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Fluorescent study of the interaction between *Staphylococcus aureus* UMP kinase and UTP

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Fluorescence-based techniques applied to the proteins are giving information on their binding sites for ligands, interactions with solvent, the degrees of flexibility, internal motions, rotational diffusion coefficients, etc. The fluorescence behaviour of one member of NMP kinase family, namely, *Staphylococcus aureus* UMP kinase, was tested. NMP kinases are ubiquitous enzymes of the living cells, very important in the cellular energetic metabolism, and also in synthesis of nucleic acid precursors. It seems that *Staphylococcus aureus* UMP kinase has a different behaviour, as compared to UMP kinases from *Streptococcus pneumoniae* or from *Escherichia coli*. The binding of a specific ligand, UTP, to this UMP kinase seems to be a cooperative process, being accompanied by about 8-fold decrease in the intrinsic fluorescence, in UTP concentration range (50 – 1,500) μM. UMP kinase from *Staphylococcus aureus* is exhibiting an intrinsic fluorescence, due to its unique tryptophan, decreasing with the increase of UTP concentration. In the hypothesis of a collisional quenching of fluorescence, the fluorescence data were fitted by the *Stern-Volmer* equation. A very large *Stern-Volmer* constant, K_D, of 5×10^3 M^{-1} was obtained. Using a mean lifetime fluorescence of tryptophan, in neutral aqueous solutions, we found a bimolecular quenching constant, k_q, of 2×10^{12} M^{-1} s^{-1}. This value is larger than that predicted for a diffusion-controlled reaction, therefore implying a complex formation between the enzyme and the ligand.

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1. Introduction

Fluorescence techniques can provide valuable parameters consistent with crystallographic results. The various microenvironments of the fluorophores of a folded protein and the unique stereochemistry of the protein chain affect the fluorophore state in many ways, allowing us to characterize and monitor the changes in the protein folding [Lakowicz, 1983].

The fluorimetric methods are frequently used in (bio)chemical and medical research due to their high sensitivity and accessibility of time scale, the fluorescence process taking place in a time interval of about 10^{-8} s, consecutive to light absorption. During this period of time, a lot of physical and chemical processes can interfere, affecting the spectral characteristics of the fluorescent compound [Valeur, 2001].

The combination between the high sensitivity and the accessible time scale are permitting to the fluoroscopic methods to be adequate for the study of proteins and even of cellular membranes during their interactions with different other molecules [Lakowicz, 1991]. Thus, by fluorimetric studies, one can approach protein folding and their behaviour in different environments, protein-ligand interactions, and protein-lipid interactions into biological membranes.

Generally, it is known that proteins could contain three peculiar amino acids which are conferring to them the property to be fluorescent in the ultraviolet spectral range: Tryptophan (Trp), Tyrosine (Tyr) and Phenylalanine (Phe), the former of them dominating, if it is present, the fluorescent property of a protein [Lakowicz, 1991; Valeur, 2001].

2. The influence of UTP on the UMP kinase fluorescence

In this study the interaction between uridine monophosphate (UMP) kinase from *Staphylococcus aureus* and the uridine triphosphate (UTP) ligand was characterised by steady state fluorescence. UMP kinase is an enzyme pertaining to the nucleoside monophosphate (NMP) kinase family, ubiquitous in all organisms, being involved in cellular energetic metabolism as well as in the synthesis of nucleic acid precursors [Serina *et al*., 1996; Bucurenci *et al*., 1998].

As concerns the interaction with ligand, the UMP kinase from *Staphylococcus aureus* presents a quite different behaviour as compared to the UMP kinases from *Streptococcus pneumonia*e and *Escherichia coli* [Fassy *et al*., 2004].

UTP is one of the specific ligands of UMP kinases [Bucurenci *et al*., 1998; Evrin *et al*., 2007]. In the case of interaction of UTP with UMP kinases from *Streptococcus pneumoniae* and *Escherichia coli,* an increase of fluorescence emission with UTP concentration increase is observed [Fassy *et al*., 2004], while the fluorescence emission of UMP kinase from *Staphylococcus aureus* is decreasing under the same conditions.

UMP kinase from *Staphylococcus aureus* is a hexamer protein, possessing 214 amino acids in its sequence, with a molecular mass of 2.83 kDa, containing one Trp and 6 Tyr per monomer [Briozzo et al., 2005].

