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ISOLATION AND CHARACTERIZATION OF ALKALINE PROTEINASE OF *ASPERGILLUS FLAVUS**

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SUMMARY

1. A simple method was developed to isolate an alkaline proteinase from a crude enzymatic product prepared from the culture medium of *Aspergillus flavus* consisting of (i) adsorption of the crude product on CM-Sephadex at pH 4.5, (ii) desorption of proteinase by 0.5 M sodium acetate at pH 7, and (iii) chromatography on DEAE-Sephadex in phosphate buffers at pH 6.0. This purification results in a 120-fold increase of proteolytic activity.

2. The proteinase obtained is homogeneous when subjected to disc electrophoresis and sedimentation analysis in the ultracentrifuge. The molecular weight was determined, and the following values were obtained: (i) 19 500 to 22 000 by gel filtration, (ii) 19 000 ± 1000 by light-scattering technique, and (iii) 17 800 when the molecular weight was calculated from the amino acid composition: Lys_{11}, His_{3-4}, Arg_2, Asp_{21}, Thr_{11}, Ser_{20}, Glu_{12-13}, Pro_{4-5}, Gly_{20}, Ala_{23}, Val_{15}, Ile_{9-10}, Leu_9, Tyr_5, Phe_5, Trp_2, amide-NH_3 17. The enzyme does not contain either disulphide bonds or cysteine; the N-terminal end-group residue is glycine; the C-terminal amino acid is alanine. The enzyme contains 1 mole of a sugar component not yet identified. Treatment of the proteinase with diisopropylphosphorofluoridate provided evidence of one active serine residue.

3. Similarities existing between analytical data on alkaline proteinase of *Aspergillus flavus* and other extracellular enzymes from Aspergilli are discussed.

INTRODUCTION

So far very little is known about the genetic relationship of proteinases from different species of Aspergilli in spite of the fact that many efforts have been made to

Abbreviations DIP-, diisopropylphosphoryl-; DNS-, dimethylaminonaphtalene sulphonyl-.

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classify and isolate these enzymes[1–12,53]. The main reason for this lack of information is the fact that only five proteinases have been isolated in a homogeneous state and characterized in detail. So far aspergillopeptidase A has been isolated by Ichishima and Yoshida[13–17] from *A. saitoi*, aspergillopeptidase B was obtained from *A. oryzae* by Subramanian and Kalnitsky[18–20,54], and aspergillopeptidase C was obtained from the same mold by NORDWIG AND JAHN[21,22]. An alkaline proteinase was isolated by HAYASHI, FUKUSHIMA AND MOGI[23,55,56] from *A. Sojae*.

The aim of this paper is to report the isolation of an alkaline proteinase from a crude enzymatic preparation obtained according to the prodceure of HANUS AND KUČERA[12] from the culture medium of *A. flavus*, to provide evidence of the physical homogeneity of this enzyme, and to characterize the proteinase by analytical data. An effort was made to compare this data with the characteristics of the four above-mentioned proteinases isolated from Aspergilli.

MATERIAL

The crude commercial enzymatic preparation prepared by ethanolic precipitation of the medium after submerged fermentation of the mold *A. flavus*[12] was obtained from the Central Research Institute of Food Industry in Prague. The preparation contained in addition to contaminants (such as inorganic salts, polysaccharides and amylase) several proteolytic enzymes. Labeled diisopropylphosphorofluoridate ([^{32}P]DFP) was purchased from the Radiochemical Centre, Amersham, England, and its radioactivity was 435 μC/mg.

