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SPECTROSCOPIC STUDIES ON THE COMPLEXATION OF PAPAIN WITH POTASSIUM POLY(VINYL ALCOHOL SULFATE)

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ABSTRACT

The complexation of papains (EC.3.4.22.2) with potassium poly-(vinyl alcohol sulfate) (KPVS) in aqueous solutions as a function of pH was studied using fluorescence spectroscopy. A maximum emission at 333 nm was observed in aqueous diluted papain solutions (concentration $< 10^{-6}$ mol/dm^3) not containing KPVS. As the concentration of papain increased, the positions of the maximum emission were blue shifted; that is, the difference in wavelength from 333 nm increased with an increase

in the protein concentration. A similar shift was observed when KPVS was added to papain solutions at a constant concentration (10^{-6} mol/dm^3) with pH values which had been adjusted to 2 and 7. However, such a shift was not observed at pH 10. In addition, the effect of KPVS on the maximum emission shift was larger at pH 7 than at pH 2. These results are discussed in connection with the protein/protein interaction within an intrapolymer papain/KPVS complex.

INTRODUCTION

Research on the binding nature of polyelectrolyte/protein complexes has attracted considerable attention due to its practical applications in addition to its general academic interest. However, few studies [1, 2] have been reported on polyelectrolyte/protein complexation in solutions because of the complexities of such systems. Even though some attempts [3] have been made to understand complexation at the macroscopic level by using colloid titration, there are few molecular level studies on the conformational and environmental changes which occur during complexation. In the present study, fluorescence and UV spectroscopic techniques [4] were used in order to elucidate the intrapolymer complexation of proteins with strong polyelectrolytes such as potassium poly(vinyl alcohol sulfate) (KPVS). Papain, which contains inherent chromophores such as tryptophan and tyrosine [5, 6], was used for this purpose. The pH dependence of complexation over a wide range (from pH 2 to pH 10) was studied. The binding nature of papain/KPVS complexes is discussed based on the UV and fluorescence experiments.

EXPERIMENTAL

KPVS was purchased from Wako Pure Chemical Industries, Ltd. The number-average molecular weight, equivalent molecular weight, and degree of esterification were 229,500 Daltons, 166, and 0.922, respectively. The polymer sample was dried with phosphorous pentoxide in vacuum for 2 days before use. Papain was purchased from Sigma Chemical Co. The molecular weight of this enzyme was 23,400 Daltons.

Water deionized and then passed through a Millipore filter was used as the solvent. Most sample solutions were bubbled with nitrogen gas before spectroscopic measurements in order to minimize the effects of oxygen on fluorescence spectra.

These spectra were measured using a SPEX Fluorolog spectrophotometer, and a Cary model 3 spectrophotometer was used for the measurement of UV absorption spectra. A high quality quartz cuvette with a 2- or 10-mm path length was used for both spectroscopic measurements.

RESULTS AND DISCUSSION

The UV spectrum of an aqueous papain solution showed an absorption band around 280 nm. In order to identify the origin of this band, the UV spectra of tryptophan/tyrosine mixtures with different ratios were also measured. It was found

that both tryptophan and tyrosine (or mixtures of the two) exhibit UV absorption at 280 nm, indicating that the observed absorption band in the spectrum for the papain solution is due to both tyrosine and tryptophan residues.

Taking into account the results described above, the wavelength for the excitation in our fluorescence spectroscopic studies was fixed at 280 nm. Prior to the examinations of the aqueous papain solutions, the emission spectra excited at 280 nm were measured using the tryptophan/tyrosine mixtures. The emission maximum was observed around 340 nm in the spectrum of free tryptophan, but at this wavelength free tyrosine had few emission bands (the spectrum of tyrosine excited at 280 nm showed the emission maximum at 307 nm). In the case of the emission spectrum for an aqueous diluted papain solution with a concentration $< 10^{-6}$ mol/dm^3, on the other hand, the emission maximum was found at 333 nm (see Fig. 1). It was thus revealed that this band was due to the tryptophan residues in the protein molecules. A difference in the emission maximum (7 nm) of the spectra of papain and free tryptophan is related to whether the tryptophan molecules exist in a bulk solution or in a peptide chain.

As shown in Fig. 2, the maximum emission positions were blue shifted when the concentration of the papain solution was increased. This observed blue shift seems to be due to an environmental change in the tryptophan residues in papain as a result of the protein/protein interaction. It is well known that the overall emission fluorescence sources of papain consist of three different classes of tryptophan residues in one protein molecule [7, 8] (see Fig. 3): Class I (Trp 26) is located near the hydrophobic center of the papain; Class II (Trp 69) and Class III (Trp 7, 177 and 181) are located at the outer surface of the papain and surrounded by a bulk

