REINVESTIGATION OF MOLECULAR WEIGHT OF ALKALINE PROTEINASE FROM *Aspergillus flavus*

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Received February 19th, 1971

The molecular weight of alkaline protease from *Aspergillus flavus* was determined both in the ultracentrifuge (23 620—27 180) and by gel filtration (22 000—24 000). The sedimentation coefficient of this extracellular enzyme $S_{20,w}$ 2·82 was found to be independent of concentration.

The total number of amino-acid sequences in peptides from the tryptic and chymotryptic digest of the alkaline proteinase from *Aspergillus flavus*, obtained during our so far unpublished studies on this enzyme, indicated that its molecular weight was higher than the value 18 000 which we had originally calculated from the amino-acid composition[1]. We repeated therefore the determination of the sedimentation coefficient and the investigation of the molecular weight in the ultracentrifuge and by gel filtration. The obtained results point to a molecular weight value of 23 000 to 27 000. The final value of the molecular weight and the exact amino-acid composition of the proteinase will be calculated after the sequential studied have been completed.

EXPERIMENTAL

Material and Methods

The alkaline proteinase from *A. flavus* and its DIP-derivative* were prepared by methods described elsewhere[1]. The French preparation of the alkaline proteinase from *A. flavus* was a generous gift of Dr P. Lallouette[2]. Sephadex and blue dextran were products of Pharmacia, Uppsala, Sweden. The sedimentation coefficients of 0·25%, 0·5%, 0·75%, and 1% solutions of the proteinase in 0·1M-NaCl were measured in Spinco Model E Ultracentrifuge under conditions similar to those reported in another paper[1]. The molecular weight was determined in the ultracentrifuge by the method of Yphantis[3]. For the partial specific volume the average value of 0·726 ml/g was employed according to Cohn and Edsall[4]. The molecular weight determination by gel filtration was carried out by a procedure analogous to that used before[1], *i.e.* on a column of Sephadex G-100, 50—120 μ (2·5 × 64 cm) equilibrated with 0·02M ammonium acetate (pH 6). Fractions 4·8 ml in volume were collected at 15 min intervals. The samples applied to the column contained 4 mg of the proteinase in 2 ml of buffer, always with the admixture of blue dextran (Fig. 1).

* Abbreviations: DIP-diisopropylphosphoryl-.

RESULTS AND DISCUSSION

Unlike in the previous studies reported, we found that the sedimentation coefficient of samples of the alkaline proteinase studied in the present work does not depend on the concentration of the sample. The values of $S_{20,w}$ for 0·25%, 0·5%, 0·75% and 1% solution of the proteinase are 2·82, 2·82, 2·82 and 2·83, respectively. The molecular weight determined in the ultracentrifuge varies in the range 23 620 – 27 180. By repeated gel filtration on the Sephadex G-100 column (Fig. 1) we obtained the value of 23 900 for the alkaline proteinase from *A. flavus* isolated by us[1], 23 900 for the DIP-derivative of our preparation, and 23 100 for the French preparation of the alkaline proteinase from *A. flavus*.

In an analogous experiment with gel filtration reported before[1] (Table II, experiment B) we obtained the value 22 000 for our preparation. The values determined in the ultracentrifuge in the previous study[1] varied between 24 000 and 36 500. Since the values of the sedimentation coefficients in our earlier experiments did not show a linear dependence on concentration, we overestimated the results of repeated light scattering analysis which indicated lower molecular weight values (19 000 ± ± 1 000)[1]. Similarly, the experiments with gel filtration in urea solutions[1] (Table II, experiment C) or guanidine hydrochloride solutions[1] (Table II, experiment D) (ref.[1]) yielded lower molecular weight values (up to 19 500) (ref.[1]). We considered at that time the lower values as being more correct also because the molar amino acid ratios determined by us were in agreement with the data on the amino-acid composition of the alkaline proteinase from *A. oryzae* (Aspergillopeptidase B) reported by Subramanian and Kalnitsky[5]. These authors reported a molecular weight of

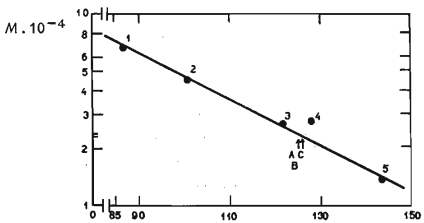

FIG. 1

Determination of Molecular Weight by Gel Filtration

Designation of standards used in the experiment: 1 human serum albumin[3], 2 ovalbumin[13], 3 beef chymotrypsinogen[14], 4 subtilisin Novo[15], 5 ribonuclease[16], A our preparation of alkaline proteinase from *A. flavus*, B our preparation of DIP-proteinase, C French preparation of alkaline proteinase from *A. flavus*.

approximately 18 000. We were of the opinion that we had isolated a similar low molecular weight proteinase and calculated[1] its amino-acid composition on the assumption of the presence of one methionine residue in the molecule. The total number of amino-acid sequences in peptides isolated from the tryptic and chymotryptic digest seemed to indicate a higher molecular weight of the substrate and we decided therefore to reinvestigate this problem.

The value of molecular weight 22 000 – 24 000 as determined by gel filtration can, however, be to a certain degree erroneous. Fig. 1 shows the elution volume of subtilisin (point 4), which lies considerably distant off the curve of standards and seems to indicate a value of 22 000. Similar molecular weight values (21 000 – 22 000) were obtained by Keay, Moser and Wildi[6] by gel filtration of all alkaline proteinases of the genus *Bacillus*, including the subtilisins. Since the molecular weight of subtilisins as calculated from their complete amino-acid sequences is 27 600, these authors report that they cannot offer any explanation of this anomaly. In view of the several times discussed similarity of the serine proteinases from the genus *Bacillus* and *Aspergillus*[7–10], we cannot exclude the possibility that the molecular weight of the alkaline proteinase from *A. flavus* is also higher. By contrast, we employed the conventionally used[4] average value of 0·726 ml/g for the partial specific volume when calculating the molecular weight of the proteinase from the results of ultracentrifugation analysis. Hayashi, Fukushima and Mogi[11] reported originally for a similar alkaline serine proteinase from *A. sojae* a molecular weight of 25 500 which they had determined by sedimentation analysis using the average value[4] of 0·726 ml/g for the partial specific volume. Later[12], after they had determined experimentally the value of the partial specific volume as 0·696 ml/g, they corrected the molecular weight to 22 600. Similarly, Subramanian and Kalnitsky[5] found experimentally a relatively low value (0·682 ml/g) for the partial specific volume of the serine proteinase from *A. oryzae* (aspergillopeptidase B). We have not determined the partial specific volume as yet. If, however, its value were lower also in the case of our proteinase, the molecular weight determined by sedimentation analysis would decrease by approximately 3000.

The interpretation given above suggests a higher molecular weight than 18000 (ref.[1]). Therefore we no longer regard the enzyme isolated by us as a relatively low molecular weight proteinase but we classify this protein as equal in size to most of the known isolated serine proteases whose molecular weights vary in the range 23 000 – 27 000.

We are indebted to Dr P. Lallouette for kindly supplying us with a sample of its preparation of alkaline proteinase from Aspergillus flavus *for our comparison experiments. We wish to thank Dr F. Franěk and Mr J. Neumann of this Institute for the determination of the sedimentation coefficients and of the molecular weight in the ultracentrifuge.*

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Translated by V. Kostka.