Glycine
Glycine
Serine 251

As the chain continues, serines play a major role. Serine 258 hydrogen bonds to serine 266 to position the coil while the hydrating series, serine-glycine-glycine-serine, permits the chain to move upward and place the hydroxyl of serine 251 in the same linear hydration plane as the the carboxylates of the two acids.

The Front View illustrates the planar positions of carboxylates 270 and 256 and serine 251 with a plane of water stabilizing them in plane 0. Once protein assembly is complete, that plane of water will be replaced by side chains of peptides which will hold substrate polypeptides in precise positions for hydrolytic cleavage.

Notice in the Top View that the inner region of this initial section is almost entirely lipid and aromatic while the ends are ionic and highly polar. As illustrated in the Front View, Plain 0 is highly ionic with an elevated ridge in the center.

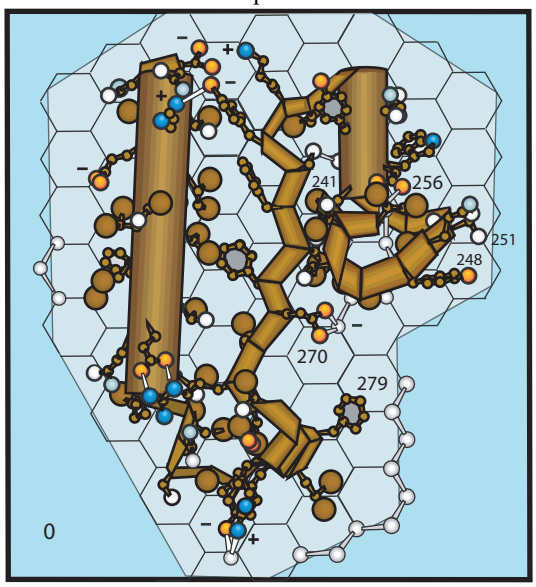
On the upper left in the Front View, the ionic groups are in a rather circular form. Further out in the polypeptide, a coil has been generated with anionic groups in complimentary positions to those shown above. In proteins containing a number of coils, most of them form early in the folding process and assemble together later to produce the final protein.[5] If coils form at a distance from each other, each serves as its own nucleating core with preferred orientations of transient linear hydration around it to guide folding and subsequent assembly. This type of hydration-coordinated assembly was illustrated in detail in the insulin article.[2]

Proteins in membranes often containing multiple coils. Sometimes, they are inserted directly into membranes from ribosomes but, often, they assemble in water with polar peptides facing outward toward water and lipid peptides facing inward toward each other. When they contact lipid membranes, the coils invert to direct lipids outward and polar groups inward to form a functional pore through the membrane. Some pores permit water to pass through but most selectively transport specific ions or molecules in and out of cells. In fact, in a recent report, an element of linear hydration permitted the selective conduction of protons through a pore in a membranal protein.[7]

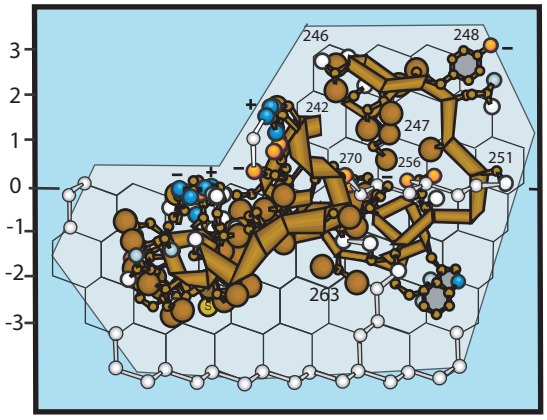
Carboxypeptidase A (307-241)

In order to maintain the stabilization of negative charge between carboxyls 256 and 270, the continuing polypeptide chain loops over the dielectric linear element of water between the acids with the methyls of threonine 246 and isoleucine 247, as illustrated in the Front View, resting on the diagonal groups below. The aromatic ring of phenylalanine 279, which is in the same plane as glutamate 270 increases the order of water on the end of that polar region.

