TABLE 1. Sorption and Desorption of the Proteolytic Enzymes of Amylorizin on a Number of Sorbents

| Sorbent | Ttl. sorption capacity (act. of moist sorbent, units/cm³ | | Protein, mg/cm³ | Total desorption, % of sorption | | |
|---|---|---|---|---|---|---|
| | aminopeptidase | proteolytic | | aminopeptidase | proteolytic | protein |
| 1. Leucyl-glycyl-glycine-support | 5,6 | 44,8 | 5,73 | 52,9 | 44,3 | 51,5 |
| 2. Leucine-support | 1,6 | 0,8 | 0,40 | 13,1 | 48,7 | 10,8 |
| 3. Leucine-support | 2,0 | — | 9,09 | 6,2 | — | 71,3 |
| 4. Leucyl-phenylenediamine-support | 0,4 | 0,7 | 0,23 | 33,0 | 83,0 | 60,6 |
| 5. Glutathione-ox.-support | 0,52 | 0,63 | 2,48 | 101,0 | 113,0 | 65,2 |
| 6. Glutathione-red.-support | 0,16 | 0,21 | 0,08 | 99,1 | 106,3 | 107,2 |
| 7. Glycyl-L-phenylalanine-support | 22,25 | 45,3 | 74,7 | 1,0 | 0,3 | 6,3 |

In evaluating the results on the sorption of the total aminopeptidase and proteolytic activities of Amylorizin one may consider as best those sorbents containing oxidized glutathione and leucylglycyl glycine as ligands. By changing the conditions of sorption of the protein on the above-mentioned sorbents and selecting the optimum conditions for desorption from the individual sorbents it is possible to achieve a specific separation of the proteinases and petpidases of Amylorizin. An important advantage of these sorbents is the possibility of their use for purifying proteolytic enzymes directly from Amylorizin extracts containing a large amount of cellulases, dextranases, and other enzymes, and also ballast proteins and pigments.

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# SOME PROPERTIES OF TWO LECTINS FROM THE SEEDS OF *Datura innoxia*


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The presence of hemagglutinating activity in extracts of the seeds of *Datura innoxia* was first detected by Boyd [1]. We have isolated and purified two forms of a lectin ($I_1$ and $I_2$) from the seeds of this plant [2] with molecular weights, evaluated by gel chromatography, of 150 and 300 kD, respectively. Lectins $I_1$ and $I_2$ are glycoproteins. $I_1$ contains 66% of carbohydrates and $I_2$ 54% (determined from total-nitrogen analysis [3]). The neutral sugars were determined by the GLC method after methanolysis and trimethylsilylation on a Chrom 5 chromatograph with a column (0.3 × 300 cm) containing 5% of SE-30 and with flame-ionization detection. In $I_1$, L-arabinose, L-fucose, D-xylose, D-mannose, D-galactose, and D-glucose were found in a ratio of 6:1:2:1:2:2, respectively. The carbohydrates of $I_2$ were L-arabinose, D-xylose, D-mannose, D-galactose, and D-glucose in a ratio of 1:2:1:1:3, respectively. The N-terminal amino acids of the lectins were blocked. As the result of methanolysis in 1 M $HCl/MeOH_{abs}$ at room temperature for eight hours and the TLC of the dansyl derivative, in both cases glutamic acid and glycine were identified. The amino acid compositions were determined on an LKB-4101 amino acid analyzer after hydrolysis in


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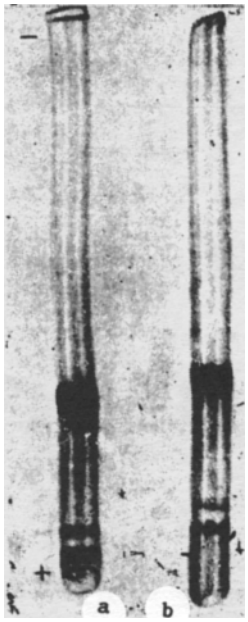


Fig. 1. Disk electrophoresis in 7.5% PAAG, pH 8.3, in the presence of 0.1% SDS, of reduced $I_1$ (a) and reduced $I_2$ (b).

