Novel Behaviour of Naphthoylmethyl Pendants Introduced into the Papain Active Site

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Spectroscopic analysis of the reactions of papain with bromoacetyl-substituted naphthalenes, which alkylate the cysteine-25 residue of this enzyme, allows us to propose that the charge-transfer complex formed between the naphthoylmethyl pendant and the histidine-159 residue is responsible for the appearance of a new long-wavelength absorption band.

In a previous study,[2g] we showed that the active site of papain remarkably promotes the formation of the ground-state dimer derived from the pyrenoylmethyl pendant attached to the cysteine-25 residue (Cys-25) of this enzyme. If we introduce a naphthoylmethyl pendant, which has a much lower association ability than that of the pyrenoylmethyl, into the papain active site, it is expected that we might be able to explore the interaction between the pendant and the surrounding amino acid residues without undergoing interference from the dimeric association process.

As shown in Fig. 2 (see full text), the reaction of activated papain with 2-bromoacetylnaphthalene (2-BAN) in an N_2-purged phosphate buffer showed first a discernible shoulder near 340 nm and then there appeared a new absorption band at 503 nm with a decrease in absorption at shorter wavelength side, giving an isosbestic point at 393 nm. The modified papain obtained from the reaction between papain and iodoacetamide was treated with 2-BAN but showed no indication of the incorporation of a 2-naphthoyl chromophore into the modified enzyme.[2g] In addition, the reference compound 2-[2-(*N*-acetyl)aminoethylthioacetyl]naphthalene (2-TAN) exhibited no long-wavelength absorption. These findings strongly suggest that the 503 nm band results from the interaction between the naphthoylmethyl pendant attached to Cys-25 and the amino acid residue(s) located at the active site. Analysis of the fluorescence behaviour of this pendant and 2-TAN demonstrates that the naphthoylmethyl pendant monomer and newly-formed species should coexist at equilibrium (Fig. 3, see full text). On the other hand, the reaction of papain with 1-bromoacetylnaphthalene (1-BAN) took place slowly to afford a new absorption at 500 nm and a shoulder near 340 nm (Fig. 5, see full text), confirming that both the reactions with 1-BAN and 2-BAN proceed by the same mechanism to give long-wavelength absorption bands.

The 1-naphthoylmethyl-*carbonyl*-^{13}C pendant attached to Cys-25 exhibited its carbonyl carbon signal at 175.4 ppm (Fig. 7, see full text). The appearance of the carbonyl carbon signal in ^{13}C-labelled 1-TAN at 203.4 ppm indicates that when the naphthoyl chromophore is introduced into the papain active site, the carbon signal is subject to a large upfield shift. It is reasonable to interpret the upfield shift of 28.0 ppm in terms of the strong hydrogen bonding (to the P_1 carbonyl oxygen) that considerably increases the contribution of the enolate-type resonance structure.[4,5] Taking into account that on forming strong hydrogen bonds the naphthalene ring has a carbonium-ion character and then serves as an electron acceptor, we are led to propose a charge-transfer (CT) complex formed between the pendant and the neighbouring amino acid residue to explain the observed long-wavelength absorption band. The three-dimensional structure of papain treated with chloromethyl ketone substrate analogues suggests that the histidine-159 imidazole ring is located near the pendant naphthalene ring so that the former ring is a most likely candidate for the electron donor that participates in the appearance of the CT complex (Fig. 9).[5] The dependence of the 500 nm absorption on reaction time reveals that the absorption at equilibrium is increased with increasing pH in the range of 6.5 to 7.5 (Fig. 10, see full text). A similar result was obtained also for 1-BAN-modified papain (Fig. 11, see full text). These observations are consistent with the involvement of the unprotonated histidine-159 residue as an electron donor. The slow reaction could be owing to the formation of the CT complex with a conformational change in the active site.

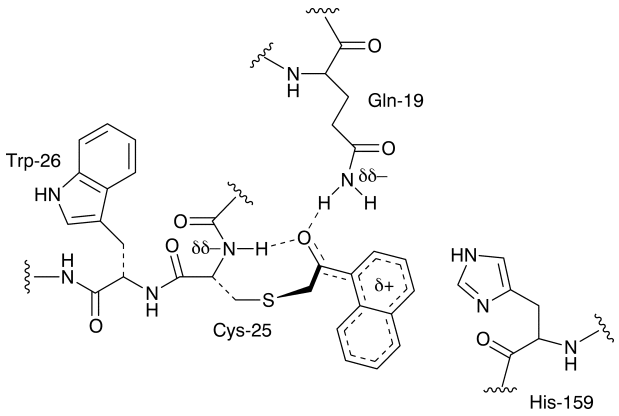

Fig. 9 Schematic illustration of the charge-transfer complex

Techniques used: UV–VIS, fluorescence, circular dichroism and ^{13}C NMR

References: 10

Fig. 1: Structure of 2-BAN, 1-BAN and 2-TAN

Fig. 2: UV–VIS absorption spectral changes of 2-BAN-modified papain and UV absorption spectrum of 2-TAN

Fig. 3: Fluorescence spectra of 2-BAN-modified papain and 2-TAN

Fig. 4: Circular dichroism spectra of native and 2-BAN-modified papain

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Fig. 5: UV-VIS absorption spectrum of 1-BAN-modified papain

Fig. 6: Structure of ^{13}C-labelled 1-TAN

Fig. 7: ^{13}C NMR spectra of 1-BAN-modified papain and 1-TAN

Fig. 8: Structure of enolates **I** and **II**

Fig. 10: Absorbance of 2-BAN-modified papain at 500 nm as a function of reaction time

Fig. 11: Absorbance of 1-BAN-modified papain at 500 nm as a function of reaction time

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