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FLUORESCENCE QUENCHING STUDY OF FIBRINOGEN-LIGAND BINDING

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Structural studies of proteins have played an important role in the development of new techniques, which have helped in the understanding of their structure and function.

The study of ligand binding to biomacromolecules is of the utmost importance to understand structure function relationships.

The study of ligand binding to Fibrinogen could provide information about the functionality of this protein during erythrocyte aggregation. In this work, we have used intrinsic tryptophan fluorescence to obtain information on the conformational changes of the Fibrinogen protein upon interaction with other molecules.

Fluorescence spectroscopy experiments were performed in order to study Fibrinogen properties in the presence of different β-Estradiol concentrations (1-77 nM). The protein shows diminished fluorescence intensities upon

binding to β-Estradiol. To evaluate the average accessibility of emissive tryptophans exposed during the fibrinogen-β-Estradiol binding, at different concentrations of the ligand, fluorescence quenching experiments were performed using cesium, iodide, succinimide and acrylamide (anionic, cationic and uncharged quenchers, respectively) at 0.8 μM – 0.160 M. The K_{sv} values obtained with acrylamide diminishes upon β-Estradiol concentration increase, while with succinimide as a quencher this phenomenon was not observed. The studies carried out with the charged quenchers indicate a β-Estradiol-dependent change in the fraction of tryptophan residues exposed to the aqueous environment. The results obtained with the different uncharged quenchers suggest that the environment can affect the conformational stability during fibrinogen-ligand binding. The quenching parameters obtained at different β-Estradiol concentrations (0, 22 and 65 nM) show alterations that can be related with possible conformational changes or a discrete reorganization of tryptophan residues during fibrinogen-ligand binding.

CONFORMATIONAL CHANGES DURING FIBRINOGEN-LIGAND BINDING FOLLOWED BY FLUORESCENCE SPECTROSCOPY

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INTRODUCTION

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The study of ligand binding to Fibrinogen could provide information about the functionality of this protein during erythrocyte aggregation. A previous *in vitro* study has shown an im-

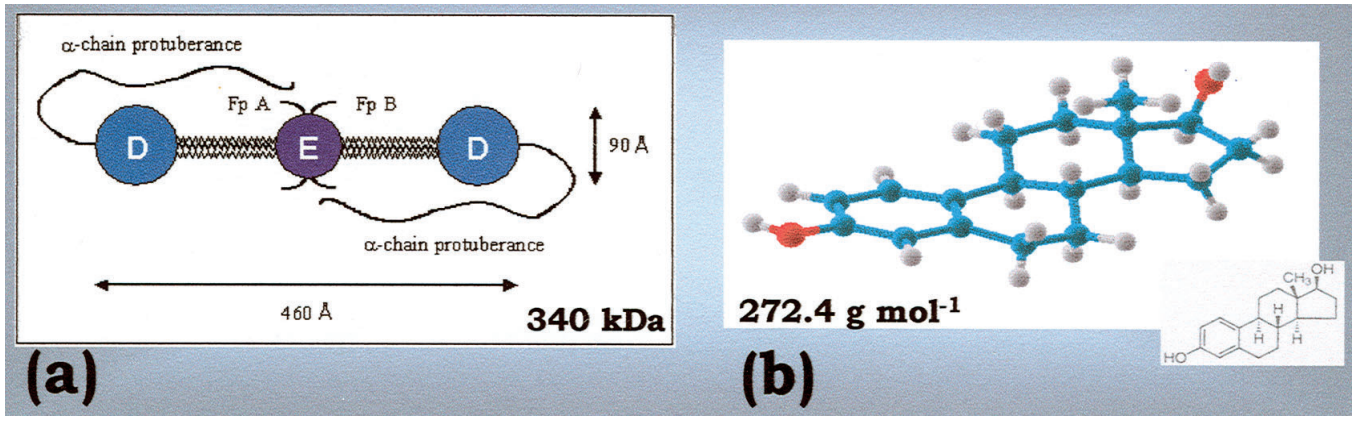

Figure 1 Structures of Fibrinogen (a) and β-Estradiol (b) molecules.