Top View



Front View



Serine 251
Alanine
Glutamine
Tyrosine 248
Isoleucine 247
Threonine
Theonine
Isoleucine
Isoleucine
Serine
Glycine 241

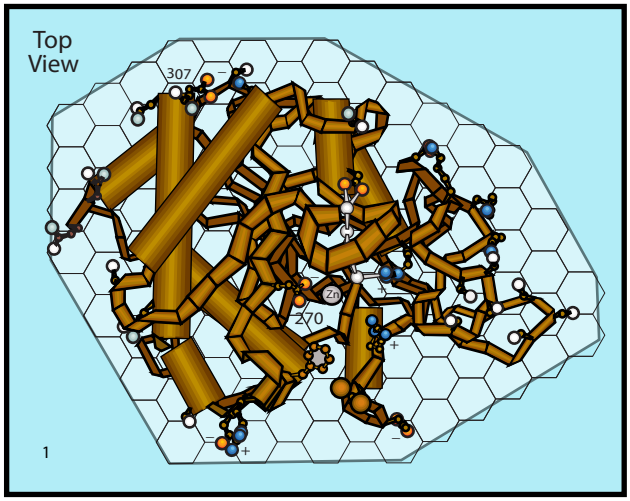
In the front view, it can be seen that the oxygens of tyrosine 248 and threonine 246, with a slight upward movement of the chain, are in reinforcing positions in plane 3. Note how the loop above glutamate 270 tends to follow the diagonal plane of cubic patterning. By moving upward, the loop can provide sufficient space for hydration stabilization of the two carboxylate anions. With serine 251 positioned in the same horizontal plane as the two anionic carboxyls, covalent linearization of water is reinforced in that plane by all three peptides.

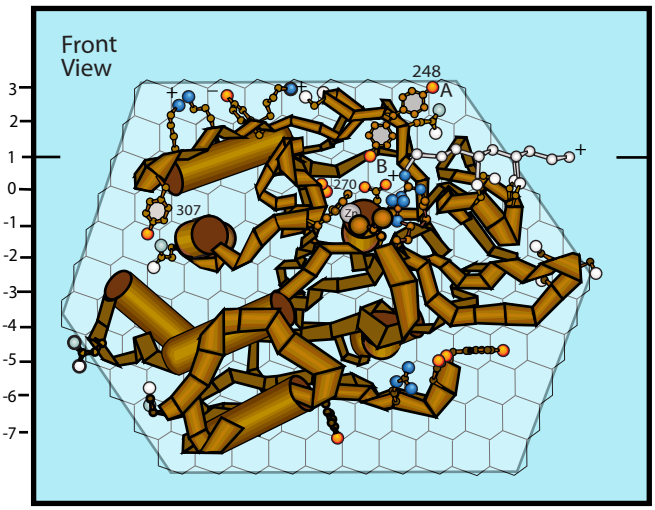
Even at this early stage of polypeptide folding, it is easy to see how the hydrated cavity, formed below the ordering methyls of isoleucine 247, might serve as a hydrophobic pocket for the aromatic rings of polypeptides which will bind for cleavage. Of course, this is only a small portion of the site which will bind substrate polypeptides. Glutamate 270 and tyrosine 248, with its phenolic ring rotated downward, will be involved in hydrolytically removing terminal aromatic peptides from the ends of chains.[8] Although a few of the peptides which will be involved in the hydrolysis are already present, many more will be needed around planes 0 and 1 to provide for guided transport and peptide cleavage.

It is imporant to realize that surfaces of polypeptides most likely become coated with water and form coils and linear segments extremely rapidly as they are released from riposomes[5] and that large lipid regions, like the one shown above with the side-chain of peptide 263 in it most likely fill almost as rapidly as they form with a compilmentary lipid region of another section of the polypeptide. To simplify this presentation, assembly was begun with the 307 coil but one of the central coils might serve as well as the nucleating core.

