AN X-RAY AND CRYSTALLOGRAPHIC STUDY OF RIBONUCLEASE

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A new crystalline protein of low molecular weight, ribonuclease, has been isolated and described by Kunitz (1, 2). A preliminary study of this protein has now been made with x-rays and with the polarizing microscope.

The material studied was crystallized from an ethanol-water solution. The crystals were dried in air without appreciable deterioration and the measurements here described were made with these air-dried crystals. They are long, thin needles, orthorhombic, elongated along the “c” axis. The prism faces are (110) and (1i0) and include an angle of 70°. The other two axes bisect the angles of the cross section, “a” bisecting the obtuse angle and “b” the acute. The extinction as observed in the polarizing microscope is, of course, straight; a is along a, b along b, and c along c. The crystal is positive and the optic axial angle is about 65° measured in air and 74° in glycerine.

X-ray oscillation films were taken about all three crystallographic axes. These give the following values for the unit cell; a = 36.6 Å, b = 40.5 Å, and c = 52.3 Å. The space group appears to be P2₁2₁2₁, 4 molecules per unit cell, each molecule without symmetry in a general position. The density of the air-dried crystals was measured to be 1.341 ± 0.002, by floating them in a mixture of methylene chloride and carbon tetrachloride. The molecular weight has been computed from these data assuming 4 molecules per unit cell. The cell volume is 77,300 Å³ and this gives a molecular weight of 15,700 ± 300. This value is an upper limit as no correction has been made for solvent of crystallization.¹

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¹ Dr. A. Rothen (3) has found the specific volume of ribonuclease to be 0.709. The density of the air-dried crystals corresponds to a value of 0.746. He suggests that this difference may be due mainly to hydration and a computation based on this difference gives a value for the hydration of 12.7 per cent. An estimate of the molecular weight of the anhydrous protein can then be obtained by reducing the x-ray value of 15,700 by 12.7 per cent. This gives 13,700 which may be compared with the molecular weights reported from sedimentation and diffusion, 13,000, and from osmotic pressure measure-
These air-dried crystals are unusually perfect and give sharp diffraction spots down to 2 Å, whereas no other dried protein crystal has thus far given reflections below 5 Å (chymotrypsin). This, in conjunction with the low molecular weight, suggests that a study of x-ray intensities, similar to that of Crowfoot on insulin (4, 5), may give useful information about the internal structure of the ribonuclease molecule. A preliminary investigation of this character is now in progress.

REFERENCES


Computations of this kind depend on comparatively small differences of specific volume and can be accepted only with reserve. They are certainly less satisfactory than direct determinations of the hydration of the crystals, but lacking such determinations do serve to give an estimate of the hydration.