paired erythrocyte aggregation in the presence of β-Estradiol [1]. The study of ligand binding to fibrinogen could provide information about the functionality of this protein during erythrocyte aggregation. ***The aim of this work is*** **to use intrinsic tryptophan fluorescence to obtain information on the conformational changes of the Fibrinogen protein upon interaction with β-Estradiol.**

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. For fibrinogen-ligand studies emission spectra were recorded by setting the excitation wavelength to 280 nm and acquired the emission spectra between 300-400 nm. For **quenching experiences** the excitation wavelength was 295 nm and the emission wavelength was 344 nm.

Analysis: The results were fitted by using the Stern-Volmer and Lehrer[2] equations and the fraction of exposed quenchers were obtained for the second case:

$$\frac{I_0}{I} = \frac{1 + K_{SV}[Q]}{(1 + K_{SV}[Q])(1 - f_B) + f_B}; f_B = \frac{I_{0.B}}{I_0}$$

were *KSV* is the Stern Volmer constant, and *fB* the fraction of exposed residues.

RESULTS

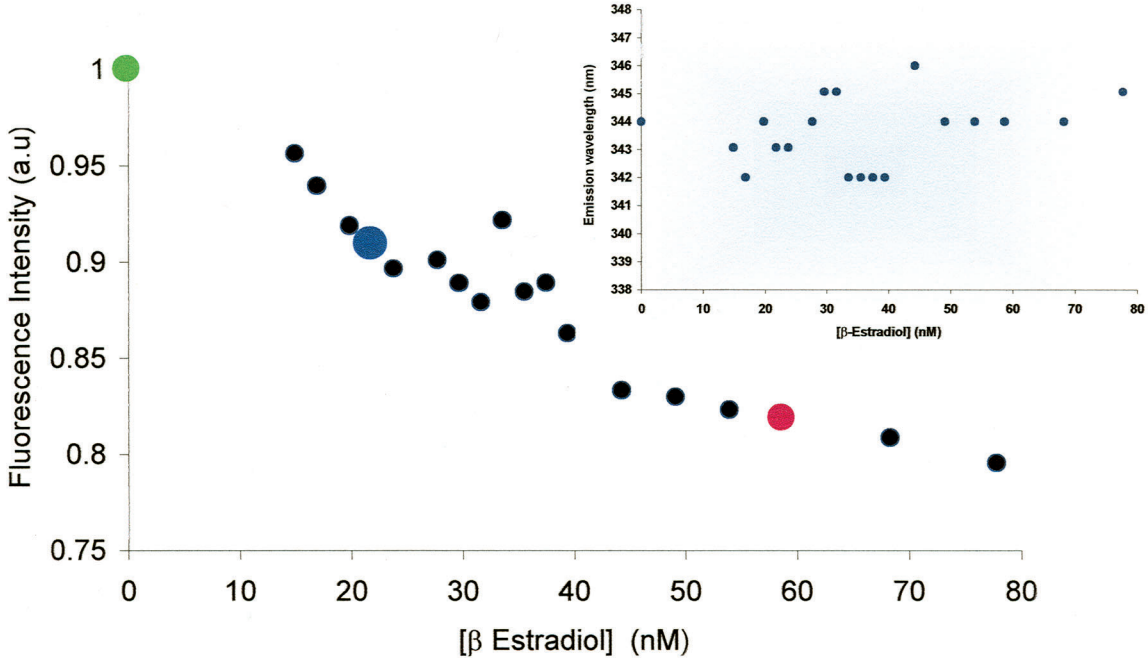

Figure 2. Fluorescence intensities for Fibrinogen-β Estradiol binding.

