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Tryptophan Modification and Fluorescence Spectrum of Hyaluronidase

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Abstract: Tryptophan residues in hyaluronidase (HAase) were modified by N-bromosuccinimide (NBS), the results indicated that there were eleven tryptophan residues in HAase, one of which was exposed and essential for the activity of the enzyme. The study on fluorescence quenching showed that KI could not quench all of the fluorescence from Trp residues in HAase, while acrylamide (Acr) could quench almost all of the fluorescence from Trp residues in HAase. The collisional quenching constants (K_D) of HAase at different concentrations of Acr were calculated in terms of Stern-Volmer equation. The results implied that some of the Trp residues were buried in the interior of HAase and the Trp residue on the surface of HAase was not located in the hydrophobic pocket.

Keywords: Hyaluronidase (HAase), tryptophan (Trp), fluorescence spectrum.

HAase is a group of *endo*-mucopolysaccharidazes. The pharmaceutical and food industry applications of hyaluronidase has been noted[1], while there are few investigations on the study of Trp modification and fluorescence spectrum. The purpose of this work is to reveal the relationship between Trp residues and the enzyme activity by means of modification of Trp residues with NBS[2] and discuss the relationship between the conformation and the properties of HAase by means of fluorescence quenching.

HAase(0.2 mg/mL) was treated by NBS(10 mmol/L) in 50 mmol/L acetate buffer (pH 5.2) at 30℃ for 30 min. The denatured enzyme was obtained by treating the native HAase with boiling urea (8 mol/L) for 5 min and then dialyzed to remove the urea. Then the denatured enzyme (0.09 mg/mL) was modified by NBS(10 mmol/L). The number of the modified Trp residues were calculated in terms of Spande's method[3]. The results indicated that there were eleven Trp residues in the denatured enzyme. When the native enzyme was modified by NBS, only one residue was modified, it indicated that the modified residues in the native enzyme was located on the surface of the enzyme, while the other ten residues were located inside of the enzyme, which could not be modified because of the tight conformation of the native enzyme..

The activity of HAase and modified HAase were assayed[4]. After modification of the exposing Trp residue, the activity of HAase was completely lost.

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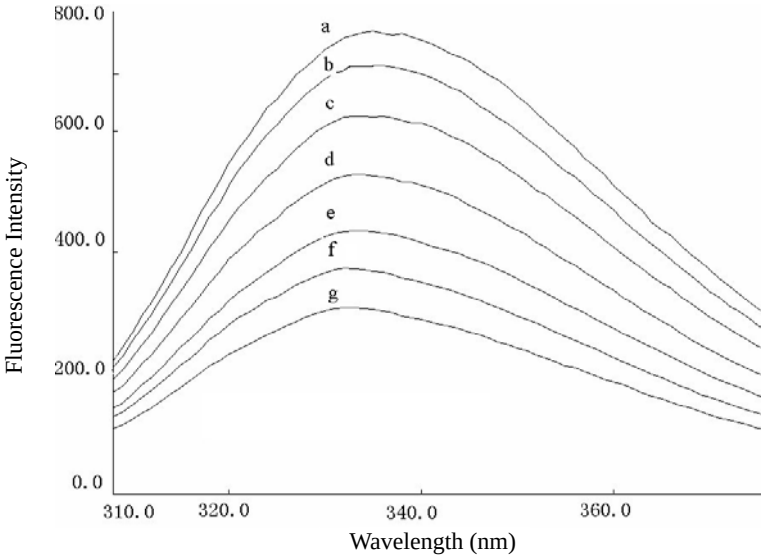
Figure 1 The influence of the adding amount of NBS to the fluorescence spectra of HAase



V_{NBS}/μL: a.0 ; b.5 ; c.10 ; d.15 ; e.20 ; f.25 ; g.30. C_{HAase}: 0.2 mg/mL

The fluorescence intensity of HAase were assayed when it was treated with different concentrations of NBS（**Figure 1**）. The results indicated that the fluorescence intensity of HAase decreased with the increase of the concentrations of NBS,that the maximum emitting wavelength had moved from 336 nm to 332 nm.