Carboxypeptidase A - The Cubic Hydration Patterning Model

In the Top View of the finished protein, it can be seen that no less than three coils overlay the terminal one with a curved plane of linear beta-sheet segments wrapping around below. Although the selection of the terminal coil as a reference unit proved satisfactory for initiating the analysis, it can be seen that a number of the other coils tend to follow cubic hydration patterning. If one of them had been chosen as the reference, the same cubic orientation would have been produced. In the Front View, it can be seen that, even though the coil/linear segment assembly in the upper left is not parallel with the horizontal planes, the ionic ends of the peptides reach high enough to hydrogen bond into plane 3.





Also, it must be realized that this protein is not produced in isolation.Thousands of molecules with the same spatial structure are produced by the ribosome. The molecules must have external structures which permit them to pack together tightly for efficient storage but with sufficient hydration space between them for rapid release. The line of polar groups on the left side in the Top View permit diagonal hydration and the binding of two molecules to form a dimer in solution.

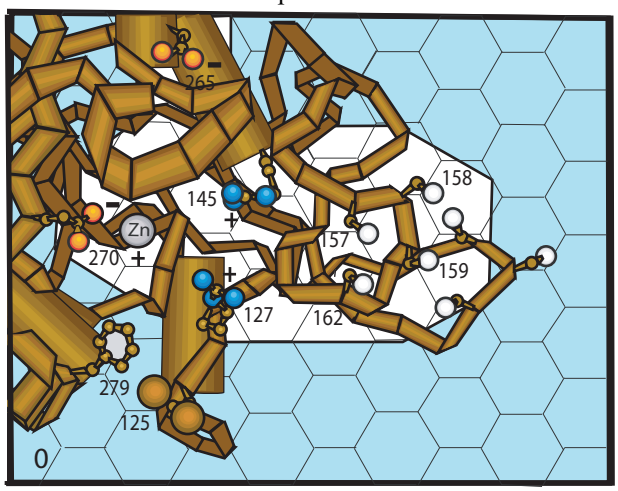
However, the most important area of residual transient linear hydration is displayed on the upper right side in plane 1 in the Front View. In all previous illustrations, atoms in the protein have been presented in their X-ray crytallographic solid-state positions. However, in aqueous solution, polar groups on outer surfaces have considerable freedom to move. For example, the phenolic ring of tyrosine 248 rotates from position A to B to participate in the hydrolytic reaction[7] and, as illustrated above and on the next page, the hydroxyls of four serines in plane 0 can be rotated by surface water to provide for the stabilization of dielectric elements of linear hydration to direct substrate polypeptides into the reaction site for cleavage.[2]

Notice that the coils appear to adopt completely different orientations in the Top and Front Views. In the Front View, it appears that the coils and linear segments are almost floating on or between layers and, based on the Transient Linear Hydration Hypothesis, they were, indeed, floating on layers of water molecules which were there only as short linear units at any instant but, when integrated over the time of polypeptide movement, produced the effect of layers. Patterning of water around natural molecules is like the patterning of electron orbitals around atoms - they define preferred quantized positions without defining precise positions.

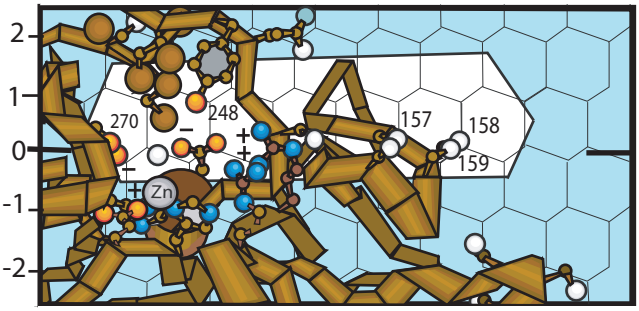
Carboxypeptidase A - The Catalytic Reaction Site

The shaded areas in both Top and Front Views outline the regions where peptide chains bind as they move into the site and are prepared for proteolysis. It is quite amazing that each of the groups in this binding area, as well as peripheral groups, appear to play a role in binding and the catalytic reaction.