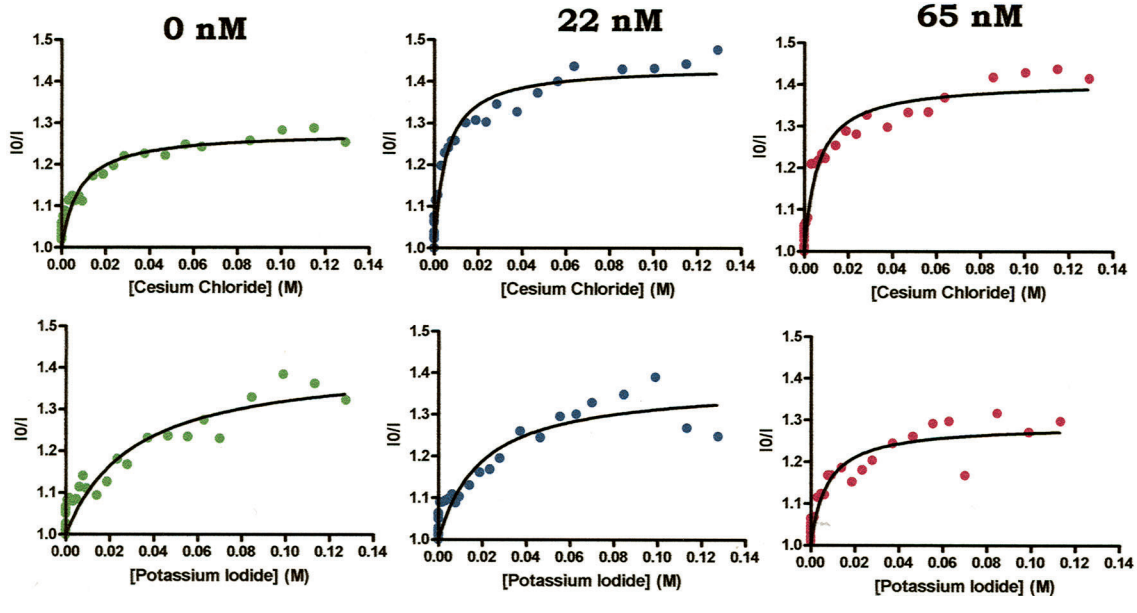

Figure 3. Fitting results obtained for the different Fibrinogen-ligand concentration.

Table 1. K_{SV} and f_B values obtained after fitting the experimental results.

Quencher	β–Estradiol concentration		
	0 nM	**22 nM**	**65 nM**
Acrylamide*	K_{SV} = 1953 ± 156	K_{SV}= 3502 ± 419	K_{SV} = 2764 ± 193
Succinimide*	K_{SV} = 981 ± 122	K_{SV} = 1758 ± 703	K_{SV}= 2212 ± 633
CsCl	K_{SV} = 158 ± 75 f_B= 0.22 ±0.02	K_{SV} = 268 ± 47 f_B = 0.30 ± 0.01	K_{SV}= 230 ± 41 f_B = 0.29 ± 0.01
KI/$Na_2S_2O_3$	K_{SV} = 46 ± 12 f_B = 0.29 ± 0.02	K_{SV}= 70 ± 17 f_B = 0.27 ± 0.01	K_{SV} = 176 ± 46 f_B = 0.22 ± 0.01

* Linear fitting

CONCLUSIONS

- In the range of the concentrations studied for Fibrinogen-β-Estradiol interaction no shifts in the fluorescence maxima were observed.
- The quenching parameters obtained at different β-Estradiol concentrations (22 or 65 nM) show alterations that can be related with either possible conformational changes or a discrete reorganization of tryptophan residues during fibrinogen-ligand binding.
- The Fibrinogen-β-Estradiol binding induces an enhanced exposure of tryptophan residues.
- There is a dependence on the K_{SV} constant with the quencher type.

REFERENCES

[1] Gonçalves, I., Saldanha, C. and Martins e Silva, J. 2001, Clinical Hemorheology and Microcirculation, 25,127

[2] Lehrer,S.S. 1971, Biochemistry, 10, 3254.