A 10 μ cubical protein crystal and a prominent ovoid starch grain are seen resting on the wall of the hollow interior of a cell from the subphellogen layer of a potato tuber. The photograph was obtained by scanning electron microscopy (x1,750) of a tissue slice without the benefit of heavy metal coating. Evidently, the cell electrolytes provide sufficient electrical conductivity to make such studies possible, but the amount of information obtained by this technique is rather limited. The high vacuum utilized causes complete dehydration of the cell with consequent collapse of internal structures. The membrane-like film surrounding the crystal is probably an artifact due to coalescing cytoplasmic protein.

These microscopic crystals have been known since 1859 to occur in potato tubers and have been given sporadic attention as the perfectly shaped cubes caught the eyes of investigators. Today we know, thanks to improved techniques in electron microscopy, that crystalline protein "inclusion bodies" occur throughout the plant kingdom and are found in the cytoplasm, in vacuoles, nuclei, mitochondria, chloroplasts and plastids. Still, the protein crystals in the potato are unique due to their large size and perfect shape. The median size appears to be 10 μ in a mature tuber but occasionally crystals as large as 25 μ can be found.

The function of the protein cubes in the cell is still an enigma. German and Russian workers observed in the 1940-50's that severe virus infections were accompanied by an increased frequency of crystals and suggested that they might represent crystallized virus particles. But crystals were also found, sometimes abundantly, in presumably virus-free clones. In the days of less sophistication in protein chemistry, when the potato was supposed to contain the simple proteins tuberin and tuberinin, the crystals were believed to represent a storage form of tuberin. The gradual disappearance of the crystals during sprouting of the tubers was used in support of this interpretation since it suggested that the proteins were broken down to mobilize amino acids needed to maintain new growth. However, the staining properties of the crystals indicated that acidic components, probably nucleic acids, were present, and suggested that the nature and function were more complex than previously thought. Various attempts by earlier workers to isolate crystals in sufficient amounts for biochemical studies of their composition were unsuccessful.

We have found several ways of isolating the crystals. Using differential sedimentation methods the crystals may be concentrated to a considerable degree (a) and when preferentially extracted with CaCl2 and dialyzed, the protein is recovered as recrystallized material in good yield (b). The crystals consist predominantly of protein with an amino acid composition mainly characterized by a relatively large amount of lysine (14%). The electrophoretic pattern on acrylamide gels indicate 2 main components, but these are broken down to a multicomponent system in the presence of urea. The presence of a relatively high-molecular RNA species (5-10%) and heavy metals (Fe) is indicated.

The etiology of the crystals is still obscure. They are found in the cytoplasm, frequently in the vicinity of the nucleus (c,d) and in the formative stages (e) a definite association is suggested. The staining behavior with acetocarmine (c, d, e) supports a close relationship with the nucleolus. It is tempting to speculate that the crystals are associated with dormancy and temporal control of cellular activity. The RNA component could represent stabilized messenger RNA needed for the burst of metabolic activity during sprouting.

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