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# FLUORESCENCE STUDIES OF PHOSPHORIBOSYLADENOSINE TRIPHOSPHATE SYNTHETASE OF *ESCHERICHIA COLI*

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## 1. Introduction

The enzyme *N*-1-(5′-phosphoribosyl)-ATP: pyrophosphate phosphoribosyl transferase (EC 2.4.2.17) catalyzes the first step of the pathway for histidine biosynthesis in *Escherichia coli,* namely the reaction between ATP and phosphoribosyl pyrophosphate (PRPP), and is feedback inhibited by the end product, histidine.

We have investigated the changes in the intrinsic fluorescence of the enzyme upon binding of histidine or PRPP, with the finding that both ligands produce an enhancement of fluorescence. In addition, the association-dissociation processes – central to the regulatory mechanisms of the enzyme – have also been studied following the variation in extrinsic fluorescence of the enzyme (free or previously incubated with histidine), complexed to $1,N^6$-etheno-adenosine 5′-triphosphate ($\epsilon$ATP) [1], in the presence of PRPP.

## 2. Materials and methods

The source of the enzyme and the purification procedure have been reported [2]. Fluorescence intensities were measured in a Perkin–Elmer MFP-3 spectrofluorimeter, at 22°C, adding increasing amounts of a concentrated solution of the ligand to 3 ml of enzyme solution. $\epsilon$ATP was purchased from P-L Biochemicals, Inc., Milwaukee, Wisconsin. Protein was determined by the method of Lowry et al. [3].

The enzyme was dissolved in 0.05 M Tris–HCl buffer, pH 8.0, 0.5 mM EDTA, 2.8 mM 2-mercapto-ethanol. When indicated, the enzyme was previously incubated at 4°C with 1 mM histidine for one hour prior to the experiments.

## 3. Results

### 3.1. *Intrinsic fluorescence*

The fluorescence was excited at 300 nm (7 nm slit) and the emission observed at 350 nm (10 nm slit), using as blank a tryptophan solution having the same fluorescence as the enzyme solution. The concentration of the enzyme was 0.1 mg/ml.

In the absence of any ligand, the peak of emission is at 337 nm. A red shift is observed in the titration with histidine; at 1 mM histidine the emission maximum appears at 341 nm. Fig.1 shows the increase in intensity of fluorescence with the concentration of histidine. A double reciprocal plot (not shown)

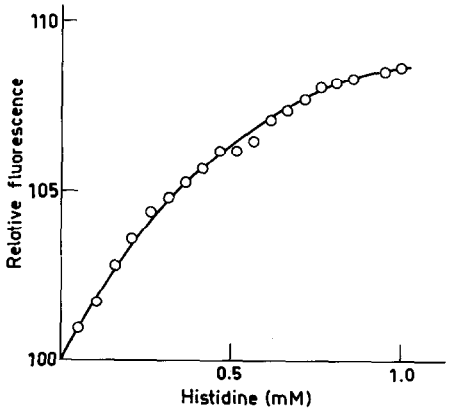


Fig.1. Fluorescence titration of phosphoribosyl-ATP synthetase with histidine.

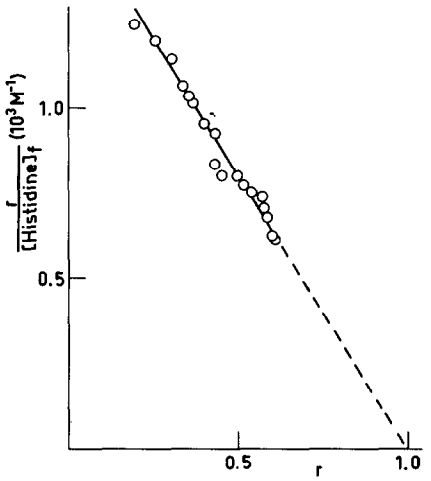


Fig.2. Scatchard plot of the data of fig.1. The symbol r refers to the increase in fluorescence at a given ligand concentration divided by the increase in fluorescence at saturation.

gives a maximum increase of fluorescence of 14.3%. A Scatchard plot [4] yields a straight line (fig.2), from which a dissociation constant of 0.61 mM is calculated (an identical Scatchard plot from a duplicate experiment gave 0.64 mM); from a Hill representation (not shown) a Hill constant of 1.03 is obtained.

