Short Communications

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Ultraviolet Optical Rotatory Dispersion of Aspergillopeptidase A

Sir:

In a previous paper,¹⁾ it was reported from the optical rotatory dispersion in the visible and near ultraviolet regions over 320 to 600 $m\mu$ that the acid proteinase of *Aspergillus saitoi* (aspergillopeptidase A, EC, 3.4.4.17)^{2~4)} has the optical rotation, $[\alpha]_D$, of -35° , the optical rotatory dispersion constant, λ_e , of 207 $m\mu$, and the values of $-a_0=211$ and $-b_0=0$ in the Moffitt-Yang parameters. The location of an amide I band at 1632 cm^{-1} was observed in the infrared spectrum of the deuterium-exchanged aspergillopeptidase A.¹⁾ This result suggested that the enzyme contains the antiparallel β structure.^{5,6)}

In this communication, an attempt has been made to clarify the internal structure of aspergillopeptidase A in aqueous solution using the optical rotatory dispersion in the deep ultraviolet region. The internal structure of other proteinases is compared and discussed.

Aspergillopeptidase A purified according to the previous method²⁾ was further purified on Sephadex G-100 at pH 4.0 for the separation of minor autodigestive products and lyophilized. Twice crystallized pepsin (EC, 3.4.4.1), No. 34046, was obtained from General Bio-

chemicals Inc., U.S.A. The mean residue molecular weight of pepsin was calculated as 101.8 from the experimental data of Rajagopalan et al.⁷⁾ The crystalline preparation of *Aspergillus oryzae* alkaline proteinase⁸⁾ (aspergillopeptidase B, kindly supplied from Dr. W. C. Crewther) was further purified by gel filtration of Sephadex G-100 at pH 7.0 and lyophilized. The mean residue molecular weight of aspergillopeptidase B was calculated to be 104.7 from the experimental data of Subramanian and Kalnitsky.⁹⁾ Crystalline *Bacillus subtilis* proteinase (subtilopeptidase A, EC, 3.4.4.16) was purchased from Nagase Co. The mean residue molecular weight of subtilopeptidase A employed was 103.6. The optical rotatory dispersion measurements of aspergillopeptidase B and subtilopeptidase A were made at pH 5.5.

The optical rotatory dispersion measurements were made in the 195 to 250 $m\mu$ wave length region with a Japan Spectroscopic model ORD/UV-5 recording polarimeter at room temperature of 22 to 25°C. All measurements were made in optical quartz cell with an optical path of 1.0 mm. The results obtained in the 195 to 210 $m\mu$ zone were checked under an anaerobic condition that the nitrogen gas was delivered to the cell chamber at a slightly positive pressure. The protein concentration was 0.09% in 0.01 M citrate buf-

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4) E. Ichishima and F. Yoshida, *Biochim. Biophys. Acta*, **110**, 155 (1965).

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fer, pH 5.5, at 212 to 250 m μ spectral zone, and it was 0.030 to 0.0075% at 195 to 210 m μ . Further dilutions of the initial solution of 0.09% were made with 0.001 M citrate buffer, pH 5.5. The corrected specific rotation $[m']$ values¹⁰⁾ were computed from the relationship

$$[m'] = [\alpha] \frac{3}{n^2 + 2} \frac{M_0}{100}$$

where $[\alpha]$ is the specific rotation and n is the refractive index of the solvent. The refractive index value of water¹¹⁾ was also used for the diluted buffer solutions. The mean residue molecular weight (M_0) of aspergillopeptidase A was calculated to be 106.0 from amino acid composition of aspergillopeptidase A.⁴⁾ All points on the curves (195~250 m μ) represent average values of measurements.

The curve in the Fig. 1 shows the optical rotatory dispersion of aqueous aspergilloptidase A. At the condition of pH 5.5, 22 to 25°C, autodigestion of aspergillopeptidase A was found to be near zero. The curve of native aspergillopeptidase A in the Fig. 1 differs strongly from the other curves of the partially α -helical proteins, which have a minimum at 233 m μ and a high positive maximum at 198 m μ .¹²⁾ The negative minimum of the

FIG. 1. Ultraviolet Optical Rotatory Dispersion of Aspergillopeptidase A.

The concentration of the enzyme was 0.09 to 0.0075% at pH 5.5, 22 to 25°C. The thickness of solution layer was 1 mm in 195 to 250 m μ spectral zone. The mean residue molecular weight of 106.0 was employed. ●—●, native aspergillopeptidase A; $\triangle$ — $\triangle$, aspergillopeptidase A with 0.02 M sodium lauryl sulfate; ○—○, aspergillopeptidase A with 8 M urea.

Cotton effect of aspergillopeptidase A was not at 233 m μ but at 220 m μ . The $[m']_{220}$ value of the negative Cotton effect was found to be

TABLE I. ULTRAVIOLET OPTICAL ROTATORY DISPERSION OF ASPERGILLOPEPTIDASE A AND OTHER PROTEINASES IN 195 TO 250 m μ ZONE.