METHODS

Protein concentration was determined from ultraviolet absorption at 280 nm. Proteolytic activity measurements were carried out by a modification of the method of ANSON[24]. To 2 ml of the hemoglobin solution at 37° (buffered with Britton–Robinson buffers) was added 0.2 ml of the enzyme solution, and the mixture was incubated 10 min at 37°. The reaction was discontinued by the addition of 5 ml of 5% trichloroacetic acid, the precipitate formed was filtered off, and the absorbance of the filtrate at 280 nm was measured. 1 activity unit was defined as that amount of proteolytic activity measured at pH 7 which, under the conditions mentioned above, would give an absorbance of 0.1. Amylase activity was determined at pH 6.9 by the method of FISHER AND STEIN[25]. Dialysis of crude proteinase and of the product desorbed from CM-Sephadex was carried out in dialysis tubing (Kalle, Wiesbaden) at +4° against three to five changes of distilled water (a 10-fold volume).

Isolation of proteinase

The solution of 100 g of the crude enzymatic preparation in 10 l of water was filtered through Zeiss asbestos pressure filters. The filtered solution was acidified to pH 4.5 by citric acid, and 80 g (dry wt.) of CM-Sephadex C-50 which had been equilibrated with 0.005 M citrate buffer at pH 4.5 was added. The mixture was allowed to stand approx. 1 h with occasional stirring and then was transferred to a glass filter coated with 20 g (dry wt.) of CM-Sephadex C-50 equilibrated with 0.005 M citrate buffer at pH 4.5. The material on the filter was washed with the same 0.005 M

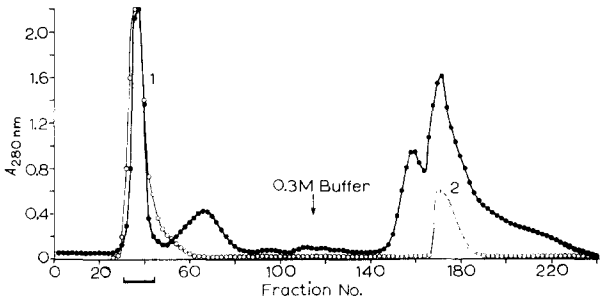


Fig. 1. Chromatography of products desorbed from CM-Sephadex on column of DEAE-Sephadex A-50 in phosphate buffers at pH 6. 0.5 g of the desorbed product was chromatographed on a 6 cm × 20 cm column in 0.01 M buffer. 10-ml fractions were collected at 10-min intervals. 1, alkaline proteinase; 2, zone of neutral proteinase (not characterized in this study) eluted with 0.3 M phosphate buffer at pH 6.0. ●—●, absorbance at 280 nm (1-cm light path); ○—○, proteolytic activity.

citrate buffer until the absorbance at 280 nm of the filtrate dropped below 0.030, and the filtrate gave a negative anthrone test[26] for polysaccharides. In order to accomplish desorption, the material on the filter was washed with ten 250-ml portions of 0.5 M acetate buffer at pH 7.0. The absorbance at 280 nm and the proteolytic activity at pH 7 of the filtrates obtained were determined, and the active fractions were pooled (as a rule fractions No. 2–7). The desorbed material was submitted first to short-term dialysis, then concentrated by freeze-drying, desalted on Sephadex G-25 and lyophilized.

The chromatography of this product (0.5 g) was effected on a 6 cm × 20 cm column of DEAE-Sephadex A-50 in phosphate buffer at pH 6.0. The details are given in the legend to Fig. 1. The pooled fractions containing the main proteolytically active components were desalted on Sephadex G-25. All operations were carried out at +4°. Yields are summarized in Table I.

Inhibition of alkaline proteinase by radioactive DFP. The proteinase (500 mg) was dissolved in 12.5 ml of 0.1 M Tris–HCl buffer at pH 8 and inhibited by the addition of 1.4 ml of an isopropanolic solution containing 4 mg of commercial [^{32}P]-

TABLE I

PURIFICATION OF ALKALINE PROTEINASE OF *A. flavus*

See MATERIAL AND METHODS for details. The proteolytic activity was determined at pH 7.

