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SPECTROSCOPIC STUDIES OF PEPSIN AND ITS COMPLEX WITH *STREPTOMYCES* PEPSIN INHIBITOR

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Summary

Binding of *Streptomyces* pepsin (EC 3.4.23.1) inhibitor to the active site of pepsin caused a characteristic ultraviolet difference spectrum having a trough around 298 nm which suggests that tryptophan residue(s) are involved in a decreased refractive index or different charge density. The fluorescence spectrum from tryptophan residues excited at 280 nm was nearly equal to that of the pepsin-inhibitor complex. Relatively large circular dichroism (CD) spectrum change at 280—310 nm was observed upon binding of the inhibitor. Solvent perturbation difference spectra of pepsin alone and the pepsin-inhibitor complex obtained with 20% ethylene glycol as perturbant showed that the exposed 2.5 tryptophan residues were not buried upon binding of the inhibitor, whereas 1.5 tyrosine residues were buried. It is speculated that the microenvironmental change around tryptophan residue(s) which are not located at the inhibitor binding site is induced upon binding of the inhibitor.

Introduction

Evidence for change in the microenvironment at the aromatic amino acid residues of the enzymes may be obtained by observing ultraviolet difference absorbance, fluorescence, and CD spectra [1]. *Streptomyces* pepsin inhibitor (acetylvalylvalyl-4-amino-3-hydroxyl-6-methylheptanoylalanyl-4-amino-3-hydroxyl-6-methylheptanoic acid) has been known to bind strongly to the active site of pepsin (EC 3.4.23.1) and other acid proteases of microbial origin [2—4]. In this communication, the interaction of pepsin and *Streptomyces* pepsin inhibitor was studied with respect to the microenvironmental change around aromatic amino acid residues in pepsin.

Materials and Method

Three-times-recrystallized porcine pepsin (EC 3.4.23.1) was purchased from Miles. The purity (80%) of pepsin was determined by titration with *Streptomyces* pepsin inhibitor at pH 5.0 using zinc(II)-dye complex as an indicator [4]. *Streptomyces* pepsin inhibitor was a gift from Professor Muaro & Dr. Oda, University of Osaka Prefecture. Ultraviolet difference spectra were measured using Shimadzu UV 200 spectrophotometer, using matched quartz cells with path length of 10 mm. For measurement of fluorescence spectra, a Uniom Giken spectrofluorimeter was used. JASCO J-20 automatic recording spectropolarimeter was used for the measurement of CD spectra. All measurements were made at 25°C. The sample solutions were prepared in 0.05 M acetate buffer of pH 5.0.

Results

A characteristic difference spectrum which had first been reported by Satoi and Murao [5] was observed upon binding of pepsin with *Streptomyces* pepsin inhibitor at pH 5.0 as shown in Fig. 1.

She solvent pertubation difference spectra with 20% ethylene glycol pertubant were measured for pepsin alone and for the pepsin-inhibitor complex. It was confirmed by difference spectrum titration that the binding of the inhibitor to pepsin took place both in water and in 20% ethylene glycol. The numbers of exposed tryptophan and tyrosine residues in pepsin and in the pepsin-inhibitor complex were calculated according to the method of Herskovits and Sorensen [6,7]. It was estimated that 2.5 tryptophan residues in a total of 5 residues are exposed both in pepsin and the pepsin-inhibitor complex, and that 13.3 tyrosine residues are exposed in pepsin, and 11.8 residues in the pepsin-inhibitor complex in a total of 16 residues.

The fluorescence spectra of pepsin and the pepsin-inhibitor complex excited at 280 nm were measured. The fluorescence emission spectra attributable to tryptophan residues were observed around 350 nm. Although a slight increase (about 3%) of fluorescence intensity was observed upon binding with the inhi-