Top View



Front View



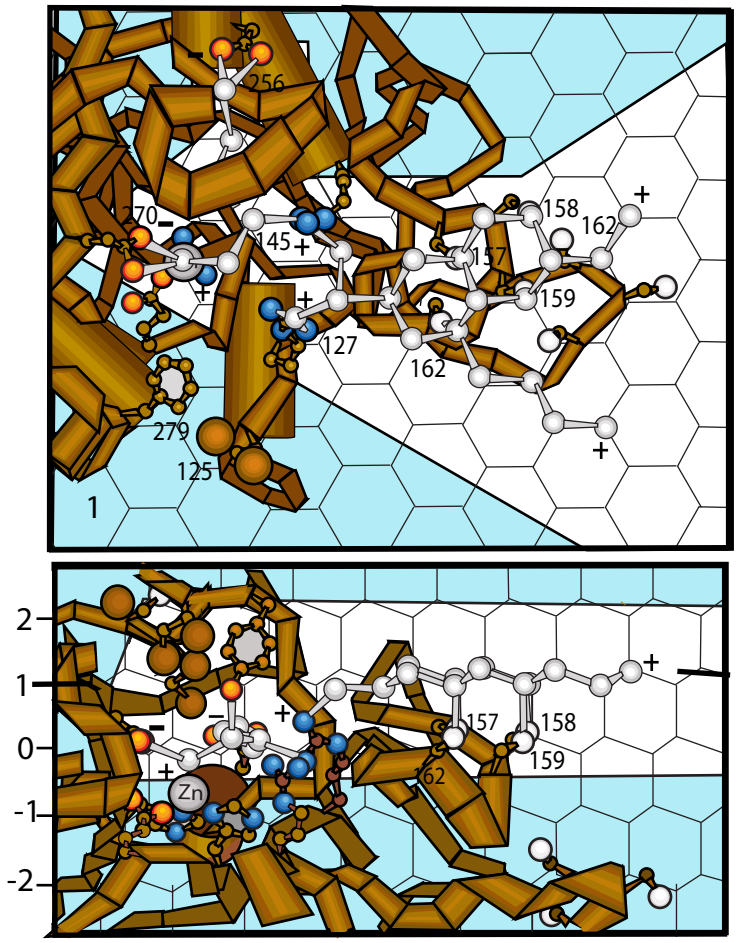
For example, the phenyl group at 279 and the methyl groups of leucine 125 provide a water-ordering lipid barrier to direct water-ordering anionic ends of polypeptide chains into the highly cationic reaction site involving a zinc ion, two arginines at 127 and 145 and the glutamates at 270 and 265. It seems rather amazing that a zinc ion binds selectively and firmly in the reaction site when other divalent ions, like magnesium and calcium, might bind instead. As shown in the Front View, nitrogen and oxygen atoms of peptides below the zinc ion are in precisely the proper positions to bind it in the proper position to participate in the catalytic reaction.

Likewise, other groups around the reaction site are in positions which permit water molecules, as well as substrate molecules, to bind in specific positions and be moved in proper directions to perform the hydrolysis reaction rapidly and efficiently. As mentioned before, the hydroxyl groups of the four serines in plane 0 in the right-hand loop and the phenolic group of tyrosine 248 are relatively free to rotate and hydrogen bond with water and substrate molecules in plane 1.

Remember, it was the anionic glutamate at 270 which played an important role in directing the formation of the loop over the aryl binding site and in gathering the cationic arginines around the reaction site. It is almost as if the site were designed by some sort of “Master Plan.”

Carboxypeptidase A - Reaction Site Hydration

Although the positioning of four serines below hexagonal sites in plane 1 seems almost preconceived, it is likely that this protein, like all natural molecules, was formed originally by trial and error. Polypeptides which formed stable functional spatial forms under the direction of water survived - those that did not, were chewed up by lytic enzymes. It is likely that random genetic changes occurred in polypeptide sequences before the one shown here appeared. The fact that slight differences in polypeptides are found in proteins in different animal species, provides evidence that numerous genetic changes occurred during biomolecular evolution.