Fig.3 depicts the effect of the PRPP on the fluorescence of the enzyme incubated with 1 mM histidine, with a maximum increase of 11.1%; a replot according to Scatchard [4] follows the pattern of negative co-

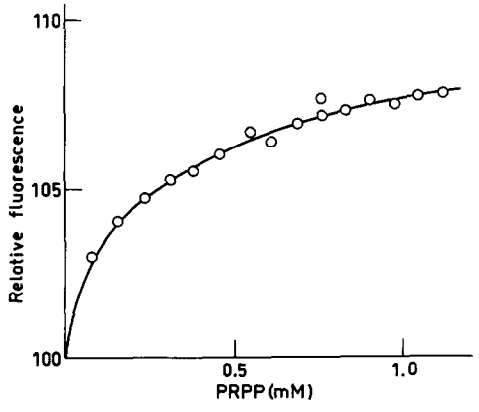


Fig.3. Titration with PRPP of the enzyme previously incubated with histidine.

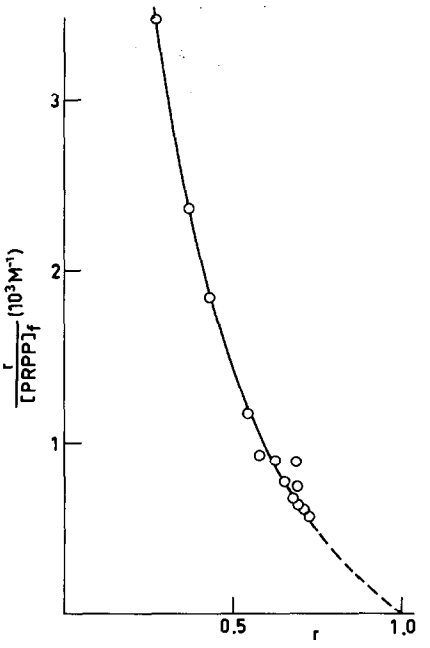


Fig.4. Scatchard plot of the data of fig.3.

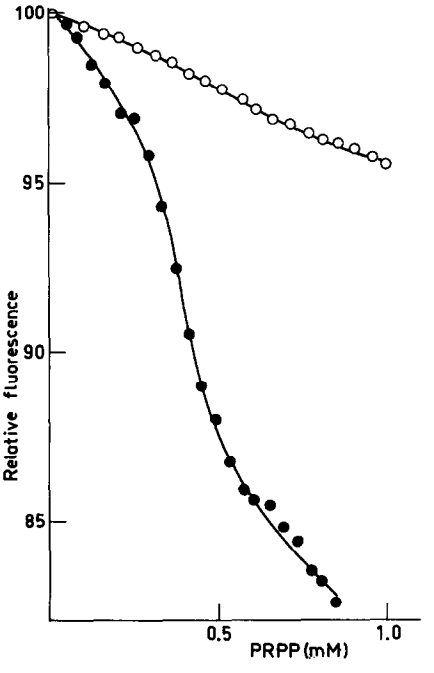


Fig.5. Fluorescence titration with PRPP of enzyme·εATP complex. Open circles, enzyme previously incubated with histidine; closed circles, enzyme not incubated with histidine.

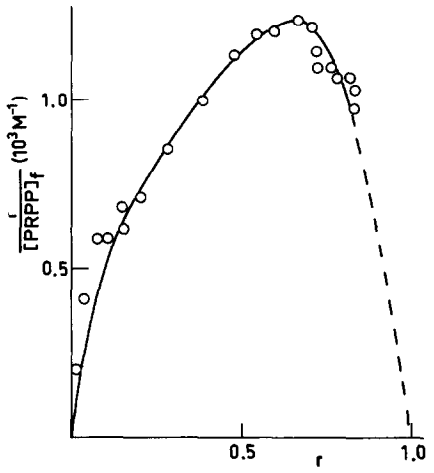


Fig.6. Scatchard plot of the data of fig.5 (closed circles).

operativity (fig.4). On the other hand, the same concentrations of PRPP have no effect on the fluorescence intensity when the enzyme is free of histidine.

