Alpha-Chymotrypsin

Chymotrypsin is synthesized on a ribosome as a single polypeptide strand of 245 amino acid residues which fold spontaneously into a finished protein called chymotripsinogen.¹ That form of the protein is inactive as an enzyme but enzymatic cleavage of peptides 15 and 16, followed by removal of several other peptides, produces the active stable enzyme, alpha-chymotrypsin.² Since the body of the enzyme is not affected by proteolytic activation and our analysis of folding begins with peptide 245 at the acid end, the presentation of wrapping and assembly is valid for both forms of the protein. An interpretation of the role of surface water in the catalytic mechanism of action of the enzyme is also included.

In order to gain a perspective of the spatial structure of the enzyme, relative to the 2.76Å cubic lattice of hydration, both Top and Front Views are presented above with hexagonal layer 0 illustrated behind the Top View.³ As usual, the analysis of this protein will begin with the longest coil with all but its upper surface hydrophobic. The coil is not parallel with any of the major axes of its X-ray crystallographic coordinates but is parallel with transient linear elements in the cubic lattice. Thus, coordinates reported for the crystalline form of the enzyme were used for the comparative analysis without changing the location of any atoms to satisfy hypothetical cubic hydration positions. Although analyses of most proteins required rotation of X-ray coordinates to provide for best fit of coils and sheets within the cubic lattice, this enzyme was analyzed directly as reported by Professor Blow.²

Substrate binding in this enzyme is complex because cleavage is in the center of polypeptide chains at aromatic peptides like phenylalanine and tyrosine. The linear elements of hydration shown in both Top and Front Views above are where covalently-bonded water molecules extend out from the enzyme to attract substrate polypeptides into the reaction site for cleavage. As you will see, the reaction site is surrounded by aromatic rings and sulfur atoms to provide for the formation of charge-transfer complexes with the aromatic rings of substrate polypeptides as they approach and bind in the site.
Sometimes, as polypeptides emerge from ribosomes, they remain as linear units but the 15 peptides on the end of chymotrypsin are so hydration-ordering that they wrap rapidly into a coil. However, the cation in arginine 230 produces so much disorder that it produces an abrupt change in chain direction. With a positive charge at the arginine end and an anionic charge at the asparagine end, a dipole is produced which assists in subsequent assembly.

Glutamines 239 and 240, with their amide oxygen and nitrogen atoms in spatial positions that do not support cubic patterning, disrupt hydration order above and behind the coil. Conversely, hydrophobic methyls of valines and leucines on the lower and front sides continually induce the formation of covalently-bonded “ice-like” linear elements of hydration which will be displaced by complimentary lipid surfaces.

Since, the continuing polypeptide chain, as shown below, does not contain a linear segment of ordering peptides of proper length to displace the water next to the coil, it follows transient linear elements away from the coil with the phenolic oxygen of tyrosine 228 fitting into a cubic water patterning position for stability. Glycine 226 turns the chain downward and proline 225 initiates a loop of glycines, threonines and serines which is terminated by tryptophane 215 with its circled flat aromatic ring resting on proline 225.
With the methyls of valine 213 positioned above tyrosine 228 and those of isoleucine 212 below tryptophane 237, the chain drops down at glycine 211, goes under the coil and forms a loop below the coil.

In the Front View, the lower loop can be seen following cubic patterning with the highly hydrated cation of lysine 202 turning the chain around at plane 5.

Again, we must be reminded that, even though surface water is displayed as extended linear elements, at an instant, they are present only as short linear segments like the trimer. Only when viewed over time, would water molecules be in the probability positions which generate cubic patterning.3

With anionic asparagine 245 at the top and lysine 202 at the bottom, another dipole is generated which might permit proton tunnelling through water following linear elements between them.4 However, the hydrophobic cavity shown by the curved segment of hydration is so large and would be so unstable that the loop might initially fold out behind the first loop and move into the position shown as more space is filled by the continuing chain. Water trapped as small droplets in crude oil turns to crystalline hydrates above 0°C as it is pumped from deep sea-wells and makes pumping extremely difficult.5

Since studies have never been reported on the folding of this short terminal segment of the polypeptide, we can only speculate on its shape but it does seem to follow orientations of transient linear elements of hydration, might provide a method for predicting probable conformations for hydrated forms of thermodynamically unstable intermediates. Until such computerized modelling techniques are available, we can only speculate based on the concept of cubic hydration patterning.
Once the chain has turned at 202 and passed back under the coil, it travels upward to proline 198 then through two glycines to serine 195, which is held tightly in plane 0 by a disulfide bond between cysteines 191 and 220.