Enzyme	ORD-Trough		ORD-Peak		Cross-over wave length (m μ)
	Position [m']		Position [m']		
	(m μ)	min.	(m μ)	max.	
Aspergillopeptidase A	220	−2400	203	+900	270
Pepsin	226	−2800	203	+4500	214~215
Chymotrypsinogen A*	221	−5200	190	+14000	205
Trypsin*	224	−3100	203	+3500	—
Aspergillopeptidase B	234	−1400	198	+4900	225
Subtilopeptidase A	232	−2500	198	+5000	220

* Experimental data of Jirgensons (1966).¹²⁾

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12) B. Jirgensons, *J. Biol. Chem.*, **240**, 1064 (1965), **241**, 147, 4855 (1966).

-2400 ± 150 (Table I). This curve has a flat peak at $203 \text{ m}\mu$, and the $[m']_{198}$ of aspergillopeptidase A is close to zero. Upon treatment with 0.02 M sodium lauryl sulfate at $22 \pm 2^\circ\text{C}$ for 15 h, which has been found as the specific inhibitor of aspergillopeptidase A,¹³ the negative minimum was shifted to $233 \text{ m}\mu$, and the amplitude of the negative Cotton effect was increased (Fig. 1). The $[m']_{233}$ and $[m']_{198}$ values were found as -3400 and $+11600$, respectively. The conformation of aspergillopeptidase A was converted by the anionic detergent into partially α -helical conformation, as judged from the ultraviolet optical rotatory dispersion curve. The similar conformational transition by the detergent had been observed with chymotrypsinogen A, trypsin, soybean trypsin inhibitor and alcohol dehydrogenase.¹²

According to the previous paper,¹¹ the $-a_0$ value in the Moffitt-Yang parameter or levorotation of the aspergillopeptidase A molecule increased markedly in the presence of urea, while the value of b_0 remained unchanged. The specific rotation and the intrinsic viscosity were -35° and 0.032 dl/g , respectively.¹¹ The decrease in the enzymatic activity of aspergillopeptidase A as a function of urea concentration was observed to be paralleled with the behavior of the $-a_0$ value.¹¹ These findings suggest that the aspergillopeptidase A is completely folded, and is devoid of α -helical conformation.¹¹ With 8 M urea at pH 5.5, 22°C , for 1 h, it was confirmed that the optical rotatory dispersion curve in the deep ultraviolet region was shifted to that of the randomly coiled form¹² (Fig. 1). It was concluded that the aspergillopeptidase A is devoid of complete α -helical strand, as judged from the ultraviolet optical rotatory dispersion curve at 233 and $198 \text{ m}\mu$.

From the data of Jirgensons¹² and Raval,¹⁴

the optical rotatory dispersions of chymotrypsinogen A, chymotrypsin (EC, 3.4.4.5), trypsin (EC, 3.4.4.4), rennin (EC, 3.4.4.3) and pepsin are similar to that of aspergillopeptidase A in the negative Cotton effect of 215 to $225 \text{ m}\mu$ spectral zone. The dispersion curves of these proteinases differ strongly not only from the curve of the α -helical type of proteins (myoglobin, serum albumin)¹² but also from the curve of disordered random chain (phosvitin,^{12,15} disordered poly- α -L-glutamic acid.)¹² The curves of these proteinases have shallow minima at 215 to $225 \text{ m}\mu$ and relatively flat maxima at 200 to 205 .¹² All of these proteinases have been shown as acid-stable proteinases.¹⁶ The rotatory dispersion at $220 \text{ m}\mu$ of chymotrypsin and trypsin was similar to that of γ -globulin, in which the existence of the antiparallel β structure had been identified by infrared spectrum.⁵ But, the curve of γ -globulin differs from all others in its positive maximum at $210 \text{ m}\mu$.¹² The previous result in the infrared spectrum¹¹ suggests that the aspergillopeptidase A contains the antiparallel β structure.^{5,6} But the content of the β structure of aspergillopeptidase A is still obscure. In spite of these findings, it is still obscure whether the internal structure of aspergillopeptidase A is characterized by a mixed conformation of the antiparallel β structure and the randomly coiled form, or by some kind of ordered structures containing the antiparallel β structure. On the contrary, aspergillopeptidase B and subtilopeptidase A have been shown proteolytic enzyme activities like those of chymotrypsin. But the two proteinases have no acid-stability. In the Table I, the optical rotatory dispersions of the two are compared with those of the acid-stable proteinases. The curves of the two proteinases have a definite minimum at about $233 \text{ m}\mu$ and a maximum at $198 \text{ m}\mu$ character-

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istics of the α -helix.

The amino acid compositions, molecular sizes and catalytic functions of these acid-stable proteinases are not similar, but these proteinases have the similar characteristic property of acid-stability.¹⁶⁾ However, a detailed interpretation of the acid-stability is impossible without further study. It is assumed that the characteristic property of acid-stability partially depends on the polypeptide-chain backbone conformation.

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Eiji ICHISHIMA
Fumihiko YOSHIDA

*Enzymology Laboratory,
Central Research Institute,
Kikkoman Shoyu Co.,
Noda, Chiba-prefecture,
Japan.*

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