Degree of purification	*Weight (g)*	*Total activity (units × 10^3)*	*Specific activity* (units/mg)*	*Yield of activity (%)*	*Purification**
Starting material	1000	700	0.7	100	1
Desalted desorbed product	2.9	110	38	16	54
Desalted material after Chromatography on DEAE-Sephadex	1	85	85	12	120

* These values are merely approximate, since they are biased by presence of other proteinases in the starting material.

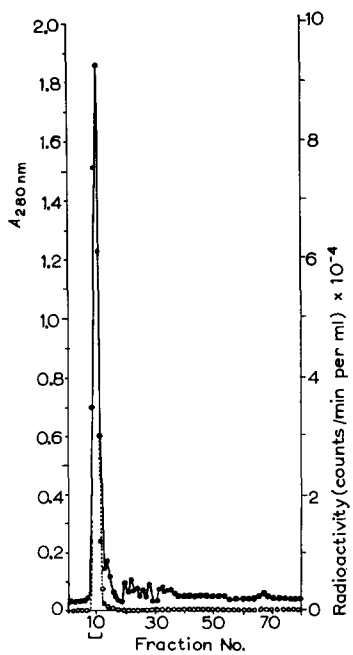
DFP and 140 μl of an isopropanolic solution containing 10 mg of nonradioactive DFP. After standing for 30 min at room temperature, 50 mg of nonradioactive DFP in 700 μl of isopropanol was added and the mixture was allowed to stand another hour. The reaction mixture was dialyzed against three 1.5-l changes of 0.01 M acetic acid at room temperature and lyophilized.

Oxidation of [^{32}P]diisopropylphosphoryl (DIP)-aspergillopeptidase. The [^{32}P]-DFP-treated material was dissolved in a mixture of 10 ml of 98% formic acid and 1 ml of methanol and then oxidized 3 h at $-7°$ with 10 ml of performic acid[27]. After oxidation SO_2 gas was passed through the reaction mixture. The solution was then dialyzed against four 2-l changes of water and lyophilized.

The details of ion-exchange chromatography of 50 mg of unoxidized [^{32}P]DIP-proteinase are given in the legend to Fig. 2. The radioactivity of dry residues of 100-μl aliquots was measured on aluminium planchets using a Geiger–Müller tube with a thin mica end-window. The bulk of the radioactivity was found in a single fraction, and this fraction was desalted on Sephadex G-25 (yield 40 mg).

Characterization of the isolated proteinase

Disc-electrophoresis in polyacrylamide gel was carried out in β-alanine buffer



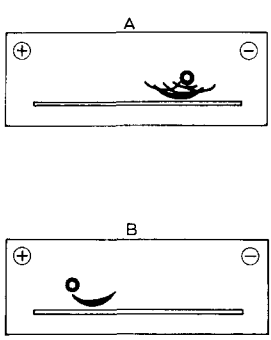


Fig. 2. Chromatography of alkaline proteinase inhibited by radioactive DFP on Amberlite IRC 50. 50-mg of lyophilized [^{32}P]DIP-derivative was chromatographed on a 0.9 cm × 72 cm column of Amberlite IRC 50 equilibrated with 0.01 M phosphate buffer at pH 6.5. The same buffer was used as eluent. 4-ml fractions were collected at 20-min intervals. ●—●, absorbance at 280 nm (1-cm light path); ○ · · · · · ○, radioactivity.

Fig. 3. Immunoelectrophoretic pattern of desorbed product and of aspergillopeptidase. Samples: (A) 200 μg of desorbed product; (B) 100 μg of alkaline proteinase. Developed in both cases with the rabbit antiserum to the desorbed product.

