LETTERS TO THE EDITORS

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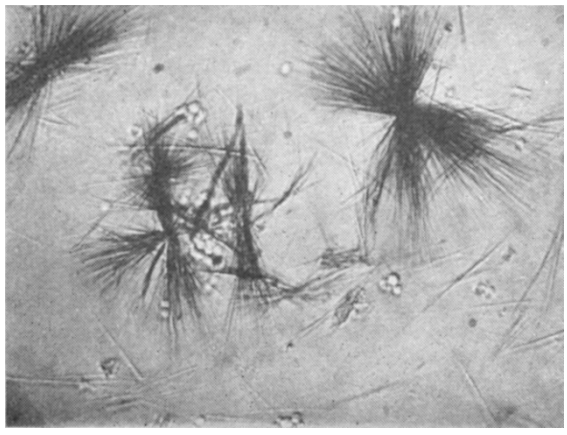
Preparation of Crystals Containing Protease from *Aspergillus oryzæ*

A STRAIN of the mould *Aspergillus oryzæ* (selected from an original culture of *Aspergillus flavus-oryzæ* 492–2795, kindly supplied by Dr. Charles Thom), grown for ten days at 21° C. on liquid medium containing sucrose, tartrate, inorganic salts and ammonium salts as the sole source of nitrogen, has been found to produce a highly active protease system in the culture medium.

Partial purification of this system has been effected in the following way: 20 litres culture filtrate was cooled to 2° C. and precipitated with 40 litres ethanol at − 25° C. After settling for 1 hr. in a chamber at − 25° C., the precipitate was recovered, suspended in 500 ml. water, dialysed with agitation for 1 hr. against running tap water, centrifuged to remove insoluble salts, and dialysed for a further 2 hr. Acetate buffer (200 ml., *p*H 4·0, 1·0 *M*) was added to the solution, which was then diluted to 2 l., cooled to 2° C., and fractionated at this temperature by adding ethanol until the concentration reached approximately 45 per cent by volume for the first fraction, and 60 per cent for the second fraction. The first ethanol precipitate contained most of the mould catalase, sucrase, and some amylase, and represented about one-quarter of the total protein. The second contained the mould proteases and most of the remaining protein. The protease precipitate was dissolved in a minimum quantity of water, treated with sufficient 4 *M* ammonium sulphate to produce cloudiness, filtered, and held at 2° C. The concentration of sulphate required to produce cloudiness was usually 2·7 *M*. Needle-shaped crystals, mostly in the form of rosettes, appeared within 24 hr. and continued to form during several days. These enzyme-rich crystals were recovered by centrifuging repeatedly and were dried *in vacuo* to yield up to 10 gm. dry material containing 60 per cent ammonium sulphate. The crystals dissolve slowly in water and may be recrystallized in the form of sheaves of extremely long slender needles by preparing a saturated aqueous solution at 30° C. and holding it for several days at 2° C.

When the enzyme is precipitated under unfavourable conditions, submicroscopic particles are formed, and these show double refraction of flow. The same property is displayed when the enzyme crystals are suspended in water, due to their dissociation into minute asymmetric particles before dissolving completely.



Recrystallized material rich in protease from *A. oryzæ*. × 300

Enzyme	Relative proteolytic activity per unit of protein nitrogen		
	Gelatin viscosity reduction method[1]	Digestion of low molecular-weight gelatin[2]	Hæmoglobin digestion method[3]
Crystalline mould protease from *A. oryzæ*	100	100	100
Crystalline trypsin (Armour)	29	100	1
Crystalline chymotrypsin (Armour)	9	1	3
Crystalline pepsin (Armour)	—	3	32
Papain (Parke Davis)	16	33	73

Comparison of once-crystallized mould protease with several other well-known proteases shows that it ranks with trypsin as the most active enzyme with respect to extensive digestion of gelatin; it is slightly more active than papain, the best of the other preparations with respect to the digestion of hæmoglobin; and is considerably more active than any of the other enzymes in reducing the viscosity of gelatin.

Solutions of the crystals probably contain at least two proteolytic enzymes, one reducing the viscosity of gelatin, the other acting on the lower molecular-weight components of gelatin. Esterase activity is also associated with the crystals, though it is not yet known whether this is due to the presence of a true esterase or to unspecific action of one of the proteases.

A preliminary examination of the crystals has been made by electrophoresis and in the ultracentrifuge at the Harvard Medical School, Department of Physical Chemistry, through the courtesy of Prof. E. J. Cohn and colleagues. By the former method, using phosphate buffer at an ionic strength of 0·1 and at *p*H 7·0, an apparently homogeneous component constituting about 83 per cent of the total protein was separated. The ultracentrifuge indicated that 90 per cent of the total protein consisted of a single component. Further physical measurements at different *p*H values and ionic strengths, and a more detailed examination of the properties of the enzymic constituents, will be necessary to determine whether the crystals contain several enzymes, and whether these components can be identified by physical methods.

So far as can be ascertained, the foregoing is the first account of the preparation of crystals containing mould enzymes.

Our thanks are due to Miss M. E. Maxwell, of this Section, who selected the strain of mould, developed the culture medium used, and supplied the culture filtrate used in these investigations. The work, which will be reported in greater detail elsewhere, was carried out as part of the research programme of the Division of Industrial Chemistry of the Commonwealth Scientific and Industrial Research Organisation.

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[1] Lennox, F. G., and Ellis, W. J., *Biochem. J.*, **39**, 465 (1945).
[2] Crewther, W. G. (unpublished).
[3] Anson, M. L., *J. Gen. Physiol.*, 22, 79 (1938).