Een röntgenografisch onderzoek van excelsine, edestine en tabakszaadglobuline

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SUMMARY

This thesis describes an attempt to obtain information about the structure of the seed proteins excelsin, edestin and tobacco seed globulin by means of X-ray diffraction and electron microscopy.

In chapter 1 a survey is given of the methods of X-ray analysis which can be used for the study of protein structures. The application of electron microscopy to the investigation of protein molecules is illustrated with some examples. The approach of Pauling and Corey to the protein structure problem is also discussed.

Excelsin, edestin and tobacco seed globulin were extracted from the brazil nut, from hemp seed and from tobacco seed respectively. The purity of the preparations was determined by electrophoresis and by either the method of solubility or an antigen-antibody reaction. None of the three preparations was found to be entirely homogeneous. The presence of impurities in the excelsin preparation also followed from its copper content.

Single molecules of the three proteins could be observed with the electron microscope. These molecules were of spherical shape with a diameter of 80-90 Å. Since the resolution obtainable with the instrument was only approximately 50 Å some deviation from the spherical shape is possible. The surfaces of small dry crystals of the proteins showed in the electron microscope a disordered arrangement of the molecules. Therefore the packing of the protein molecules in their crystal lattices could not be observed in this way. The disorder may be due to an amorphous deposit of protein molecules on the crystal faces or to a collapse of the crystal structure as a result of the loss of water.

X-ray diffraction of protein crystals requires a narrow beam to prevent overlap of neighbouring reflexions. Simple geometrical considerations showed that the best way to realize this beam is to use the whole tube focus with a long collimator (Huxley, 1953). It also appeared that rotating anode X-ray tubes are better suited for X-ray work on proteins than micro-focus tubes.

A flat film oscillation camera and an integrating precession instrument were designed. Some details of their construction are described. The precession instrument was used for accurate intensity measurements.

Edestin crystals were found to have a face-centred cubic unit cell (the true primitive cell is a rhombohedron with α_{rh} =

60°). Excelsin and tobacco seed globulin crystals are not exactly cubic since one of the body diagonals of the "cubic" unit cell is slightly shorter than the other three. The crystal lattice of these two proteins is therefore rhombohedral with α_{rh} a little larger than 60°. The cell dimensions and densities of the crystals were measured as functions of the humidity. From the values obtained for the dry state the molecular weight of the proteins could be calculated because their magnitudes were already approximately known. The results are:

	molecular weight	standard deviation
excelsin	303,000	10,000
tobacco seed globulin	350,000	20,000
edestin	360,000	20,000

From the cell dimensions and densities in the wet states the water content of the crystals could be deduced. For excelsin crystals this was done for eight different states of humidity. The results agreed well with the values for the water content measured in a direct way.

From the fact that the rhombohedral unit cells contain one protein molecule, it followed that these molecules are arranged in a cubic closest-packing. Their size could be estimated from the dimensions of the unit cells. For the dry state the following data were obtained.

excelsin	ellipsoidal molecules with axes 83, 83 and 80 Å
tobacco seed globulin	ellipsoidal molecules with axes 88, 88 and 82 Å
edestin	spherical molecules with a diameter of 86 Å.

The crystals of excelsin gave better X-ray pictures than those of the other two proteins. Therefore attention was especially directed on this protein. As is also the case with other crystalline proteins, the dried crystals showed a less ordered structure than the wet ones. It was remarkable to find that excelsin crystals in equilibrium with a medium of a relative humidity of 97-100% showed a badly ordered structure too. High quality X-ray photographs of excelsin crystals could be obtained only at a relative humidity between 91 and 97%. These photographs showed reflexions as far as $d = 2{,}8$ Å.

From the aforementioned behaviour of excelsin crystals and from their specific volume as a function of the water content the following conclusions were drawn. At 91% relative humidity the protein molecules are in contact with each other and the water fills the interstices. From 91 to 97% relative humidity more water is taken up by the crystal; the molecules

are pushed a little apart but still keep well defined positions relative to each other. Above 97% however, the high water content prevents a good linkage between the protein molecules. Below 91% the crystal structure collapses by deformation or shrinkage of the protein molecules.

Since the space group of the excelsin crystals is R32 and the unit cell contains one protein molecule, these molecules have to be composed of six identical parts. This requires for each reactive group a number of six or a multiple of six to be present per excelsin molecule. Six -SH groups and six $-NH_2$ groups were found indeed. The number of $-NH_2$ groups was determined with 4-iodophenylisothiocyanate. The -SH groups reacted with silver and mercuric ions and with the organic mercurial 2,5-di-(acetatomercurymethyl)-dioxane (DMD). From these reactions it appeared that the six -SH groups were present in the excelsin molecule as three pairs of two neighbouring groups. The symmetry of the molecule requires these pairs to be located on the three two-fold axes.

A more detailed structure determination of excelsin failed. The Patterson function for the centro-symmetrical projection showed several maxima. A number of these maxima have a mutual distance of 17 Å. Therefore this seems to be an important distance in the centro-symmetrical projection of the excelsin structure. No other features of the protein structure could be deduced from this Patterson projection. "Trial and error" with various models did not yield a good agreement between calculated and observed intensities.

It was also attempted to apply a direct method of structure determination. Perutz and his collaborators, in their study of horse haemoglobin, used two such methods, namely the transform method and the isomorphous replacement technique. The first one is not applicable to excelsin because the unit cell dimensions do not change sufficiently on drying the crystals. The isomorphous replacement method was tried with the protein-mercury compounds: excelsin. 3Hg and excelsin. 6DMD. The crystals of these compounds showed the same cell dimensions as the crystals of excelsin itself, whereas those of the compound excelsin. 3DMD were slightly larger. To locate the positions of the heavy atoms difference Patterson functions were calculated. However, the results were not in accordance with the conclusion from the reactions of the -SH groups, that these groups and the mercury atoms must be located on or near the two-fold axes. Evidently the intensities had not only been changed by the contribution of the heavy atoms, but also by other unknown factors, perhaps a distortion of the protein structure as a result of the introduction of the heavy atoms. Moreover the mercury atoms contributed less to the structure factors of the reflexions than was to be expected from statistical arguments.

The high-angle diffraction pattern of excelsin shows relatively strong reflexions corresponding with d = 4.7 and 3.3 Å. This indicates some similarity in structure with the fibrous proteins of the β-type for which Pauling and Corey (1951) proposed the "pleated sheet" model. If this model really represents the protein structure, the direction of the polypeptide chains in the excelsin molecules is perpendicular to the three-fold axis.