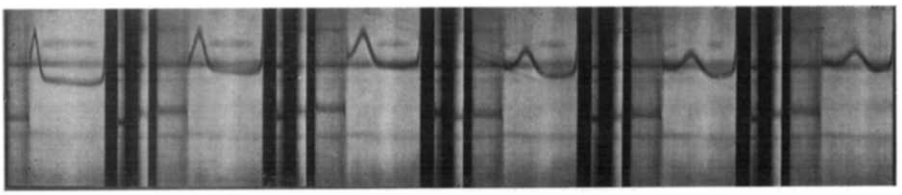


Fig. 4. Sedimentation pattern of alkaline proteinase. A 0.5% solution of the enzyme in 0.1 M NaCl was used. The photographs were taken at 59 780 rev./min in Spinco Model E ultracentrifuge after 30, 46, 62, 78, 94 and 110 min.

at pH 4.5 (ref. 28) and in Tris–glycine buffer at pH 8.3 (ref. 29). Immunoelectrophoresis (*cf.* Fig. 3) was performed by the technique of GRABAR and co-workers[30,31] in the Imunophor LKB 6800 apparatus according to the manufacturer's directions (LBK, Stockholm). The antiserum of the desorbed product was prepared by immunization of rabbits according to the scheme of LESKOWITZ AND WAKSMAN[32] (*cf.* NORDWIG AND JAHN[22]). The sedimentation coefficient of a 0.5% solution of proteinase in 0.1 M NaCl was measured in a Spinco Model E ultracentrifuge (*cf.* Fig. 4).

The N-terminal amino acid was determined with 1 mg of protein by the di-

TABLE II

EXPERIMENTAL CONDITIONS AND RESULTS OF DETERMINATION OF MOLECULAR WEIGHT OF ALKALINE PROTEINASE BY GEL FILTRATION

The molecular weights were read from the graph in Fig. 5. The column used in Expt. A was silicone coated[42]; in Expts. B, C and D, columns without the coating were used.

Expt.	*Sample (wt/v*)*	*Xerogel (particle size)*	*Medium (pH)*	*Dimensions of column (cm × cm (volume in ml))*	*Fraction (volume/ interval)*	*Mol. wt. read from graph*
A	Aspergillo-peptidase (2.5 mg/2 ml)	Bio Gel P-100 (50–150mesh)	0.05 M Tris HCl + 0.1 M KCl (pH 7.4)	1.8 × 31 (79)	5 ml/30 min	22 000
B	Aspergillo-peptidase (4 mg/2 ml)	Sephadex G-100 (100–200 mesh)	0.02 M ammonium acetate + NH_4OH (pH 6)	2.7 × 60 (295)	4.5 ml/ 15 min	22.000
C	DIP-aspergillopeptidase (5 mg/2 ml)	Sephadex G-50 (medium)	0.02 M ammonium acetate + 8 M urea + + NH_4OH (pH 6)	2.0 × 65 (210)	3 ml/60 min	21 000
D	DIP-aspergillopeptidase (5 mg/2 ml)	Sephadex G-50 (medium)	6 M guanidine · HCl adjusted by NaOH to pH 6	2 × 59 (186)	5 ml/60 min	19 500

* The protein was always dissolved in the given volume of buffer and allowed to soak into the top of the column.

methylaminonaphtalene sulphonyl(DNS-)technique according to GRAY AND HARTLEY[33]. The DNS-amino acid was identified by thin-layer chromatography on silica gel in systems S_1 (methyl acetate–isopropanol–28% NH_4OH, 45:35:20, by vol.) and S_2 (chloroform–methanol–acetic acid, 75:25:5, by vol.) according to the description of SEILER AND WIECHMANN[34]. For C-terminal end-group analysis, 1 mg of the protein was subjected to hydrazinolysis according to the method of AKABORI, OHNO AND NARITA[35] and the amino acid released was identified as its DNS-derivative[33,34,36]. Quantitative amino acid analysis was carried out with 0.025 μmole of protein in the automatic amino acid analyzer according to SPACKMAN, STEIN AND MOORE[37]. Trypto-