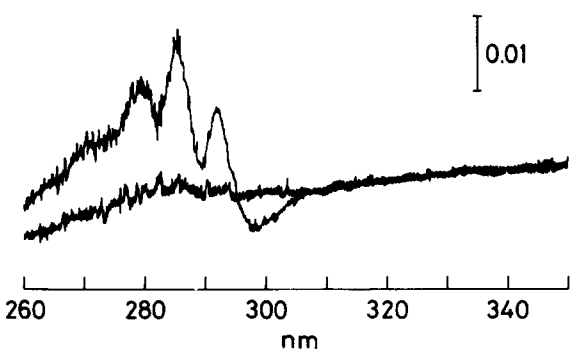

Fig. 1. Ultraviolet difference spectrum caused by the binding of *Streptomyces* pepsin inhibitor to pepsin at pH 5.0. The concentrations of pepsin and the inhibitor are $1.14 \cdot 10^{-5}$ M and $2.50 \cdot 10^{-5}$ M, respectively. Baseline is scanned with the cells containing pepsin only.

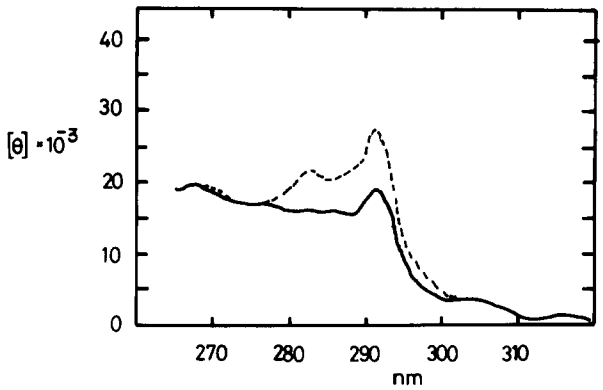

Fig. 2. Change in CD spectrum of pepsin upon binding with *Streptomyces* pepsin inhibitor at pH 5.0. The concentrations of pepsin and the inhibitor are $1.80 \cdot 10^{-5}$ M and $5.00 \cdot 10^{-5}$ M, respectively. Solid line: pepsin; dotted line: the pepsin-inhibitor complex.

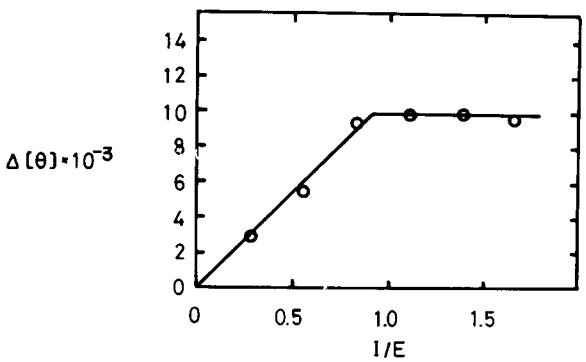

Fig. 3. Plot of difference ellipticity of pepsin $\Delta[\theta]$ at 291 nm vs. the molar ratio (inhibitor/pepsin). Enzyme concentration is fixed ($1.80 \cdot 10^{-5}$ M) and the inhibitor concentration is varied.

bitor, the effect of the inhibitor binding upon the fluorescence spectrum of pepsin may be negligible.

CD spectra in the near-ultraviolet region of pepsin alone and its complex with the inhibitor are shown in Fig. 2. The ellipticity of pepsin increases upon binding with the inhibitor. The plot of ellipticity at 291 nm against the molar ratio (inhibitor/pepsin) gives evidence of a 1 : 1 complex (Fig. 3).

Discussion

The difference spectrum caused by binding of *Streptomyces* pepsin inhibitor to pepsin has a trough around 298 nm which is characteristic of a change in the decreased refractive index or a different charge density [8]. Although 1.5 tyrosine residues are buried upon binding with the inhibitor, the number of exposed tryptophan residues is not changed upon binding with the inhibitor, i.e., exposed tryptophan residues are not buried upon binding with the inhibitor. The above results may indicate that exposed tryptophan residues are not located at the binding site for *Streptomyces* pepsin inhibitor.

CD spectral change at 280—310 nm upon binding with the inhibitor shows a microenvironmental or a conformational change around the tyrosine and/or tryptophan residues.

The fact that the microenvironment around the tryptophan residue(s) not located in the inhibitor-binding site of pepsin is changed upon binding with the inhibitor may suggest a ligand-induced conformational change of pepsin or a combination of conformational changes and direct shielding of some of tyrosine residues by the inhibitor.

Acknowledgment

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