UMP kinase and the ligand, UTP, were dissolved in the following buffer solution: 50 mM Tris-HCl, 100 mM NaCl at pH = 7.4. UMP kinase concentration was kept constant (2 μM), while the UTP concentration was steadily increased from 0 to 1,500 μM (Table 1).

3. Results

The fluorescence measurements were performed with a Perkin Elmer LS 55 spectrofotofluorimeter, using a 1 cm pathway cuvette. The fluorescence emission spectra were recorded in the spectral range 290 - 600 nm, at the excitation wavelength, λ_{ex} = 285 nm.

UMP kinase from *Staphylococcus aureus* manifests an intrinsic fluorescence, the emission intensity maximum being situated around the wavelength, λ = 350 nm (Fig. 1A).

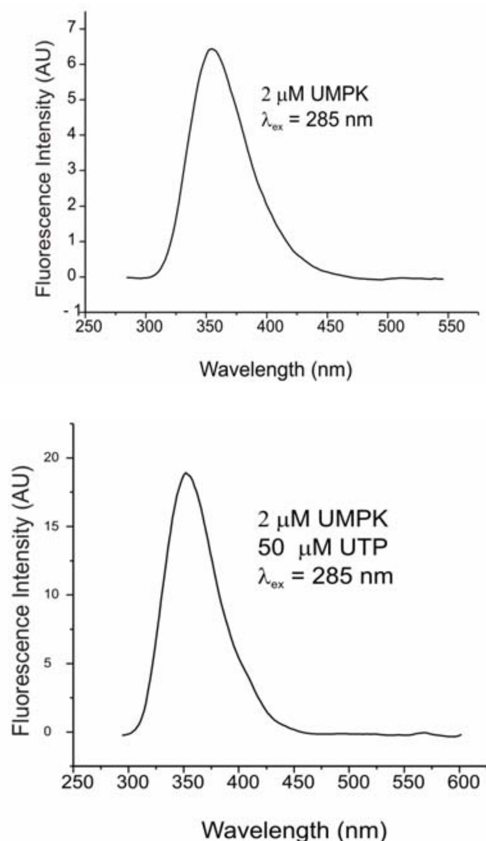

Fig. 1. A. Emission fluorescence spectrum of UMP kinase. AU: arbitrary units. B. Emission fluorescence spectrum of UMP kinase in the presence of the ligand, UTP, with the concentration of 50 μM.

It was noticed that the UMP kinase fluorescence intensity is decreasing as far as the UTP concentration is increasing (Fig. 1B and Fig. 2).

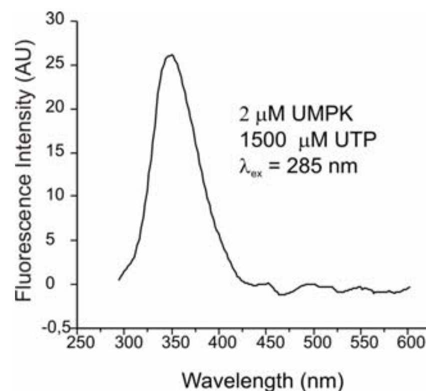

Fig. 2. Emission fluorescence spectrum of UMP kinase in the presence of the ligand UTP in the highest used concentration: 1,500 μM. AU: arbitrary units.

By recording a set of fluorescence spectra of UMP kinase in the presence of UTP at different concentrations, (Table 1 and Fig. 3) one observes a decrease of about 8 times of its fluorescence for UTP concentration of 1.500 μM.

Table 1. The evolution of the maximum of fluorescence intensity, F, and of ratio, F_0/F, where F_0 represents the maximum of the intrinsic fluorescence of UMP kinase.

No.	[UTP] / μM	Emission maximum, *F*	F_0/F
1	0	21.4	1.00
2	50	18.8	1.14
3	100	14.1	1.52
4	150	11.4	1,88
5	200	10.9	1.96
6	300	8.8	2.43
7	400	4.8	4.46
8	600	4.1	5.22
9	800	5.1	4.20
10	1,000	2.9	7.38
11	1,500	2.6	8.23

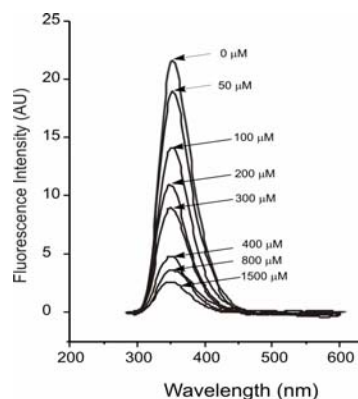

Fig. 3. The evolution of the fluorescence spectra of UMP kinase, in the presence of UTP of increasing concentrations, in the range (0-1.500) μM. AU: arbitrary units. For the sake of clarity, only a part of the recorded spectra are presented.