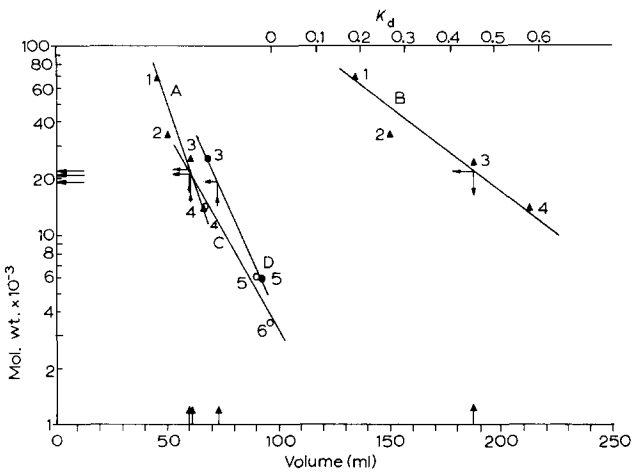


Fig. 5. Determination of molecular weight by gel filtration. Lines A–D were obtained by plotting the results of experiments designated by the same letter in Table I. On the lower horizontal axis the scale of elution volumes for all experiments is given. The upper horizontal axis is a scale of K_d values for Expt. B. The molecular weights are read from the ordinate. Designation of standards used in all experiments: 1, human serum albumin[44]; 2, pepsin[45]; 3, bovine chymotrypsinogen[46]; 4, ribonuclease[47]; 5, insulin[48]; 6, insulin B-chain[49].

phan was determined by the method of SPIES AND CHAMBERS[38], the content of amide nitrogen according to RAJAGOPALAN, MOORE AND STEIN[39] and the sugar content by the phenolsulphuric acid method of DUBOIS *et al.*[40]. The phosphorus content of the [^{32}P]DIP-derivative was estimated according to the method of LOWRY AND LOPEZ[41] with aliquots containing 0.375 or 0.636 μg of the lyophilized proteinase.

The determination of the molecular weight by gel filtration[42,43] was performed by four different procedures and the experimental conditions are summarized in Table II. The elution volumes found and K_d-values[42], respectively, were plotted *versus* the known molecular weights of standards in a graph (Fig. 5) from which the molecular weights of alkaline proteinase was read. The determination of the molecular weight by the light-scattering technique was carried out on the Sofica photoelectric apparatus Model 4200. The intensity of scattered light was measured at $\lambda = 436$ nm and an observation angle $\Theta = 30$–$135°$ with a series of solutions of enzyme (concentration $c = 0.36$–$1.43\ g \cdot ml^{-1} \times 10^{-3}$) in 0.1 M NaCl.

RESULTS AND DISCUSSION

Isolation of alkaline proteinase of A. flavus

In our preliminary experiments, evidence was obtained of the presence of at least three proteolytic enzymes with pH-optima in the basic, neutral and acid pH regions in the crude enzymatic preparations used as starting material. These enzymes can be fractionated by chromatography on DEAE-cellulose (*cf.* ref. 12), they cannot, however, be obtained in a pure state by this operation. We attempted to adopt some of the procedures used for the preparation of enzymes from *A. oryzae*[4,9,18,19,21,22,53,54]. The most effective procedure for our purpose, however, is direct adsorption of the crude protease from a sufficiently diluted (1%) solution on CM-Sephadex C-50 equilibrated with 0.005 M citrate buffer (pH 4.5). The basic proteinase and the greatest part of the neutral proteinase is readily adsorbed. During filtration and washing with 0.005 M citrate, proteins of low proteolytic activity are washed off together with nonprotein components. As can be seen in Table I a 54-fold purification of protein was achieved by adsorption and desorption. The major advantage of our approach is the selective enrichment of the proteinase, and also the simple and complete removal of polysaccharides and other nonprotein components which would cause considerable difficulties if the material were treated by other procedures.

