Note

Ultraviolet Difference Spectroscopic Analysis of the Binding of a Substrate Analogue to an Active Lysozyme Derivative: Kyn 62-Lysozyme

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X-Ray crystallographic data for hen egg-white lysozyme (EC 3.2.1.17) and its complexes with substrate analogues have indicated that the indole NH groups of Trp 62 and Trp 63 are hydrogen bonded to O (6) and O (3) of the GlcNAc residue at subsite C in the oligomer of GlcNAc, respectively, and that there is a nonpolar interaction between Trp 62 and the GlcNAc residue at subsite B.1,2 Chemical modification data have also suggested the importance of Trp 62 in enzymatic function of lysozyme.3,4 On the other hand, there is evidence suggesting that the hydrogen bond between Trp 62 and GlcNAc is weak and that it does not significantly contribute to the binding energy at subsite C.3,4 Recently, we found that a modified lysozyme (Kyn 62-lysozyme), in which Trp 62 was selectively converted to kynurenine, showed fairly high enzymatic activity.5,6 Because of its spectroscopic characteristic due to the kynurenine, Kyn 62-lysozyme was expected to be a good derivative for shedding further light on the structure and function relationship of lysozyme. We report here UV-difference spectroscopic data for the binding of (GlcNAc)3 to Kyn 62-lysozyme.

Hen egg-white lysozyme (6 x crystallized) was obtained from Seikagaku Kogyo Co., Tokyo. Kyn 62-lysozyme was prepared as reported previously.5,6 (GlcNAc)3 was prepared by the method of Rupley7 and Rafetry et al.8 The concentrations of intact lysozyme and Kyn 62-lysozyme were determined spectrophotometrically using absorption coefficients of 26.9 at 280 nm9 and 3.29 at 360 nm,10 respectively. The concentrations of the proteins and (GlcNAc)3 were 2.8 x 10^{-5} M and 9.5 x 10^{-3} M, respectively. The details were given in the text. Plots of the magnitude of the difference spectra versus the concentration of (GlcNAc)3 are shown in the inset. (●), at 293 nm for intact lysozyme; (○), at 293 nm for Kyn 62-lysozyme; (△), at 390 nm for Kyn 62-lysozyme.

Fig. 1. UV-Difference Spectra of Kyn 62-Lysozyme and Intact Lysozyme Induced by (GlcNAc)3.

The difference spectrum of a mixed solution of Kyn 62-lysozyme (or intact lysozyme) and (GlcNAc)3 was measured versus the same concentration of Kyn 62-lysozyme (or intact lysozyme). The spectra for Kyn 62-lysozyme (A) and intact lysozyme (B) were taken in 0.1 M acetate buffer (pH 5.5) and at 25°C. The concentrations of the proteins and (GlcNAc)3 were 2.8 x 10^{-5} M and 9.5 x 10^{-3} M, respectively. The details were given in the text. Plots of the magnitude of the difference spectra versus the concentration of (GlcNAc)3 are shown in the inset. (●), at 293 nm for intact lysozyme; (○), at 293 nm for Kyn 62-lysozyme; (△), at 390 nm for Kyn 62-lysozyme.

Abbreviations: Kyn 62-lysozyme, an active lysozyme derivative, in which Trp 62 is converted to kynurenine; (GlcNAc)3, β(1 → 4) linked n-mer of N-acetyl-D-glucosamine; UV, ultraviolet.

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with increasing concentration of (GlcNAc)$_3$ until the binding site of the modified lysozyme was saturated with the ligand (inset in Fig. 1), although the magnitude of the peak at 293 nm was approximately 60% that in the case of the difference spectrum generated by intact lysozyme. These observations suggest that the kynurenine at position 62 in Kyn 62-lysozyme interacts with the substrate analogue not individually but cooperatively with other tryptophan residue(s). Taking into consideration the fact that Trp 62 and Trp 108 are dominant chromophores contributing to the peak at 293 nm in the difference spectrum of lysozyme induced by the saccharide binding, the peak at 293 nm observed in the difference spectrum of Kyn 62-lysozyme would be attributable to the perturbation of Trp 108.

From the changes in the magnitudes of the peaks at 293 and 390 nm with the saccharide concentration, the binding constants ($K_{ES}$) for the interaction of Kyn 62-lysozyme and intact lysozyme with (GlcNAc)$_3$ were calculated by means of Scatchard plots. The binding constants for Kyn 62-lysozyme and intact lysozyme were determined to be $7.9 \times 10^3$ M$^{-1}$ and $1.8 \times 10^5$ M$^{-1}$, respectively, at pH 5.5 and 25°C. These values showed good agreement with those obtained on fluorescence spectroscopy. Assuming that Kyn 62-lysozyme interacts with (GlcNAc)$_3$ at subsites A, B and C, similar to intact lysozyme, the smaller $K_{ES}$ value for Kyn 62-lysozyme might be attributable to the difference in structure between tryptophan and kynurenine at position 62. On X-ray crystallographic analysis of N-acetylkynurenine, Kennard et al. showed that the carbonyl oxygen is hydrogen bonded to the aromatic amino group of kynurenine to form a structure like tryptophan. There is other evidence suggesting that oxidation of Trp 62 in lysozyme by N-bromosuccinimide affects the proton resonance of Trp 62. There is a possibility that conversion of Trp 62 in lysozyme to kynurenine caused the change in the state of Trp 63 that led to the decrease in the binding ability as to (GlcNAc)$_3$ at subsite C. From the results of circular dichroism measurement, Teshima et al. suggested that the side chain of kynurenine at position 62 in Kyn 62-lysozyme moves toward Asp 101 to interact with the GlcNAc residue at subsite B. It can be concluded that such an interaction of kynurenine with the GlcNAc residue in the substrate analogue brought about the change in the state of kynurenine at position 62 in the modified lysozyme that led to the UV-difference spectrum with a maximum at 390 nm.

REFERENCES