STRUCTURAL ALTERATIONS IN THE 30 S RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI* OBSERVED WITH THE FLUORESCENT PROBE *N*-(3-PYRENE) MALEIMIDE

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1. Introduction

Ribosomes are known to undergo structural alterations when subjected to various treatments, such as magnesium depletion [1,2], exposure to urea [3], and heat reactivation [4,5]. The study of these alterations can provide useful information on the structure of the ribosome. It is necessary to use a variety of techniques in these studies, since different techniques are sensitive to different structural parameters. We have shown that the intrinsic tryptophan fluorescence of the ribosomal proteins is sensitive to structural changes induced by all of the treatments mentioned above [6]. We have also shown that the extrinsic fluorescent probe *N*-(3-pyrene)maleimide [7], covalently bound to the ribosomal proteins, is sensitive to changes occurring during heat activation [8]. In the present communication we report additional observations on the fluorescence of NPM**-labelled 30 S ribosomes. The fluorescence is affected both by urea and by the changes induced by magnesium depletion. When bound to the ribosome, NPM forms an energy transfer system with tryptophan, quenching the intrinsic tryptophan fluorescence of the ribosome.

2. Materials and methods

NPM was synthesized according to Weltman et al. [7] and was reacted with *E. coli* 30 S ribosomal subunits at reagent concentrations of 1 μM or 5 μM. Excitation was at 330 nm for NPM fluorescence and 290 nm for tryptophan fluorescence. Emission spectra were recorded at 2°C unless otherwise stated. Ribosome concentrations are given as the concentration of ribosomal protein. Where the fluorescence intensities of different solutions are compared, the solutions either contained identical ribosome concentrations or else the results were calculated for solutions of the same concentration. The experiments are described in the legends. All materials and methods have been fully described elsewhere [6,8].

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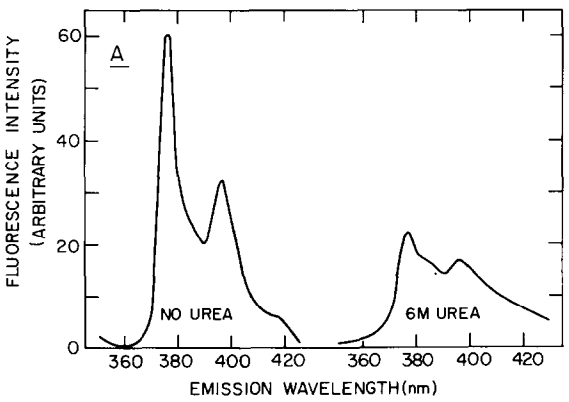
** *Abbreviation*: NPM, *N*-(3-pyrene)maleimide.

3. Results

3.1. *The effect of urea*

NPM by itself is not fluorescent, but becomes so after reacting covalently with the sulfhydryl group of a large or a small molecule [7]. Emission spectra of both kinds of NPM adduct are shown in fig.1, the large



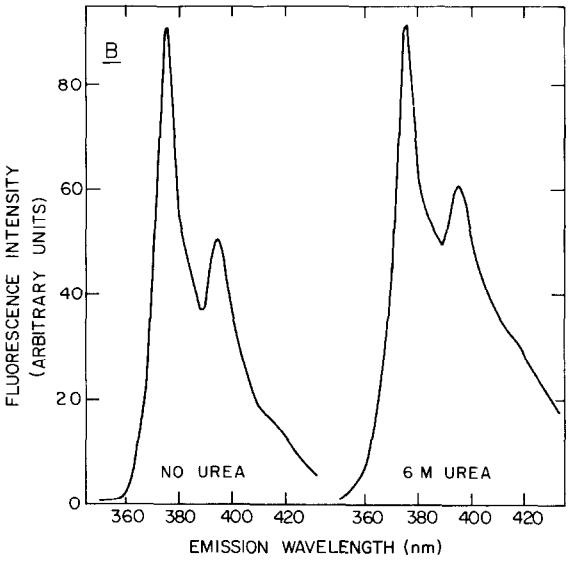


Fig.1. Effect of 6 M urea on the fluorescence spectrum of NPM-labelled 30 S ribosomes. The medium was 1 mM magnesium acetate, 10 mM Tris–HCl (pH 7.4) or the same plus 6 M urea. (A) 30 S ribosomes (1.2 mg protein/ml). Spectra recorded at 2°C. (B) NPM adduct of 2-mercaptoethanol. 10 μl of 2-mercaptoethanol and 5 μl of 0.1 mM NPM in ethanol were pipetted into 5 ml of cold medium and left overnight at 2–4°C. Spectra recorded at 20°C.

molecule being the 30 S ribosome (fig.1A) and the small, 2-mercaptoethanol (fig.1B). The spectra in the absence of urea resemble those reported by Weltman et al. [7] but are slightly shifted to shorter wavelengths. Free ribosomal proteins labelled with NPM showed a similar spectrum.