The subsequent step is chromatography on DEAE-Sephadex A-50 in phosphate buffers at pH 6 (*cf.* Fig. 1). The alkaline proteinase with pH optimum of activity at 8–9 is eluted by 0.01 M phosphate, provided the material contained in the first peak has been desalted on Sephadex G-25. The material desalted by dialysis yields a proteinase with pH optimum at 7. Both procedures give preparations of identical amino acid composition. The cause of the shift of the pH optimum during dialysis is being studied and has not yet been explained. When the column is eluted with phosphate buffer of higher molarity and the same pH, proteinases with pH optima of proteolytic activity in the neutral (Fig. 1-2) and or acid pH region emerge. The study of these proteinases lies outside the scope of the present paper. Part of the protein present in the desorbed product is eluted only during the regeneration of the column. As can be seen in Table I, the proteolytic activity of the preparation is 120-times higher after chromatography on DEAE-Sephadex. In our opinion, the purification procedure described here is simpler than those cited above. The removal of amylolytic activity present in the starting material is a further indication of its efficacy. Similarly the value of the absorbance ratio ($A_{280\ nm}/A_{260\ nm} = 1.85$) indicates that the purified preparation is free of any significant amounts of nucleic acid components.

Homogeneity tests

The purification of the proteinase was checked by disc-electrophoretic assay of aliquots of individual preparations. When assayed in the β-alanine buffer[28], the desorbed product showed the presence of at least five zones which were separated best at pH 4.5. The material which had been purified by chromatography on DEAE-Sephadex moved as one zone when examined at different concentrations and for different periods. The absence of neutral and acidic proteins was proved by disc-electrophoresis in the Tris–glycine buffer[29]. Immunoelectrophoresis gave identical results (Fig. 3). For the preparation of antisera the desorbed product was selected as

the best source of antigen because it is rich in proteinases and free of nonprotein contaminants. As can be seen in Fig. 3A, this product contains at least five proteins and alkaline proteinase is obviously the most basic one among them. The result shown in Fig. 3B indicates the homogeneity of the preparation and that all less basic products can be removed by chromatography on DEAE-Sephadex. As shown in Fig. 4, only one symmetrical peak was apparent when the sedimentation coefficient of the preparation was studied in the ultracentrifuge. This indicates the physical homogeneity of the preparation. The value of the sedimentation coefficient is $s_{20,w} = 3.05$ S. In our opinion, the above results of disc electrophoresis, immunoelectrophoresis and sedimentation analysis provide evidence in favor of the physical homogeneity of the enzyme prepared by us.

Characterization of alkaline proteinase

The determination of the molecular weight of alkaline proteinase by gel

TABLE III

AMINO ACID COMPOSITION OF ALKALINE PROTEINASE OF *A. flavus* AND OF ITS [^{32}P] DIP-DERIVATIVE

Number of residues per mole (mol. wt., 19 000).

Amino acid	*Preparation of alkaline proteinase*									*Nearest integer*
	Active proteinase			*Trichloroacetic acid-precipitated protein*			*[^{32}P]DIP-derivative*			
Time of hydrolysis (h):	*20*	*70*	*oxid., 20*	*20*	*70*	*oxid., 20*	*24*	*48*	*96*	
Lys	9.55	9.73		10.65	11.00	11.10	10.28	10.78	10.49	11
His	3.32	3.23		3.5	3.91	2.2	3.32	3.36	3.45	3–4
Arg	2.13	2.13		2.56	2.34	2.26	2.08	2.29	2.15	2
$CySO_3H$	—	—		—	—	—	—	—	—	0
Asp	19.3	19.85	21.45	20.90	21.00	20.4*	19.92	20.28	19.03	21
Methionine sulfone	—	—	1.24	—	—	1.0*	—	—	—	(1)
Thr	10.6	9.72	10.74	10.2	10.05	10.3	10.53	10.35	9.30	11**
Ser	19.66	16.20	19.91	18.80	16.35	19.30	19.22	17.56	15.38	20**
Glu	11.93	11.59	12.10	11.90	12.30	11.80	12.34	12.49	12.03	12–13
Pro	4.46	4.44	4.85	4.0	4.04	4.22	5.71	4.96	5.03	4–5
Gly	18.93	18.80	19.28	20.00	20.40	20.00	18.92	18.90	18.69	20
Ala	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23***
Cys	—	—	—	—	—	—	—	—	—	0
Val	11.58	13.32	11.67	11.00	13.90	12.20	11.47	13.18	14.40	15†
Met	1.19	1.26	—	1.12	1.26	—	1.19	1.17	1.10	1
Ile	8.75	9.39	9.03	8.63	9.3	8.6	8.39	9.18	9.30	9–10†
Leu	9.08	9.16	9.37	8.83	9.11	8.80	9.09	9.12	8.19	9
Tyr	5.29	5.09	—	5.16	5.28	—	5.11	5.01	4.98	5
Phe	4.71	4.8	2.18	4.97	5.14	0.73	4.92	5.07	4.74	5
Trp							2.1			2
Amide-NH_3							17.0			(17)
Total										173–177

