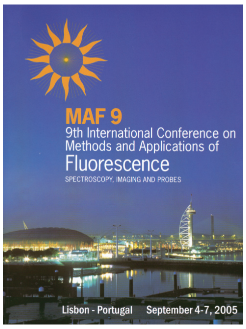

FIBRINOGEN-β–ESTRADIOL BINDING STUDIED BY FLUORESCENCE SPECTROSCOPY

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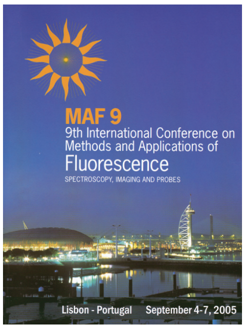
ABSTRACT

Fibrinogen is a blood plasma protein that plays a crucial role in hemostasis. It is known that erythrocyte aggregation increases in the presence of fibrinogen and that β estradiol decreases the erythrocyte aggregation with a constant fibrinogen concentration. In this work we have used intrinsic tryptophan fluorescence to obtain information on the conformational changes of the fibrinogen protein upon the recently proposed interaction with β estradiol. To evaluate the effect of the conformational changes during fibrinogen β estradiol binding fluorescence experiments were performed by using Gdm HCl (0 6M) as denaturant at different pH values. The results obtained with the different pH values shows no effects for pH 6.5 and 8.0. The main differences were encountered when pH is equal to 4.2 and 7.4 in absence and in presence of two different denaturant concentrations (1 M and 5 M). Also a red shift from 344 nm to 354 nm is observed when denaturant concentration is above to 3 M, this phenomenon occurs when the protein, in presence of denaturant, lost its compact structure and the *Trps* residues are exposed to the environments altering fibrinogen-β-estradiol binding. These results suggest the existence of conformational states in the fibrinogen molecule during the interaction with others molecules.

METHODS

Measurements Absorbance measurements were carried out in a Genesys 10 UV ThermoSpectronic at 280nm. Fluorescence measurements were carried out in a Varian Carry Eclipse Fluorescence Spectrophotometer. The excitation wavelength was set to 280 nm and emission spectra were acquired between 300 400 nm. For fibrinogen-β-estradiol studies emission spectra were recorded in absence and presence of different denaturant concentrations (0 6 M) at different buffers: Acetate buffer pH 4.2, Phosphate buffer pH 6.5, 8.0 and Trisbase HCl buffer pH 7.4.



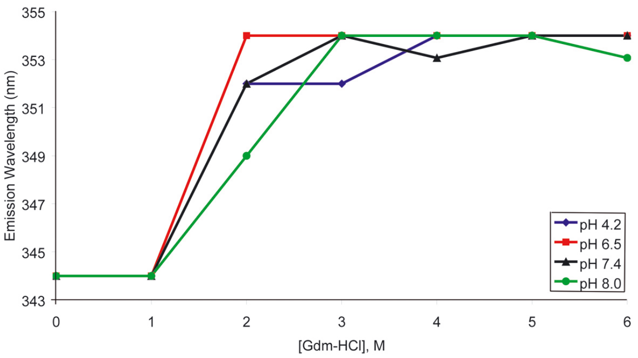


Figure 1. Emission wavelength values as a function of Gdm HCl concentration for fibrinogen at different buffers: pH 4.2 (Acetate buffer); pH 6.5 and 8.0 (Phosphate buffers), pH 7.4 (Trisbase HCl buffer, λ_{exc}=280).

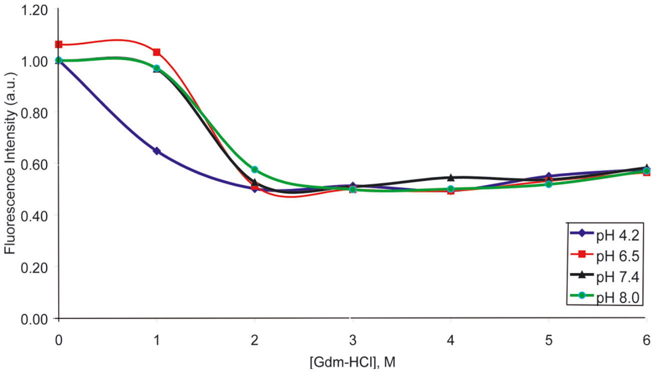


Figure 2. Fluorescence intensity values as a function of Gdm HCl concentration for Fibrinogen at different buffers: pH 4.2 (Acetate buffer); pH 6.5 and 8.0 (Phosphate buffers), pH 7.4 (Trisbase HCl buffer, λ_{exc}=280).

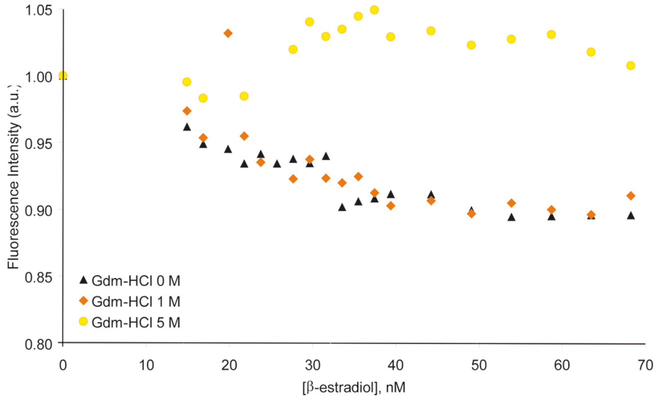


Figure 3. Fluorescence intensities for fibrinogen-β-estradiol binding in presence of three different Gdm HCl concentrations, pH 4.2 (Acetate buffer, λ_{exc}=280).

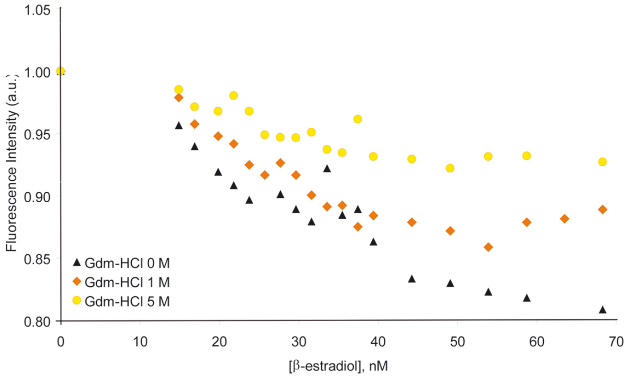
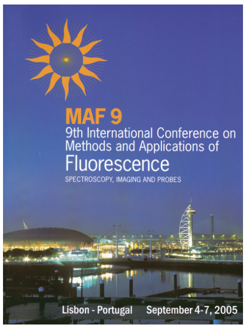


Figure 4. Fluorescence intensities for fibrinogen-β-estradiol binding in presence of three different Gdm HCl concentrations, pH 7.4 (Trisbase HCl buffer, λ_{exc}=280).



CONCLUSIONS

- In the range of Gdm HCl concentrations studied Fibrinogen maxima emission wavelength suffers a red shift from 344 nm to 354 nm as a result of a change in the compact structure of the protein.
- The presence of different denaturant concentrations shows during fibrinogen-β-estradiol binding alterations that can be related with either possible conformational changes or a discrete reorganization of tryptophan residues during fibrinogen-ligand binding.

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