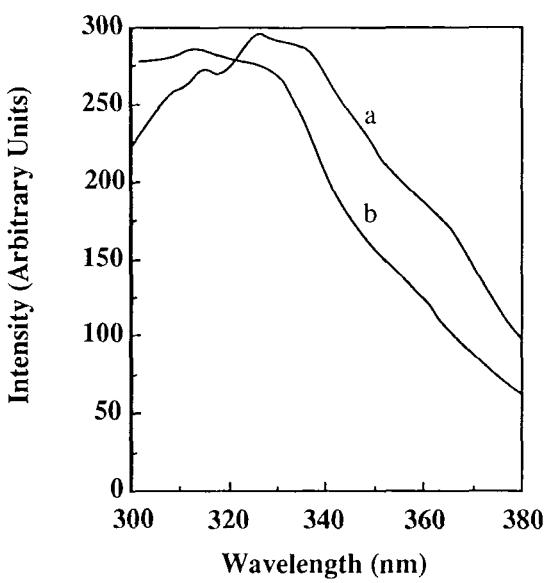

FIG. 1. Fluorescence emission spectra of native papain (a) and papain/KPVS complex (b) excited at 280 nm. Papain concentration, 10^{-6} mol/dm^3; KPVS concentration, 0.0025 mol/dm^3 (based on sulfate groups); papain:KPVS = 1.5:0.8 (volume ratio).

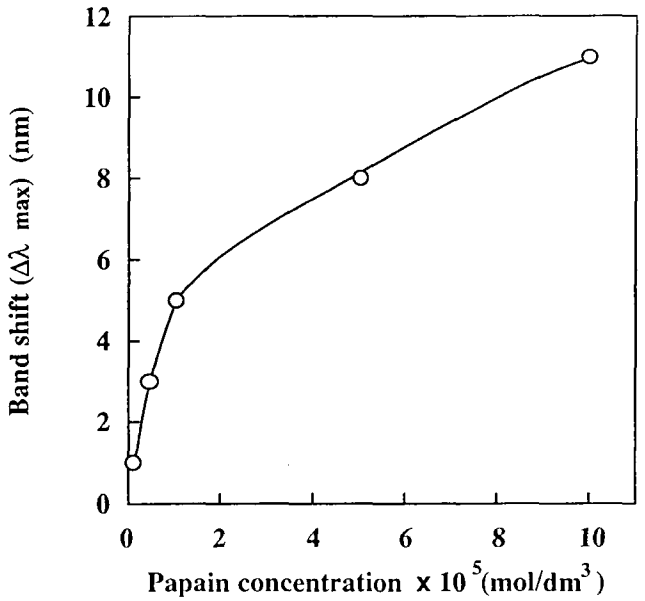

FIG. 2. Change in shifting of maximum emission wavelength ($\Delta\lambda_{max} = \lambda_{max} - \lambda_{333}$) with papain concentration.

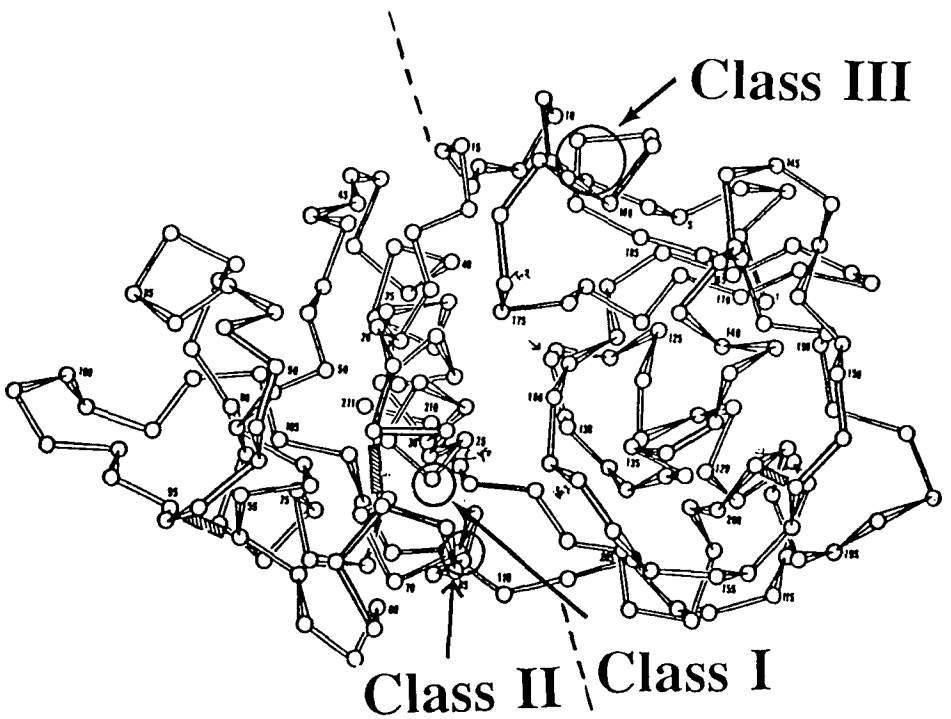

FIG. 3. Inherent fluorescence chromophores due to tryptophan residues in papain. The shaded areas represent S—S linkages in papain.