* The curves were insufficiently separated, the total value is 21.4 residues.

** Graphically extrapolated to zero time of hydrolysis.

*** Since the value of alanine did not vary with the time of hydrolysis, it was used as a standard for the calculation of amino acid composition from different analyses.

† Graphically extrapolated to total hydrolysis.

filtration (Fig. 5, Table II) gave a value of 19 500–22 000. When the gel filtration was carried out in solutions of urea or guanidine at concentrations sufficient to suppress aggregation, values lying close to the lower limit of this range were obtained. When the molecular weight was examined by the light-scattering method, a value of 19 000 ± 1000 was obtained. We used both these methods because the analysis in the ultracentrifuge by the method of YPHANTIS[50] gave values ranging from 24 000–36 500. Similarly the profile of the dependence of the sedimentation coefficient on the concentration of the proteinase seemed to suggest dimerization of the molecule. A more detailed analysis of this phenomenon would require a separate study. The value of 19 000 ± 1000 is in good accordance with the value 17 700–18 172 calculated from the amino acid composition given in Table III. The results given were obtained with three preparations. We also analyzed a great number of other preparations, both oxidized and unoxidized, and similar results were obtained.

The molecule of alkaline proteinase is relatively small. A characteristic feature of its amino acid pattern is the absence of both cysteine and cystine and a relatively high content of alanine, serine, aspartic acid and glycine. One residue of methionine is the only sulphur-containing amino acid present. These data are in accordance with the results of POLLOCK AND RICHMOND[51] who found that most extracellular microbial enzymes do not contain disulphide bridges. The profile of the ultraviolet spectrum of the enzyme in 0.1 M NaOH, 0.1 M HCl, and 0.1 M acetate buffer at pH 5 is very similar to that of the ultraviolet spectrum of aspergillopeptidase B measured by SUBRAMANIAN AND KALNITSKY[54]. $E_{1\,cm}^{1\%}$ (pH 5) of the native enzyme is 9.04 at 280 nm. The proteinase is very hygroscopic. Since the analysis by the phenol–sulphuric acid method[40] gave a positive result, we assume that the enzyme contains a small amount of a sugar component in addition to amino acids. The spectrum of the reaction product showed a maximum at 480 nm. The calculations were therefore based on the assumtion that ribose was present. Assuming the molecular weight of the enzyme to be 17 800, the content of the sugar component determined at three different concentrations becomes 1.08, 1.1 and 0.9 mole per mole of proteinase. The sugar component has not been identified so far.

