DISK ELECTROPHORESIS OF THE AMINOPEPTIDASES OF *Aspergillus oryzae* AND *Asp. flavus*

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We have reported previously [1] that in concentrated enzyme preparations of *Aspergillus oryzae* and *Asp. flavus* aminopeptidases have been found isolated and purified. The subject of the present communication is an investigation of the nature of these aminopeptidases by means of electrophoresis in polyacrylamide gel (PAAG) and determinations of the molecular weights in PAAG in the presence of the anionic detergent sodium dodecyl sulfate.

The proteins were investigated in an alkaline gel (7.5%, pH 8.3) by Davis's method [2]. Parallel columns of gel were used to determine the localization on them of the aminopeptidase activity (with leucyl-β-naphthylamine as substrate). We used the method of Pearse and Nachlas [3], which was somewhat modified — the time of incubation was lengthened and the pH of the incubation mixture was raised to 7.8.

In the initial concentrated enzyme preparations from *Asp. oryzae* and *Asp. flavus* 10-11 electrophoretic fractions were detected, but activity appeared in only some of them. Intense coloration was found in zones with relative electrophoretic mobilities of 0.47, 0.64, and 0.86 for the first material and 0.30 and 0.90 for the second (Fig. 1a, b). The same mobilities are shown by some protein zones revealed with the aid of Amido Black 10 B. These

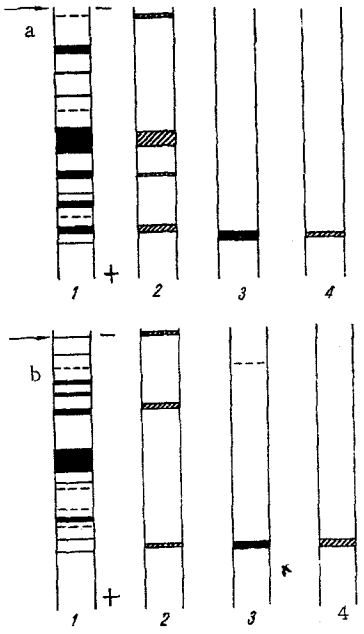

Fig. 1. Electrophoretograms (1, 2) and isoenzyme spectra (3, 4) of the aminopeptidases of *Asp. oryzae* (a) and *Asp. flavus* (b): 1) initial concentrated enzyme preparation; 2) aminopeptidase; 3, 4) localization of the activity with respect to L-leucine β-naphthylamide.

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are probably isoenzyme spectra of the aminopeptidase. The aminopeptidase preparation isolated from the materials mentioned were found to be homogeneous and each showed one protein band in the gel. Intense coloration was observed in zones with relative electrophoretic mobilities of 0.86 and 0.90 for the aminopeptidases of *Asp. oryzae* and *Asp. flavus*, respectively (see Fig. 1, a and b, columns 3 and 4). The molecular weight of the aminopeptidase from *Asp. oryzae* was determined in PAAG by the method of Weber and Osborn [4] in the modification of Fairbanks et al. [5] in the presence of the anionic detergent sodium dodecyl sulfate. The following proteins were used as markers: ribonuclease (13,500), egg protein lysozyme (17,500), chymotrypsinogen (25,700), pepsin (35,000), egg albumin (43,000), and bovine serum albumin (68,000). The determinations were performed in 10% gel in 0.01 M tris acetate buffer, pH 7.2. The mobilities (calculated by a standard formula [3] were plotted on a graph against known molecular weights expressed on a semilogarithmic scale. The molecular weight of the aminopeptidase was 60,000. It has been shown previously that the molecular weight of the enzyme from *Asp. flavus* determined by gel filtration on Sephadex G-2000 was 59,000 [6]. Thus, the molecular weights of the two aminopeptidases are practically identical.

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THE INTERACTION OF HYDROLYSIS LIGNIN WITH PHENYLENEDIAMINES

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Hydrolysis lignins of the pods of the seeds of the cotton plant (HL-I) and from the sawdust of coniferous trees (HL-II) are waste materials from hydrolysis factories. As compared with dioxane lignins [1, 2], obtained by a milder method, they contain smaller amounts of methoxy and hydroxy groups and larger amounts of oxygen, hydrogen, and carbonyl groups. This shows that the HLs are highly condensed.

To expand the range of practical utilization of lignin, we have obtained a number of nitrogen-containing derivatives (III-VI) from HL.

Products (III) and (IV) were formed by heating HL-I with o-phenylenediamine in a ratio of 2:1 (by weight) in dimethylformamide (DMF) at 145-148°C for 4-6 h. Substance (IV), unlike (III), was readily soluble in DMF. Under similar conditions the reaction of lignin with p-phenylenediamine gave a product (V). Ammoniated lignin (VI) was obtained by moistening HL with a 5% solution of ammonia at room temperature.

HL-I. $C_9H_{11.48}O_{3.88}(OCH_3)_{0.37}(OH)_{0.59}(O_{CO})_{0.56}$,

HL-II. $C_9H_{11.41}O_{3.95}(OCH_3)_{0.49}(OH)_{0.79}(O_{CO})_{0.38}$,

III. $C_9H_{10.47}O_{3.03}(OCH_3)_{0.22}(OH)_{0.68}(O_{CO})_{0.26}N_{0.36}$,

IV. $C_9H_{11.78}O_{1.57}(OCH_3)_{0.19}(OH)_{0.65}(O_{CO})_{0.19}N_{0.78}$,

V. $C_9H_{10.41}O_{3.34}(OCH_3)_{0.25}(OH)_{0.63}(O_{CO})_{0.37}N_{0.29}$,

VI. $C_9H_{9.61}O_{3.69}(OCH_3)_{0.21}(OH)_{0.61}(O_{CO})_{0.37}N_{0.23}$.

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