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Porosity of the cell wall as related to the Secretion of proteolytic enzymes in N. crassa

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Abstract

Secretion of proteolytic enzyme

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Hyphae of N. crassa secrete two acid proteases into culture media which contain protein as the only nitrogen source (Matile 1965 Z. Zellforwh. 65: 884). These enzymes ore localized intracellularly in small sacs, protease particles, which upon the supply of a proteinaceous nitrogen source are transferred to the outside of the plasmalemma (Matile et al. 1965 Z. Zellforsch. 68: 205). This process leads to the occurrence of free extracellular proteases which subsequently cross the cell wall and appear in the culture medium. Thus, the sire of the secreted enzyme molecules must be of the some order of magnitude as the dimensions of the pores of the cell wall. In contrast, another secretion product, aminopeptidase, has never been observed outside the cells. This enzyme is localized somewhere between the plasmalemma and the cell wall (Motile 1964 Naturwissenschaften 51: 489). Travithick and Metzenberg (1966 J. Bacteriol. 92: 1010) have described a process of molecular sieving in Neurospora cell walls with respect to secreted invertase; this finding suggests the existence of a similar mechanism resulting in the retention of secreted ominopeptidose molecules. In order to prove the validity of the above hypothesis, the approximate molecular size of both secreted proteases and aminopeptidase has been determined, using the gel filtration method of Whiteaker (1963 Anal. Chem. 35: 1950).

For isolation of cell walls, 50 g of wet mycelium (strain chol-1 (34486), FGSC #485) were mixed with on equal volume of gloss beads (0.45 mm) and a small volume of 1% NaCl. The cells were broken by the action of a vibration mixer (1 hour). The resulting suspension, containing less than 1% of intact cells, was diluted with 1% NaCl and the glass beads were allowed to settle. Centrifugation for 15 min at 100 x g yielded a white pellet which contained the cell walls. This pellet was subjected to washings in 1% NaCl until the supernatant was perfectly transparent. After 20 washings, only clean fragments of cell walls could be observed in the phase contrast microscope.

Gel filtmtion with Sephadex-150 was carried out in a 15 x 300 mm column. Columns of isolated cell walls ore completely impassable for the solvent: in order to obtain a homogeneous working column (11 x 65 mm), a suspension consisting of isolated cell walls and gloss beads (0. 18 mm) was added in small portions under continuous gentle stirring. IN NaCl in 0.1M Tris-HCl buffer pH 7.2 was used for the elution of both Sephodex and cell wall columns. Blue dextrane (Pharmacia Uppsala) was used for the determination of void volumes. Bovine serum albumin (Colbiochem. A-grade), cytochrome c and tyrosine (Fluka) served as reference substances. The proteolytic enzymes subjected to gel filtration were contained in a culture filtrate (acid proteases, pH optima 4.2 and 6.4) or in a high speed supernatant of a cell-free extract (aminopeptidase).

Both acid proteases were eluted froma Scphadsx-150 column at a Ve/Vo value of 2.9, the aminopeptidase at Ve/Vo = 2.0. The corresponding molecular weights ore co. 22,000 and 85,000. Most probably these values don't represent true molecular weighk since it has not been established that the secreted proteolytic enzymes ore pure proteins. It moy be assumed that the ominopeptidose is a glycoprotein like invertase (Metzenberg 1963 Arch. Biochem. Biophys. 100: 503) and other extracellular enzymes of Neurospora and yeast (Lampen 1965 Symp Soc. Gen. Micmbiol. 15: 115). However, the Ve/Vo values point to a considerably different size of protease and ominopeptidose molecules. This finding supports the hypothesis that the smaller molecules of acid proteases con, while the larger ones of ominopeptidose cannot, penetrate the pores of the Neurospora cell wall.

However, it appears from a comparison of the molecular sizes of invertase (which is partially released into the medium) and of aminopeptidase (which is not released) that the situation is more complex. Invertase molecules should be smaller than

aminopeptidase molecules if molecular sieving alone is responsible for their different behaviour with respect to the wall. Gel filtration with Sephadex, however, indicates that the invertase molecule (monomer) is larger than the aminopeptidase. Therefore, in addition to molecular sieving, binding between cell wall constituents and aminopeptidase molecules must be considered.

The elution pattern from a cell wall column of acid proteases clearly demonstrates that these enzymes are able to penetrate the pores of the cell wall. The bulk of enzyme activity occurs in a broad peak at Ve/Vo = ca. 3.5; a small fraction of activity is eluted together with the blue dextrane. Since from columns of Sephadex the same enzymes are eluted in a uniform peak, it must be concluded that for possible reasons of the geometry of the cell wall fragments a fraction of enzyme molecules never enters the pores during the movement through the column. The broad peak of the bulk of protease activity points to the fact that isolated cell walls don't allow an ideal distribution of the molecules between the inner (pores) and the outer volume. Therefore, only qualitative results can be obtained from molecular sieving experiments with isolated cell walls.

Invertase filtered through isolated cell walls appears in an almost uniform peak together with the excluded blue dextrane. The small fraction of enzyme activity which is eluted after the dextrane indicates a limited penetration of the cell walls which is possibly responsible for the appearance of the enzyme in the culture medium. Similarly, the elution pattern of aminopeptidase regularly shows a tail of activity which does not move with the front. The bulk of aminopeptidase seems to be excluded and is therefore eluted together with the blue dextrane.

Since the aminopeptidase is not secreted into the culture medium, its partial retention in a cell wall column must be due to reversible binding rather than to penetration of the cell wall. The sizes of pores in the Neurospora cell wall determined by Trevithick et al. (1966 J. Bacteriol. 92: 1016) would permit the passage of even larger molecules than those of aminopeptidase and isozymes of invertase. The fact that the latter enzymes are released into the medium according to the principles of molecular sieving, whereas the former, smaller molecule never occurs outside the cell, cannot be explained by molecular sieving alone.

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