The other substrate (ATP), up to 1 mM, has no effect on the fluorescence intensity of the enzyme incubated with 1 mM histidine.

3.2. *Extrinsic fluorescence*

0.2 ml of a 0.4 mg/ml solution of the enzyme were incubated at 4°C for 30 min with 0.3 ml of a 5 mg/ml $\epsilon$ATP solution; the free ligand was separated from the ligand–protein complex on a G-25 Sephadex column, giving then an enzyme solution of 0.02 mg/ml. The blank was a solution of $\epsilon$ATP having the same fluorescence as the enzyme · $\epsilon$ATP complex. Excitation was at 257 nm (10 nm slit) and emission at 410 nm (10 nm slit).

In fig.5 can be seen the fluorescence quenching due to the binding of PRPP to the complex, showing a maximum of 20% and positive cooperativity (fig.6).

If this experiment is repeated with enzyme previously incubated with histidine, the same pattern emerges (see fig.5); the cooperativity is also of the positive type (not shown), but the maximum quenching is now only 8.3%.

## 4. Discussion

From fig.2, it is clear that the binding of histidine to the *E.coli* enzyme, followed by fluorescence changes in the range 0.05 to 1 mM, is not a cooperative process. This is in agreement with the findings of Bell and Koshland [5] in *S. typhimurium*: the activity of the enzyme vs histidine concentration follows also a Michaelis-Menten curve and gives a slope of 1 in the Hill plot. The value of 0.62 mM for the $K_i$ of histidine binding is in the same order of magnitude (0.2 to 0.8 mM) as that reported by Bell et al. [6] in *Salmonella* by fluorescence enhancement titration studies.

In *S. typhimurium* phosphoribosyl-ATP synthetase is composed of 6 subunits of 36 000 daltons [5,7–9] and Klungsöyr and Kryvi report similar results with the *E.coli* enzyme [10]. It is known that histidine in the range 0.05 to 1 mM associates the *E.coli* enzyme [2,10]. We have previously shown by gel filtration [2] that the main species of the enzyme in the absence of ligands is the dimer, and that 0.4 mM histidine displaces the equilibrium dimer-tetramer-hexamer towards the hexamer. The same results are found by ultracentrifugation studies in the presence of 0.4 mM histidine (A. R. Tébar, unpublished experiments). From the data presented here can be deduced that the association of the enzyme is mediated by histidine without appearance of cooperativity among the subunits.

The red shift observed is indicative of an increase in the polarity of the microenvironment. So, we infer that histidine causes an exposure of tryptophan residues. Blasi et al. [11] studying the effect of histidine on the *S. typhimurium* enzyme also observed an increase of the relative fluorescence and a red shift, but taking into account additional spectroscopic data, they favored the explanation that histidine produces a burying of chromophores.

Tébar et al. [2] studied the effect of PRPP on the quaternary structure of the enzyme; PRPP totally reversed the association produced by histidine, displacing the pattern toward the dimeric form. Here, when the synthetase is free of histidine, it exists preferentially as a dimer, so PRPP has no effect on the fluorescence quantum yield. When the enzyme is in advance incubated (associated) with histidine, the binding of PRPP renders successive dissociations difficult (i.e. negative cooperativity).

We reported [2] that ATP associates the enzyme, although not so highly and specifically as histidine

does. It is conceivable that $\epsilon$ATP could substitute for ATP in association of the enzyme, and this would explain the appearance of positive cooperativity in the binding of PRPP (fig.5). If the enzyme is associated prior to the formation of the $\epsilon$ATP·enzyme complex, it is protected against dissociation by PRPP.

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