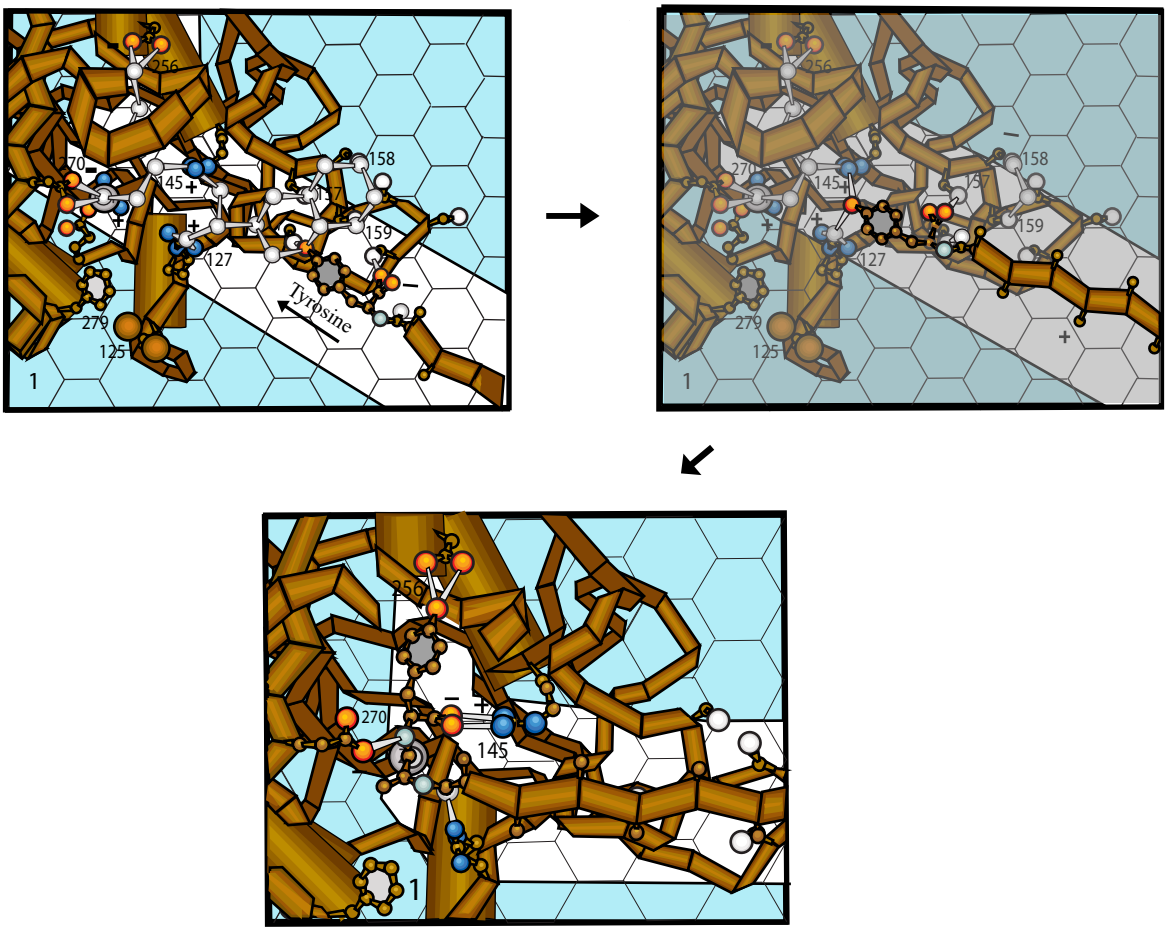


Although water molecules are pictured in specific locations, they are only probability positions. Water extends in multiple lines out from the reaction site. Often, regions leading into reaction sites are hydrophobic. In this case, hydrocarbon peptides in plane 1 at 125 and 279 provide a hydrophobic side to increase hydration order leading into the reaction site but it is serines at 157, 158, 159 and 162 which bind water molecules in linear and hexagonal forms leading into reaction site. It is those charged dieelectric linear elelments which guide anionic peptides into the site.