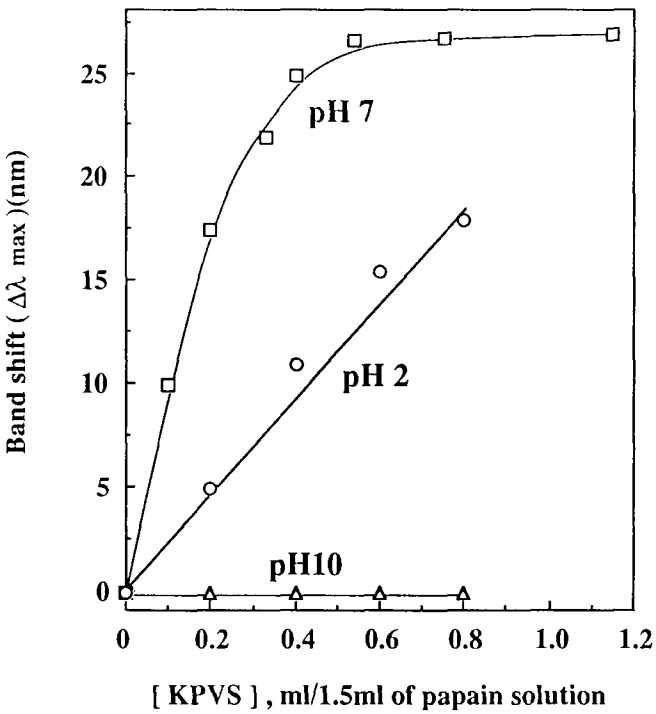

FIG. 4. Changes in $\Delta\lambda_{max}$ with KPVS concentration at different pH levels.

aqueous medium. At low concentration the papain molecule behaves as a monomer; thus, Classes II and III are exposed to the aqueous phase. However, at high concentration, a protein/protein interaction, i.e., multimer formation, may also be possible. As a result, Classes II and III may be covered with hydrophobic residues of other papain molecules. Similar trends can be expected even at a low protein concentration not bringing about such multimerization if the papain molecules are bound together by one KPVS anion to form an intrapolymer complex.

As shown in Fig. 1, a blue shift was observed in an aqueous solution containing both papain and KPVS. This finding is in conformity with the above expectations and indicates the formation of an intrapolymer papain/KPVS complex. Thus, a change in the emission maximum caused by the KPVS concentration was studied as a function of pH. The results obtained are summarized in Fig. 4. A band shift was observed in emission spectra at pHs 7 and 2, whereas at pH 10 there was no shift in the spectra. The shift at pH 7 showed an initial increase due to the addition of the KPVS solution, an increase which later leveled off. At pH 2, however, a linear relationship was found over a wide range of KPVS concentrations.

Our recent studies on the colloid titration of papain with KPVS have shown that the degree of binding of $-O_3SO^-$ groups in KPVS to one molecule of papain increases with a decrease of pH from 8 to 2 and reaches a constant value at pH < 2 [9]. Such a pH change in KPVS binding was found to depend upon the degree of protonation of the basic groups in the protein. Contrary to KPVS binding, the number of papain molecules bound to (or complexed with) one molecule of KPVS should increase with a increase in pH. This is because 1) the number of papain-attached, protonated basic groups increases with a decrease in pH, but 2) the state

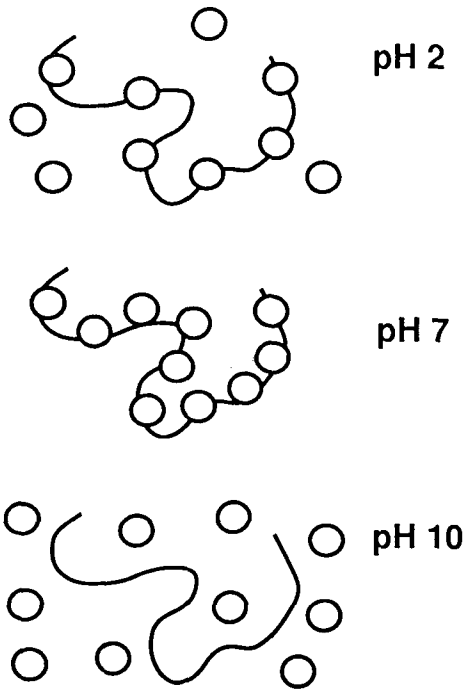

FIG. 5. Schematic illustration for understanding the complexation between papain and KPVS as a function of pH.

of dissociation of OSO_3^- groups in KPVS as the strong polyanion is unchanged over a wide range of pH. As a result, a larger number of papain molecules is necessary to neutralize the total of the anionic charges in KPVS at pH 7 than at pH 2 (see Fig. 5). It is reasonable to consider that an increase in the number of the protein molecules complexed with the polyanion enhances the protein/protein interaction, the features of which are similar to the increase in papain concentration described above; that is, a decrease in pH seems to bring about an environmental change in the tryptophan residues in papain within the intrapolymer complex.

At pH 10, the papain free of the protonated basic groups will not bind to KPVS; therefore, there was no shifting in the emission maximum caused by the KPVS concentration.

On the basis of the results obtained here and those from previous studies with other strong polyanions [10], it may be concluded that fluorescence spectroscopy is a good tool for the study of protein/polyelectrolyte complexation.

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