The end-group analyses of different preparations having the same amino acid composition gave different results, in spite of the fact that all these preparations were homogeneous from the physical viewpoint (sedimentation coefficient, disc electrophoresis, immunoelectrophoresis). All preparations showed the presence of N-terminal glycine and C-terminal alanine. In some preparations, however, we also found N-terminal alanine and smaller amounts of other N-terminal amino acids (serine, threonine, aspartic acid and glutamic acid). We have proved that these preparations had undergone partial autolysis. In order to prevent autolysis we prepared the DIP- and [^{32}P]DIP-derivatives of the enzyme, which were proteolytically inactive. During the chromatography of the latter derivative, the proteolytic activity measurement of effluent fractions was replaced by radioactivity measurement (Fig. 2). The preparation obtained by this approach from freshly prepared enzyme showed only N-terminal glycine and C-terminal alanine, and its amino acid composition was identical to that of the unsubstituted preparation (Table III). The inhibition experiment also showed that the enzyme prepared by us is a serine proteinase. The result of the phosphorus analysis (0.9 atom per mole of protein of mol. wt. 17 800) indicates that only one serine-containing active center is present in the molecule.

Comparison of alkaline proteinase of A. flavus with other proteinases from Aspergilli

The essential differences among these proteinases, from the analytical point of view (Table IV), concern the content of sulphur-containing amino acids. While aspergillopeptidase A and the proteinase from *A. sojae* each contain one disulphide bridge, aspergillopeptidases B and C and alkaline proteinase of *A. flavus* do not contain disulphides. As to the methionine content, the alkaline proteinase from *A. sojae* contains two residues, but aspergillopeptidase C and the enzyme prepared by us contain one residue each. The analyses of aspergillopeptidase A and B reported do not mention methionine. SUBRAMANIAN AND KALNITSKY[54] found a small amount of methionine in aspergillopeptidase B, however they consider the value found to be

TABLE IV

AMINO ACID COMPOSITION, TERMINAL AMINO ACIDS AND MOLECULAR WEIGHTS OF PROTEINASES ISOLATED FROM MOLDS OF THE GENUS ASPERGILLUS

Amino acid composition	*Aspergillopeptidase A (A. saitoi)*	*Aspergillopeptidase B (A. oryzae)*	*Aspergillopeptidase C (A. oryzae)*	*Alkaline proteinase (A. sojae)*	*Alkaline proteinase (A. flavus)*
References:	*13–17*	*18–20, 54*	*21, 22*	*23, 55, 56*	*This paper*
Lys	11	11–12	12	14	11
His	3	4	4	5	3–4
Arg	1	2	3	3	2
Asp	34–35	21	21–22	31	21
Thr	25	11	13	18	11
Ser	42–43	19	23	28	20
Glu	22	12	13	19	12–13
Pro	10	4	5–6	6	4–5
Gly	31–32	19	21	27	20
Ala	20	23	23	32	23
Cyś	2	0	0	2	0
Val	22	15	16	18	15
Met	0	0	1	2	1
Ile	11–12	9–10	10–11	14	9–10
Leu	19–20	9	10	14	9
Tyr	17–18	5	4–5	8	5
Phe	13	5	6	7	5
Trp	1	2	2	2	2
Amide-NH_3	31–32	15		20	17
Total	283–289	171–173	187–191	250	173–177
N-terminal amino acids	Ser	Gly			Gly
C-terminal amino acids	Ala	Ala			Ala
Mol. wt.	34 800	17 800	19 650	25 750	18 000

an artifact. In view of the high degree of similarity in the composition of aspergillopeptidase B and the enzyme prepared by us and in the amino acid sequence around the active center (*cf.* the subsequent paper[52]) it is possible that these two proteinases are identical. It would appear from the presence of a sugar component reported[54] also in aspergillopeptidase B that these proteins are very similar. So far we cannot

determine the extent to which aspergillopeptidase C resembles these proteins. The differences in the amino acid compositions of these three enzymes are not great. Moreover, all three aspergillopeptidases are enzymes containing one active serine residue. Even though the strains producing them are not identical, the molds *A. flavus* and *A. oryzae* are so similar that it would not be at all surprising if they produced identical proteins. This problem requires separate study, and an investigation of the specificities of the isolated enzymes may be of interest. Any conclusions concerning the degree of similarity of proteinases from Aspergilli must of course await the determination of the primary structures of these enzymes.

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