In this protein, it is the hydroxyls of serines on a planar surface which provide the hydration order required for rapid catalytic function. In the feet of penguins, it is amides and acids on planar polypeptide and membranal surfaces which disrupt transient linear order to prevent freezing.[8] In fact, as mentioned in the introduction, Dr. Davies at Queen's University in Canada recently reported that a protein, with as many as 400 water molecules trapped in fixed ordered positions between coils, exhibited antifreeze properties.[8] In this case , disorder in surface water was not produced by randomness in atoms in the contact surface but because the order was pentagonal rather than hexagonal and cubic.

Carboxypeptidase A - Substrate Binding

As the anionic end of a polypeptide chain moves into the cationic reaction site, the phenolic oxygen of a terminal tyrosine most likely binds by hydration bridging to the serines in several alternative positions and then into a binding position between arginines 127 and 145.

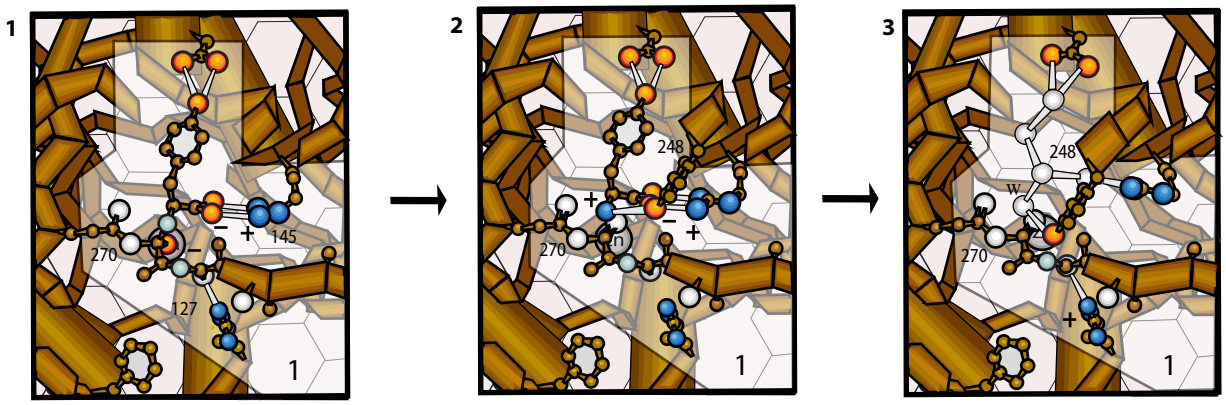


In the reaction site, as illustrated above, the carboxylate anion of tyrosine is bound tightly to arginine 145 with the phenol ring hydrogen bonded to aspartate 256.[9] (The loop has been removed for clarity.) The carbonyl oxygen of the second peptide is bound to the zinc ion. In binding, the polypeptide chain has released most of the water in the binding site to increase hydration entropy and structural stability.[2]

All atoms of the reacting molecule must be held in proper spatial positions to permit reactions to proceed rapidly and spontaneously.

Carboxypeptidase A - Substrate Cleavage

In Figure 1 below, the O-C bond which initially forms between carboxylate 270 and the carbonyl carbon of the second peptide, breaks the C-N bond to release tyrosine as the free amino-acid leaving the carbonyl of the second peptide bonded to glutamate 270 as an anhydride. In Figure 2, the phenolic ring of tyrosine 248 is shown hydrogen bonding to the amine of tyrosine to escort it out of the binding site. In Figure 3, it is shown holding water molecule "w" precisely over the anhydride.[9]



Of course, reaction of the water molecule with the anhydride is rapid, forms the two acids and tyrosine 248 transports the shortened polypeptide out of the site.[9]

Although detailed studies of the reaction described above have been reported in the literature, this is the first time that a possible role of water has been included. Time will tell whether the concepts presented here are valid or not.[2]

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