To complete this section, the chain turns 120 degrees, passes below the first two linear segments to form a water coupling between anionic cysteine 182 and cationic arginine 230.

In Top and Front views, it can be seen that the polypeptide chain, by wrapping back and forth, has filled a rectangular space, displacing all of the water. It is as if serine 195, which positions its hydroxyl precisely in a cubic water position at the corner of a solid block of atoms, might serve some important function.

And, indeed it does - as protein assembly continues on the right side, a linear segment of substrate polypeptide will replace water shown leading into the hydroxyl group of serine 195 for binding and cleavage. Serine 195 must be held by the block of atoms in precisely the proper position to serve a primary role in the catalytic hydrolysis of the amide portion of aromatic peptides in the center of polypeptide chains. The open space, designated as A, is the cavity into which the aromatic rings of peptides are inserted and tightly bound.

As you know, enzymes increase reaction rates thousands of times faster than normal. To do that, binding sites must hold reacting regions in precisely the proper positions and at precisely the proper angles for quantized units of energy to be transferred between atoms with minimal activation energy.

The left side of this enzyme, as shown above, holds reacting aryl peptides in precisely the proper position to react with the serine 195 hydroxyl. The right-hand side, which will be shown on page 6, is involved in directing polypeptides with aromatic rings into the reaction site. Based on analyses of a number enzymes, it appears that it is covalently-bonded linear elements of hydration which are induced to form adjacent to specific sets of ordering peptides which drive and direct substrates, in multiple steps, into reaction binding sites. Selection of substrates for reactions often occurs at entrances to those sites as well as within those sites.
**Alpha-Chymotrypsin (245-133)**

Since some proteins do not contain coils, alpha-carbon plots may be used to select the best orientation to view transient covalent linear elements and cubic patterning. Viewing the plot of alpha-chymotrypsin from asparagine 245 to glycine 133, it can be seen that the chains, in wrapping back and forth, appear to follow linear elements in cubic hydration patterning.

Following the placement of cysteine 182, the chain moves up vertically to plane 1 and then down at glycine 173 to form a coil, including cysteine 168, which bonds with cysteine 182 to tie the coil horizontally in Plane 1. In the Top View, it can be seen that peptides 147 and 148 have been removed catalytically to activate the enzyme.

In the Front View of the total protein shown below, the molecule is divided into six spatial units which correspond to units of cubic patterning. It would be interesting to see if natural proteins are composed of a finite number of cubic space-units.

As illustrated in the Front View above, individual chains do not necessarily follow the orientations of ordering surface water but the assemblies which develop in the internal regions of proteins do reflect cubic patterning. Whenever linear chains change direction, there is usually a glycine, serine or proline at the position of change to hydrogen bond into surface water, disrupt order, increase hydration entropy and drive the chain in an alternate direction. Although an understanding of the role of transient linear hydration might assist in providing more viable interpretations of folding and assembly, the question of whether or not it will assist in providing more accurate predictions of final protein structures from polypeptide sequence information must wait until appropriate mathematical models are available.
Alpha-Chymotrypsin - TLH Model

In these views of the total protein, surface groups are included to illustrate that many of them are not in positions to support cubic hydration patterning but, as mentioned before, in positions to disrupt the formation of transient linear hydration and coordinated cubic hydration in order to increase both stability and solubility.

Although surface groups in the crystalline state, as illustrated here, are held in relatively ridged positions, in aqueous solution, they have a good deal of freedom. For example, in the Front View, the loop above the coil has a degree of freedom to move upward but the one bearing histidine 57 in front of the coil is tied by a disulfide link to the lower unit and is relatively ridged. The histidine ring can rotate as it participates in the hydrolysis reaction, but the peptide itself is in a relatively ridged position to provide conformational control.

Since this molecule is composed of only two coils, it is the disulfides, formed by the oxidation of cysteine sulfides during assembly which provide for much of the stability. As will be illustrated on subsequent pages, it is the probability positioning of transient linear water in and around the binding site, which directs substrates into it.

