Preliminary Crystallographic Data for a New Crystalline Form of Abrin*

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SUMMARY

A new crystalline form of abrin has been obtained from the most toxic constituent of the seed extract of Abrus precatorius. An x-ray diffraction study shows that the toxin crystallizes in a monoclinic unit cell of probable symmetry P21 and parameters a = 113 A, b = 72 A, c = 71 A, and β = 103°. The asymmetric unit contains 2 protein molecules of molecular weight 63,800 and has a solvent content of approximately 40% by volume.

Since the first reports that abrin, present in the seeds of Abrus precatorius, affords therapeutic protection against various tumors in rats and mice (1, 2), this toxic plant protein has been studied intensively in several laboratories. It has been shown that abrin prevents peptide chain elongation (3), and thus inhibits protein synthesis in vitro (4) as well as in vivo (5). Although the mechanism of this toxic effect is not well understood, it has been attributed by Lin et al. (6) to the degradation of polyribosomes of liver or tumor cells. Olsnes and Pihl (4, 7) have studied the properties, including inhibition of protein synthesis and binding to specific sites on the cell surface, of their abrin preparation and of subunits which they prepared from it.2

Recently we have developed a procedure by which two major toxic proteins have been isolated from the seeds of A. precatorius and purified to homogeneity by chromatography on DEAE Sephadex A-50 and CM- and DEAE-cellulose (13). Although the molecular weights of these two abrins are similar (60,100 for one species and 63,800 for the other, as determined by sedimentation equilibrium), they behave quite differently in several aspects (13). Our results strongly suggest that the abrin preparations previously described by Lin et al. (14) and by Olsnes and Pihl (7) are not identical. In fact, one of our abrins (hereafter designated abrin A) possesses properties similar to those of the preparation of Lin et al. (14), and the other of molecular weight 63,800 (hereafter designated abrin C) closely resembles that of Olsnes and Pihl (7).

Abrin A, the less toxic of the two, and the more positively charged fraction obtained from the DEAE-cellulose chromatography, has been crystallized in the orthorhombic space group P212121 with unit-cell parameters a = 75, b = 270, and c = 70 A, as communicated earlier (15). We now report the crystallographic data of the more toxic abrin C and preliminary x-ray data for these crystals.

MATERIALS AND METHODS

The abrin C preparation used was obtained from seeds of A. precatorius and purified by successive chromatography on DEAE-Sephadex A-50 and CM- and DEAE-cellulose (13). Crystallization was by the free interface diffusion technique described by Salemme (16). Crystals were grown at 37° in Pyrex tubes (5 X 30 mm) by layering 50 μl of protein solution (22 mg per ml) over 100 μl of unbuffered 70% saturated ammonium sulfate solution.

The x-ray diffraction patterns were recorded at room temperature (24°) with nickel-filtered CuKα radiation from an Elliott rotating-anode generator operated at 40 kv and 40 ma. A Nonius precession camera with a crystal-to-film distance of 75 mm and a 0.25-mm collimator was used.

RESULTS AND DISCUSSION

Unlike abrin A, which gives rise to crystals up to 1 mm long within 2 days under analogous treatments (15), crystals of abrin C required up to 2 months to grow to a maximum length of 0.5 mm. Although most of the crystals were poorly shaped, good rod-shaped specimens, elongated along the c axis, could be selected for x-ray diffraction.

The x-ray diffraction patterns showed the crystals to be monoclinic with unit-cell parameters a = 113 A, b = 72 A, c = 71 A (± 0.5%), and β = 103° (±1°). The volume of the unit cell is 563,000 A³. The only observed systematic absences were 00l for k odd, indicating the probable space group to be P21. As shown in Fig. 1, diffraction patterns extended to 3-A spacings, indicating that a high resolution study could be...
made. (The larger abrin A crystals diffracted only to 6-A spacings.) However, the crystals seemed somewhat sensitive to x-radiation; after 30 hours of exposure considerable weakening of the diffraction pattern was observed.

The number of protein molecules per unit cell is \(2n\), where \(n\), the number of molecules per asymmetric unit, can be estimated by comparing values of \(V_M\) (defined as the ratio of crystal volume to protein weight), based on various assumed values of \(n\), with the normal range of values (1.68 to 3.53 \(\text{A}^3\) per dalton) compiled by Matthews (17) for crystals of globular proteins. For \(n = 1\) and 3, the \(V_M\) values are 4.41 and 1.47 \(\text{A}^3\) per dalton, respectively, which lie outside the normal range. For \(n = 2\), \(V_M = 2.21 \text{A}^3\) per dalton, which is close to the most commonly observed value. We conclude that the abrin C crystals contain 2 molecules per asymmetric unit, as do the orthorhombic crystals of abrin A. The partial specific volume of abrin C is 0.731 \(\text{cm}^3\) per g, as calculated from the amino acid composition.

With the assumption that \(n = 2\), therefore, the solvent content of the crystal is 45% by volume, which is not very different from 43%, the most frequent value (17).

Recently, another crystal form of abrin has been reported by McPherson and Rich (18). Their abrin preparation (obtained by a procedure modified from that of Tomita et al. (19)) is similar to our abrin C and to the preparation of Olsnes and Pihl (7) in its behavior in Sepharose 4B chromatography and in its electrophoresis pattern on sodium dodecyl sulfate polyacrylamide gel. The molecular weight as measured by gel filtration was approximately 280,000, corresponding to a tetrameric species of a monomer with molecular weight 65,000. The crystals were orthorhombic with space group \(P2_12_12_1\) and unitcell parameters \(a = 138, b = 142,\) and \(c = 178 \text{A}\). The authors concluded that the asymmetric unit contained one tetramer.

The asymmetric unit of abrin C crystals contains only 2 (monomer) molecules, rather than a tetramer (as above). The resolution of the data for abrin C crystals was found to be much superior to that obtained with crystals of abrin A (see above). Therefore, of all three crystal forms of “abrin” reported to date, that described here appears to be the most favorable for x-ray structural investigation.

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