HAase(0.033 mg/mL) was treated with the typical collisional quencher KI. The results indicated that the fluorescence intensity of HAase decreased with the increase of the concentrations of KI(**Figure 2**), but KI could not quench all the fluorescence of HAase. It also implied that some of the Trp residues were buried in the interior of Haase and the Trp residue on the surface of HAase was not located in the hydrophobic pocket. The activity of HAase also decreased with the increase of the concentrations of KI, when the concentration of KI reached 2.33 mol/L, the activity remained 42.37% and changed no longer with the increase of KI. The impact of KI on enzyme activity can be proposed that KI change the enzyme conformation in collision.

In addition, we tried to quench the fluorescence of HAase (0.033 mg/mL) with Acr, a polarized quencher without electric charge. The fluorescence intensity of HAase also decreased with the increase of the concentrations of Acr, the curve of F_0/F-C_{Acr} was linear, when C_{Acr} was in the range of 0-1.07, which implied that the fluorescence of HAase with Acr obeyed Stern-Volmer equation[5]:

$$F_0/F=1+K_D[Q]$$

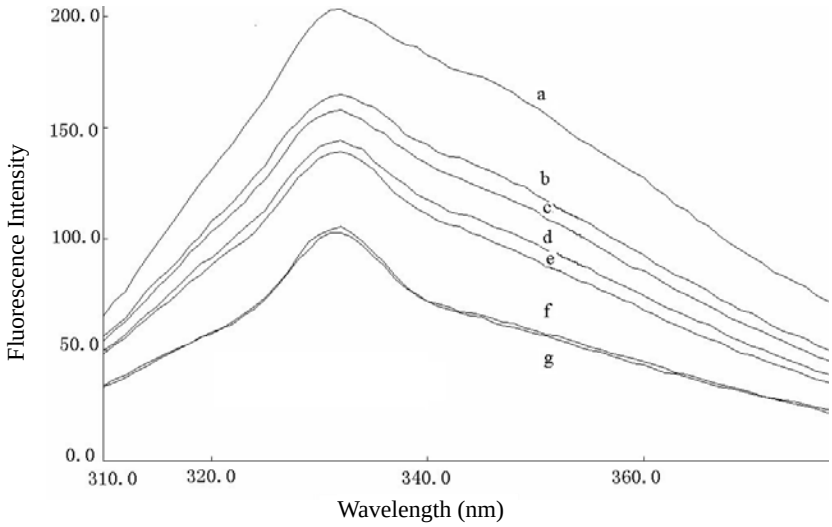
Where [*Q*] stands for the concentration of the quencher; K_D stands for the collisional quenching constant. K_D of HAase at different concertrations of Acr were calculated in terms of Stern-Volmer equation and the results indicated that increase of K_D was non-linear with the increase of the concentrations of Acr. Modified Stern-Volmer

equation can be deduced from Stern-Volmer equation[6] as follows:

$$F_0/\triangle F=1/(fa \times K[Q])=1/fa$$

Where K stands for Stern-Volmer constant; $\triangle F$ stands for the decrease of fluorescence intensity with quencher; fa stands for the percentage of the chromogenic residues which could be quenched. Constructing a plot of $F_0/\triangle F$-1/[Q], the intercept 1/fa= 1.104 and fa=0.986, which implied that Acr could quench almost all the fluorescence of HAase. The activity of HAase also decreased with the adding of Acr, while the impact was ruleless, it may be caused by the partial change of the enzyme conformation.

Figure 2 The influence of the adding amount of KI to the fluorescence spectra of the modified HAase



V_{KI}/mL: a.0 ; b.0.2 ; .0.4 ; d.0.6 ;e.1.0 ; f.2.0 ; g.3.0. C_{HAase}: 0.033 mg/mL

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