Differences appeared when 6 M urea was present in the medium. The emission of NPM-labelled ribosomes was markedly quenched (fig.1A, table 1). The different

Table 1
Effect of 6 M urea on the fluorescence of NPM-labelled 30 S ribosomes

Expt.	Concentration (μg protein/ml)	Medium	Fluorescence intensity at 375 nm (arbitrary units)		
			No urea	6 M urea	Ratio: 6 M urea/no urea
1.	Free proteins				
	105	a	84	35.5	0.42
	Ribosomes				
	30	a	68	23	0.34
	30	b	64.5	30.5	0.47
	171	c	92	44	0.48
2.	Ribosomes				
	700	d	150	55	0.37
	700	e	147	57	0.39
	700	f	150	52	0.35
	700	g	150	55	0.37
	700	b	154	55	0.36

Stock solutions of isolated total 30 S ribosomal proteins in 10 mM HCl or of 30 S ribosomes in 100 mM KCl, 1 mM magnesium acetate, 10 mM Tris–HCl (pH 7.4) were diluted with salt–buffer solutions to give the indicated concentrations and media. The media contained the following concentrations (mM) of KCl, magnesium acetate and Tris–HCl (pH 7.4), respectively, (a) 350, 20, 10; (b) 0, 1, 10; (c) 10, 1, 10; (d) 300, 20, 10; (e) 100, 1, 10; (f) 0, 10, 10; (g) 0, 5, 10. Labelling was done in 5 μM NPM in expt. 1 and 1 μM NPM in expt. 2. Fluorescence intensities are directly comparable in expt. 2 but not in expt. 1, where different recorder scales were used.

Table 2
Effect of magnesium depletion on the fluorescence of NPM-labelled 30 S ribosomes

NPM concentration during labelling	1 μM		5 μM	
Ribosome concentration (μg protein/ml)	22	11	22	11
Fluorescence intensity at 375 nm (arbitrary units)				
Depleted of magnesium	22.5	11	55	34.5
Not depleted	33	16.5	92	44
Ratio: depleted/not depleted	0.69	0.67	0.60	0.78

30 S ribosomes (0.5–1.0 mg protein/ml) were dialyzed at 2–4°C. Ribosomes not depleted of magnesium were dialyzed 2 days against 100 mM KCl, 1 mM magnesium acetate, 10 mM Tris–HCl (pH 7.4). Those depleted of magnesium were dialyzed 1 day against 1 mM EDTA, 10 mM Tris; then an additional day against 1 mM Tris. Protein concentration was determined and adjusted to the indicated values by dilution with the same buffer.

emission bands were quenched unequally. The major band at 375–6 nm was reduced to less than half its former intensity; the 395–7 nm band and the shoulder at about 385 nm were also quenched, but less. The degree of quenching was independent of ribosome concentration over a wide range (table 1). Free proteins extracted from NPM-labelled ribosomes were quenched to the same extent as proteins in the ribosome. In contrast, the emission of the NPM adduct of mercaptoethanol was not quenched by 6 M urea (fig.1B). In other experiments we have sometimes observed a small degree of quenching, but never as great as is invariably seen with NPM-labelled ribosomes. The quenching by urea of ribosomal NPM fluorescence cannot, therefore, be due only to solvent-fluorophore interaction, but must be at least partly due to urea-induced conformational changes in the ribosome.

3.2. *The effect of magnesium depletion*

When depleted of Mg^{++} ions, the 30 S ribosome undergoes extensive structural alterations at low ionic strength [1,2]. These alterations are accompanied by changes in the intrinsic tryptophan fluorescence of the ribosomal proteins [6]. The data of table 2 show that this is also the case when NPM is the fluorescent indicator.

3.3. *Quenching of tryptophan fluorescence by ribosome-bound NPM*

When illuminated at 290 nm, ribosomes show a strong tryptophan fluorescence with the emission maximum at about 340 nm [6]. NPM is excited at this wavelength, and the two fluorophores could constitute an energy transfer system if close enough to each other in the ribosome. Table 3 shows that this is the case. The tryptophan emission intensity was diminished when NPM was present, the effect increasing at the higher NPM concentration. At the higher level of NPM (5 μM), not only was the intrinsic tryptophan fluorescence quenched but also the characteristic NPM emission bands at 375 nm and 395 nm could be detected when the solution was illuminated at 290 nm.

Since the degree of energy transfer is a function of the distance between the fluorophores, we have tried to see if changes in distance could be detected when the ribosomal conformation was altered by magnesium depletion or exposure to urea. The initial results have been inconclusive.

Table 3
Effect of bound NPM on the intrinsic tryptophan fluorescence of 30 S ribosomes

	Relative fluorescence intensity at 338 nm (arbitrary units)		
NPM concentration during labelling	0 μM	1 μM	5 μM
Expt. 1	100	83.2	77.4
Expt. 2	100	82.9	77.3

30 S ribosomal subunits labelled with NPM at the levels indicated were dialyzed in the cold against 350 mM KCl, 20 mM magnesium acetate, 10 mM Tris–HCl (pH 7.4), and their protein concentrations were determined. Tryptophan fluorescence was excited at 290 nm. Fluorescence intensities are given as units per mg protein, normalized to the unlabelled values.

4. Discussion

The results presented here, together with others reported elsewhere [8], indicate that NPM is an environmentally sensitive fluorescent probe that can be used to investigate ribosomal structure. The observed response has been a change in fluorescence intensity on rearrangement of the ribosomal components, which determine the microenvironment of the proteins that carry the bound NPM molecules. In certain experiments not reported here we have also observed shifts in the NPM emission spectrum, but this has not been reproducible and is now under investigation.

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