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The three-dimensional structure of subtilisin novo

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SUMMARY

The three dimensional structure of the enzyme subtilisin Novo has been determined at a resolution of 2.8 Å by means of X-ray diffraction of single crystals.

Subtilisin Novo

Subtilisin Novo is a proteolytic enzyme excreted by *Bac. Subtilis.* Its molecular weight is 26.500 and it consists of 275 amino acids. The amino acid sequence has been determined by other investigators. In the introduction the physico-chemical and enzymatic properties are discussed and compared with those of other enzymes.

Structure determination

The crystals needed for the X-ray investigation were grown from a (1 : 1) mixture of acetone and 0.05 M glycine-NaOH buffer, pH 9.1. Prior to crystallization the enzyme was inhibited with di-isopropylfluorophosphate (DFP) in order to prevent autolysis. The structure was determined using the "standard" isomorphous replacement technique with inclusion of the anomalous dispersion differences. The three heavy atom derivatives contained resp. TlF, K_2PtCl_4 and Na_3IrCl_6. The intensities of the reflections were measured with a triple channel Hilger Watts linear diffractometer. Two of its counters were used and the rotation axis was not a reciprocal lattice axis. An electron density map with a resolution of 2.8 Å was calculated. A model of the protein molecule was built using an optical model building device, which was also used to measure the atomic coordinates.

Results of the structure determination

The subtilisin Novo molecule is roughly a sphere with a diameter of 46 Å. The molecule is folded in a helical way at seven places; the helices comprise 86 residues. Six strands run parallel to each other and form a twisted pleated sheet composed of 33 amino acids. Ten amino acids are part of an anti-parallel β-structure. The remaining 146 amino acids are folded irregularly. Most of the hydrophobic side chains are buried in the interior of the molecule; most of the hydrophylic residues are found at the surface. The active centre is not a cleft like in some other enzymes but is a shallow depression at the surface of the molecule. It is interesting that subtilisin Novo binds ions at two sites at the surface. At one of these sites monovalent cations are bound, a phenomenon not earlier observed in the proteins investigated by X-rays.

Comparison of the structures of subtilisin Novo and BPN′

During the course of this study it appeared that another group at La Jolla, California, U.S.A. had proceeded quite far with the structure determination of the protein subtilisin BPN′. This protein has the same amino acid sequence as subtilisin Novo, but the subtilisin BPN′ crystals were grown from a medium quite different from ours (BPN′: 2.1 M ammonium sulphate pH 5.9, Novo: acetone-water (1 : 1) pH 9.1). This was an unique possibility to compare in detail the structure of the same protein molecule in two quite different media. This comparison has been performed at the MRC-laboratory in Cambridge, England. It appeared that the two structures were strikingly similar in spite of the difference in surrounding media. (It should be mentioned that some proteins are readily denatured in mixtures of organic solvents and water). No large rotations of aromatic side groups have been observed; surprisingly even, the high motility of an external loop did not appear to be affected by the change in surrounding medium.

Small structural differences of 1-3 Å are found at the surface; these are mostly rotations of side groups. Many of the differently orientated side groups are involved in intermolecular contacts which are different in both crystal forms. At the active site two side groups

have taken different positions in the two structures, this due to the presence of the inhibiting group in the Novo-molecules. The binding of ions in subtilisin BPN′ is much less apparent than in subtilisin Novo. This can be explained by the difference in the solvents surrounding the subtilisin Novo and subtilisin BPN′ molecules. In the neighbourhood of the ion sites some distinct differences in the positions of side groups are observed.