Remember, the cubic lattice is displayed behind the protein only to illustrate the orientation of linear elements of surface water involved in directing folding and assembly. Several proposals have been presented that water forms clathrate-type cages around proteins but they don’t seem viable because of large losses in entropy that would occur in surface water. Instead, as mentioned before, it is most likely the formation of short covalently-bonded linear elements of hydration, like the trimer, which form kinetically, in an integrated fashion, on hydrophobic and poly-ionic surfaces. They last only about $10^{-10}$ seconds, by forming and degrading, generate a three-dimensional matrix of cubic hydration around the molecules. Some oxygen atoms in serines and threonines are in proper positions and at proper angles to bond into transient linear elements and reinforce cubic patterning while others usually disrupt local hydration order.
**Alpha-Chymotrypsin - The Catalytic Reaction Site**

As illustrated below, the primary role of alpha-chymotrypsin in digestion is to hydrolyze polypeptides at aromatic peptides like tyrosine and phenylalanine. The cavity designated as “A,” which was left open and hydrated during the folding process, is precisely the proper size to fit the aromatic rings of bound peptides as they are held in position for hydrolytic cleavage.²

In the Front View on the lower left below, it can be seen that the reaction site is surrounded by peptides with aromatic rings at 215, 41, 39, 141 and 146 to increase hydration order around the binding site and bind the aromatic rings of substrate polypeptides. Methyl groups on two corners of the site provide even greater order in surface water. As you will see, histamine 57 and serine 195 will be intimately involved in the cleavage reaction.

In the Top View on the right, a number of peptide carbonyl oxygens, as well as the amide nitrogen of peptide 192 are identified because they provide specific sites for binding substrate polypeptides. Again, Region A will bind aromatic rings, Region B will bind the peptide to be cleaved and C, D and E will bind the polypeptide chain.
Alpha-Chymotrypsin - Reaction Site Hydration

The X-ray diffraction pattern of the crystalline enzyme reveals a number of water molecules in binding cavity A, but their high energy makes it difficult to define precise locations. Dissolved in aqueous medium, water in the binding site must be extremely dynamic but must have preferred probability locations to direct substrate molecules into proper binding positions. In order to locate those positions, physical and computerized models of the site were constructed with water molecules bridging between surface oxygen atoms at acceptable angles and distances. The distribution shown is one of several alternative arrangements.

Some of the bridging water molecules are in cubic water positions but most are not. However, the carbonyl oxygens and bridging water molecules tend to be aligned between hydration levels 0 and 1 in cubic patterning to aid in binding incoming strands of polypeptides. Note that the aromatic rings of phenylalanines 39 and 41 are positioned above and below planes 1 and 0. This permits the aromatic rings of incoming polypeptides to be positioned parallel to either of these rings while amide nitrogens of peptides bind to carbonyl oxygens in the site.

Often we forget that reaction substrates, as they approach a binding site, move in quantized steps through a series of binding relationships with the site, each involving small exchanges of energy to permit spontaneity.

For example, methionine 192, with its peptide carbonyl hydrogen-bonded upward into the binding site will play a critical role in the catalytic reaction while the sulfur atom in its side chain is in precisely the proper spatial position, as shown on the right above, to form charge-complexes with the aromatic rings of incoming substrates as they move into cavity A.
The carbonyl of the peptide to be cleaved is held next to the oxygen of serine 195 at a 90-degree bend in the chain. As illustrated above, the nitrogen atom of the phenylalanine peptide is hydrogen bonded to the histadine 57 ring at B ready to catalyze the hydrolysis.

As shown below, the carbonyl of the tyrosine peptide is bound in the site so closely to the oxygen atom of serine 195 that a tetrahedrally-bonded intermediate, TI, forms immediately to break the C-N bond and release the right-hand portion of the polypeptide chain as a free cationic amine (A). This leaves the serine oxygen bonded to the tyrosine carbonyl as the ester (E).

Peptide 192, which is shown below the ester in the Hydration Figure above, holds the carbonyl oxygen of the ester in precisely the proper position with water molecule, w, above it to form a second tetrahedral intermediate, TI, break the ester bond and release the tyrosine end of the polypeptide chain as the anionic acid.
**Alpha-Chymotrypsin - Summary**

In this Front View of the ester hydrolysis, it is clear that water molecule w, which is hydrogen bonded to the nitrogen of histadine 57, is close enough to the carbonyl carbon of the ester to bond with it, form the acid and release the left side of the chain.

What is not clear in the illustration is that, in forming the initial reaction complex, stress is imposed on the bonds which is relaxed in forming the ester and conversion to the acid. However, it must be remembered that a driving force in the formation of the reaction complex is the release of ordered water and the increase in hydration entropy.

Based on the current concept that the distribution of water around the molecules in living cells is totally random, it is impossible to understand how they can function with such extreme levels of order and efficiency. Furthermore, it is impossible to understand how they could have evolved spontaneously from random distributions of small molecules which were present on the early earth to yield the orderly self-replicating phenomenon we know as life without some sort of guiding order. However, natural molecular evolution did not occur in the randomness of air, it occurred in an environment which possesses the properties of both randomness and order - randomness to provide for motion and change, order to provide for efficiency and reproducability.

Thanks to Professor Blow and his coworkers for the detailed studies they performed on alpha-chymotrypsin.

**References**