4. Discussion

The observed quenching of the fluorescence can be the result of many independent or concurrent/synergic mechanisms: a) dynamic collisional quenching; b) static quenching; c) quenching by energy transfer; d) quenching by charge transfer.

The dependence of fluorescence intensity, F, on the quencher concentration, $[Q]$, is given by the *Stern-Volmer* equation:

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

where K_D represents the *Stern-Volmer* constant.

On the basis of the mathematical relation between the *Stern-Volmer* constant, K_D, and the fluorescence lifetime, τ_0, one can determine the bimolecular quenching constant, k_q:

$$k_q = K_D / \tau_0$$

Under the hypothesis of a dynamic collisional quenching of UMP kinase fluorescence, induced by the interaction with UTP, the experimental data were fitted using the *Stern-Volmer* equation (Fig. 4).

From the data fit a very high value of *Stern-Volmer* constant ($K_D = 5 \times 10^3$ M^{-1}) was obtained.

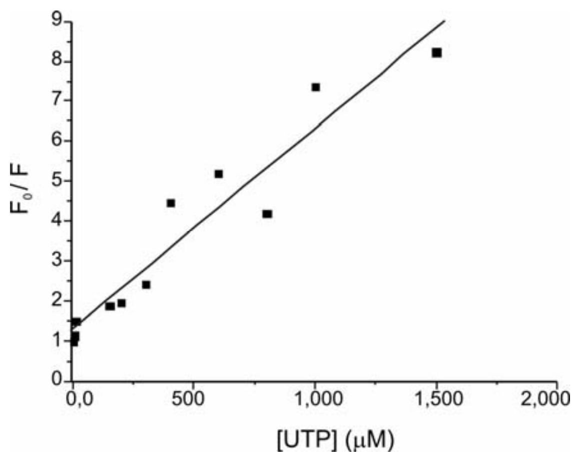

Fig. 4. The linear regression fit of the ratio, F_0 / F, versus the concentration of UTP. Black squares are representing the experimental data.

Using a value of fluorescence lifetime, τ_0, of 2.5 ns, observed in the case of protein with Trp, in neutral solutions, a bimolecular quenching constant was calculated, resulting the quenching constant value, $k_q \approx 2 \times 10^{12}$ M^{-1}s^{-1}. This high value is, two orders of magnitude, over the quenching constants of the processes limited by diffusion, which are of the order of 10^{10} M^{-1}s^{-1}.

The results confirm the different spectrofluorimetric behaviour [Gagyi *et al.,* 2003] of UMP kinases from Gram-negative bacteria (e.g., for *Escherichia coli*, λ_{em} = 332 nm) and those from Gram-positive bacteria (e.g., for *Bacillus subtilis*, λ_{em} = 348 nm and for *Staphylococcus aureus*, λ_{em} = 350 nm).

Moreover, the effect of UTP on UMP kinases from two different Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) is also different: in the case of *Bacillus subtilis*, the maximum of fluorescence is shifted, but its intensity is not affected, while, in the case of *Staphylococcus aureus,* the fluorescence intensity is decreasing, its maximum position being preserved [Ervin *et al.*, 2007].

5. Conclusion

The maximum of fluorescence spectra of UMP kinase under increasing UTP concentrations is decreasing (e.g., about 8 times for UTP concentration of 1,500 μM).

Using the fluorescence lifetime, observed in the case of proteins with Trp, a bimolecular quenching constant was calculated, resulting a very high value of this constant with two orders of magnitude, over the quenching constants of the processes limited by diffusion.

The high value of the quenching constant is demonstrating that quenching process is not the result of an intimate contact between the fluorophore (i.e., UMP kinase) and the quencher (i.e., the UTP ligand). Therefore the supposition is that, in this case, the fluorescence quenching could be the result of the following mechanisms: a) long range energy transfer; b) protein conformational changes; c) various intramolecular changes of UMP kinase, consecutive to its interaction with UTP.

Further fluorimetric studies of the interaction of *Staphylococcus aureus* UMP kinase with other ligands as ATP and GTP are needed in order to find out if the UMP kinase behaviour is similar or not to that manifested in the presence of UTP.

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