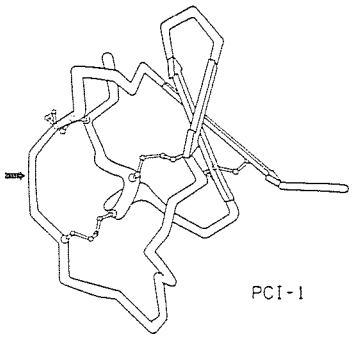
02.4-8 PROTEIN-PROTEIN RECOGNITION: THE INTERFACES OF PROTEIN INHIBITORS AND THE SERINE PROTEINASES. By Michael N.G. James, Anita R. Sielecki, Randy J. Read, Masao Fujinaga, Catherine A. McPhalen and Harry Greenblatt, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

There are 10 known families of protein inhibitors of the serine proteinases. Many of the inhibitors are relatively small proteins (50-60 amino acids). Others are small domains of larger multidomain proteins. We have determined the crystal structures of four of these inhibitors complexed to their cognate enzymes: the third domain of the turkey ovomucoid (OMTKY3) complexed to SGPB (1) and to α-chymotrypsin (2), the chymotrypsin inhibitor CI-2 from barley seeds complexed to subtilisin NOVO (3), eglin-c from leeches complexed to subtilisin Carlsberg (4) and the potato chymotrypsin inhibitor 1 (PCI-1) complexed to SGPB (see Figure). Eglin-c and CI-2 are from the same inhibitor family and have similar three dimensional structures. They are distinguished by the absence of disulfide bridges. The inhibitors of the serine proteinases form one of the best examples of convergent evolutionary pressures giving rise to a common function but with distinctly different 3-dimensional tertiary structures. All of these inhibitors are characterized by extremely tight binding ($K_d < 10^{-9}$ M) to their cognate enzyme. The common structural feature among the inhibitors is a segment of polypeptide chain spanning 6-7 residues which binds to the active site region of the enzyme. This segment has a very similar tertiary structure among all the inhibitors and resembles the conformation of an enzyme-substrate Michaelis complex. There is one peptide bond, between residues P_1 and P_1', which can be cleaved but only at a very slow rate, $k_{cat} \sim 10^{-6}$ sec^{-1}. There are several common hydrogen-bonding and electrostatic features that the different inhibitor families display in this region which contribute to these molecules acting as inhibitors and not good substrates. Water molecules play important roles at the interface between the inhibitors and the enzymes and most likely contribute to the recognition.



Potato chymotrypsin inhibitor. The 4 disulfide bridges in the molecule are indicated. The scissile bond between Leu-Asn is indicated by a solid arrow.

References

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(3) McPhalen et al., Proc. Natl. Acad. Sci. 82, 7242 1985.
(4) McPhalen et al., FEBS Lett. 188, 55, 1985.

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02.5-1 STRUCTURAL STUDIES OF DNA FRAGMENTS WITH BASE PAIR MISMATCHES. By O. Kennard and W. N. Hunter, University Chemical Laboratory, Lensfield Road, Cambridge, U.K.

The concept of base pair mismatches as a mechanism for introducing variations into genetic coding is embedded in the concept of DNA as the carrier of genetic information. The fidelity of transmitting the genetic code rests on specific pairing between components of the double helix: the Watson-Crick hydrogen pairs of normal DNA. Different combinations of the bases, however, if not detected and excised can result in modification of the genetic code and may eventually lead to mutational changes. The paper presents the results of X-ray diffraction studies of DNA fragments containing different types of base pair mismatches. It discusses the molecular structure of the ismatched base pairs, the way they influence the conformation of the double helix and possible mechanisms whereby such errors are recognised and corrected.

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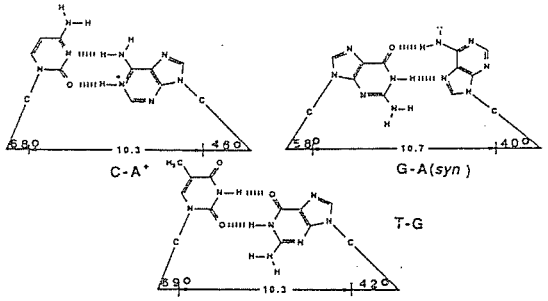

02.5-2 POSSIBLE ROLE OF PROTONATION - DEPROTONATION IN NUCLEIC ACID BREATHING: A HALF-SANDWICH OF WATER MOLECULE IN A TRINUCLEOTIDE COMPLEX OF ($A^+pAp A^+$).($Ap A^+p A^+$). R. Parthasarathy, T. Srikrishnan and S. M. Fridey, Center for Crystallographic Research and Department of Biophysics, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY, 14263.

Crystallization of A3'p5'A3'p5'A (ApApA) at acidic pH yields two crystalline forms I and II in space groups $P4_12_12$ and $P4_1$ ($Z=8$ in both forms), respectively. An earlier study (D. Suck, P.C. Manor & W. Saenger, Acta Cryst. B32, 1727, 1976) of I and a preliminary study of II indicated that the molecule exists in the crystal as $Ap A^+p A^+$. Our detailed examination of II shows that this crystal form actually contains the dimeric complex ($A1^+p A2p A3^+$).($A4p A5^+p A6^+$), one of the six possible combinations of protonated dimers. The assignments of these charges are based on a combination of criteria involving difference electron density maps, ring angles at N(1) and the stereochemistry of hydrogen bonding. The two molecules of ApApA have significant conformational differences which are directly coupled to the differences in protonation of the two molecules in the complex. The microenvironment around the adenine bases decides which two of the three bases in each molecule will be protonated. The dimer is stabilized by two self pairs between adenines A2:A6 and A3:A5 and stacking of A1, A2, A5 and A4 on top of one another. The A2:A6 self pair is between a charged and uncharged adenine and the A3:A5 self pair is between two charged