5.7 N HCl at 110°C. The amino acid compositions of $I_1$ and $I_2$ as moles/mole of protein are given below:

| | $I_1$ | $I_2$ | | $I_1$ | $I_2$ |
|---|---|---|---|---|---|
| Asp | 14.7 (15) | 20.7 (21) | Ile | 1.9 (2) | 4.8 (5) |
| Thr | 12.1 (12) | 15.2 (15) | Leu | 3.3 (3) | 8.4 (8) |
| Ser | 25.3 (25) | 28.7 (29) | Tyr | 4.4 (4) | 5.9 (6) |
| Glu | 17.9 (18) | 25.2 (25) | Phe | 1.8 (2) | 4.3 (4) |
| Pro | 11.7 (12) | 32.3 (32) | His | 1.2 (1) | 2.4 (2) |
| Gly | 23.8 (24) | 29.1 (29) | Lys* | 3.4 (3) | 7.7 (8) |
| Ala | 8.1 (8) | 15.6 (16) | Orn† | + | + |
| 1/2Cys | 23.2 (23) | 23.3 (23) | Arg | 7.4 (7) | 10.9 (11) |
| Val | 3.0 (3) | 7.0 (7) | Hy P | 18.4 (18) | 64.6 (64) |
| Met | 1.3 (1) | 2.1 (2) | Trp ‡ | 1.3 (1) | 2.3 (2) |

*Determined without taking ornithine into account.
†Determined qualitatively from the reaction with vanillin [5].
‡Determined after hydrolysis in trifluoromethanesulfonic acid for 24 hours [6].

As can be seen, there is much in common in the amino acid compositions of $I_1$ and $I_2$: a low level of aromatic amino acids and a high level of HyPro and 1/2-Cys, Gly, Ser, Asp, and Glu. In spite of their similar natures, certain difficulties are observed in the ratios of the amino acid residues in $I_1$ and $I_2$. By the atomic-absorption method zinc was found in both lectins ($\approx$0.04%). $I_1$ and $I_2$ were subjected to isoelectric focusing in plates with 5% polyacrylamide gel containing 1% of ampholytes in a pH 3.5-10.5 gradient on a Multiphor instrument (Sweden).

The proteins were stained with Coomassie Bright Blue R-250. Both lectins gave clear narrow zones corresponding to isoelectric points of 3.9 for $I_1$ and 6.0 for $I_2$. Disk electrophoresis in 7.5% polyacrylamide gel, pH 8.3, in the presence of SDS gave different results for samples reduced with mercaptoethanol and unreduced samples. The unreduced lectins migrated as broad bands with approximately the same mobilities. After reduction at 100°C (in the water bath) for 3-5 min, three zones were observed in each case, corresponding to molecular weights of 27, 13, and 10 kD for $I_1$ and 28, 13.5, and 11 kD for $I_2$. The zones corresponding to the 27 kD protein band for $I_1$ and the 28 kD protein band for $I_2$ gave positive colorations for carbohydrates (by Racusen's method [7]). The CD spectra of $I_1$ and

$I_2$ were similar, each having a positive band at 223 nm and a negative one at 204 nm.

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# ISOLATION OF THE 5S rRNA of Phaseolus aureus. DETERMINATION OF THE STRUCTURE OF THE OLIGONUCLEOTIDES OF THE PYRIMIDYL-RNase FROM A HYDROLYSATE OF 5S rRNA


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The total rRNA from 12-hour shoots (30°C) of mung beans [1] was separated into two fractions by precipitation with 2 M NaCl in 0.25 M NaOAc buffer, pH 6. The high-molecular-weight rRNAs precipitated in this way were separated off by centrifugation and the concentration of NaCl in the supernatant was brought to 4 M, after which it was left at −10°C for 12 h. The resulting precipitate was removed, and low-molecular-weight RNAs were precipitated from the supernatant with ethanol. This salt-containing fraction was subjected to electrophoretic separation in 10% polyacrylamide gel in 0.05 M Tris-borate buffer with pH 8.3, 0.001 M EDTA, and 7 M urea. The RNA zones were stained with Methylene Blue without incubation of the gels in 1 M $CH_3COOH$. The zone corresponding to the 5S rRNA was cut out of the gel, homogenized in the elution buffer, and eluted by the phenol method [2]. The eluate was diluted fivefold with 0.01 M Tris-HCl, pH 7, and 7 M urea and was deposited on a microcolumn (1 × 40 mm) of DEAE-cellulose, after which the microcolumn was washed with water, ethanol, and water again and stepwise elution was performed with 0, 0.2, 0.65, and 1 M LiCl in 0.01 M Tris-HCl with pH 7, 7 M urea. The 5S rRNA was precipitated with three volumes of ethanol from the eluate after it had been concentrated in vacuum. The nucleotide composition of the mung bean 5S rRNA was determined by alkaline hydrolysis with 0.3 N

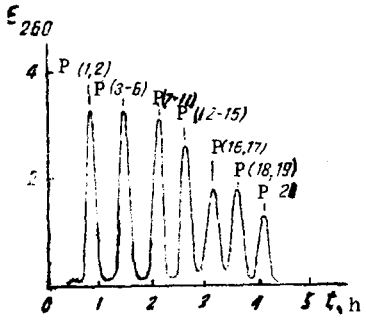


Fig. 1. MCLC separation of a pyrimidyl-RNase hydrolysate of mung bean 5S rRNA into isopleths.


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