FLUORESCENCE STUDIES OF *E. COLI* RIBOSOMAL PROTEINS S-4 AND S-7 IN REGARD TO RECONSTITUTION CONDITIONS

G. LEMIEUX

Institut de Biologie Moléculaire et Cellulaire, C.N.R.S., 67000 Strasbourg, France

and

D. GERARD

Laboratoire de Biophysique, U.E.R. Des Sciences Pharmaceutiques, Université Louis Pasteur, 67083 Strasbourg, France

Received 1 September 1973

1. Introduction

The possibility of reconstituting the *E. coli* 30 S ribosomal subunit from 16 S RNA and total 30 S proteins opened a new field for the study of specific protein–nucleic acid interactions [1]. However, in order to understand the role of each parameter in the optimal reconstitution conditions (0.35 M KCl; 0.02 M $MgCl_2$; 0.005 M KPO_4, pH 7.4, 30 min at 42°C)measurements with individual ribosomal components must be undertaken.

In a recent paper it has been shown that most proteins undergo important conformational changes upon heating [2]. Among the 21 different proteins present in the 30 S subunit, five can bind directly to RNA in absence of other proteins: S-4, S-7, S-8, S-15, S-20 [3–6].

In this work we started our studies with proteins S-4, S-7, S-8, and S-20. We then selected the two tryptophan-containing proteins, namely S-4 and S-7. The main intrinsic fluorescence properties (emission spectra, quantum yield, decay time and fluorescence polarization) of these two proteins were studied at various temperatures in optimal reconstitution solvent.

No evidence of structural variations upon heating could be obtained from emission spectra and quantum yield measurements. On the other hand, fluorescence polarization enabled us to show that conformational changes occur at a temperature higher than 32°C and 23°C for S-4 and S-7 respectively.

2. Materials and methods

2.1. *Ribosomal proteins*

Ribosomal proteins were prepared from *E. coli* MRE 600 and characterized as previously described [2]. Protein solutions were done in PMK buffer (0.35 M KCl; 0.02 M $MgCl_2$; 0.005 M KPO_4, pH 7.4). Lyophilized proteins (100–250 μg) were dissolved in 10 μl of guanidinium chloride (6 M) and diluted to 1 ml with PMK. Ultra pure guanidium chloride was obtained from Mann Research.

2.2. *Tryptophan and tyrosine content*

Tryptophan content of ribosomal proteins S-4 and S-7 was determined spectrophotometrically in denatured protein solutions [7]. Tyrosine content was determined from amino acid composition and mol. wt. data [8, 9].

2.3. *Fluorescence measurements*

Emission spectra and quantum yields were obtained as previously described [10]. Decay times of fluorescence were measured with the single photon technique [10]. Fluorescence polarization determinations were done with a laboratory built spectrophotometer [11], according to the Weber model [12]. Protein solutions were illuminated at 280 nm. Fluorescence was detected at 340 nm for decay time and polarization determinations.

North-Holland Publishing Company – Amsterdam

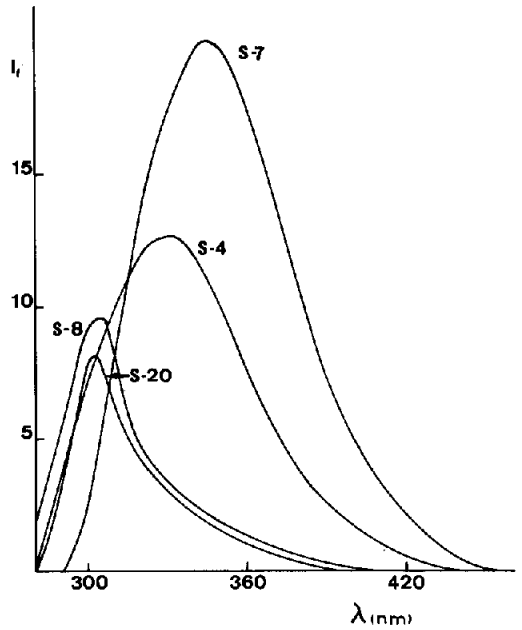

Fig. 1. Fluorescence spectra of proteins S-4, S-7, S-8 and S-20.

2.4. *Rotary diffusion coefficient determination*

The D_r values of fluorescent protein residues were calculated from the Perrin's relation [13]:

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_o} - \frac{1}{3}\right)(1 + 6 D_r \tau) \qquad \text{(eq. 1)}$$

where:

p = Degree of fluorescence polarization;
p_o = Fundamental fluorescence polarization (determined in rigid solution: glycerol at −70°C);
D_r = Rotary diffusion coefficient;
τ = Life time of excited state.

This relation has been established for spherical molecules. However, it can be also applied to ellipsoidal molecules [14]; in that case it has been shown that D_r is defined as D_{r_h}:

$$D_{r_h} = \frac{1}{3}(D_{r_1} + 2D_{r_2}) \qquad \text{(eq. 2)}$$

where:

D_{r_1} and D_{r_2} are the two rotary diffusion coefficients about the axis of the molecule.

2.5. *Relation between D_r and temperature*

D_r is related to the temperature by the following equation:

$$D_r = \frac{kT}{c\eta} \text{ [15]} \qquad \text{(eq. 3)}$$

where:

k = Boltzmann constant;
T = Temperature (°K);
η = Solvent viscosity;
c = Parameter related to the shape and size of the molecule.

3. Results and discussion

3.1. *Fluorescence spectra*

Fig. 1 shows the corrected emission spectra of ribosomal proteins S-4, S-7, S-8 and S-20 in PMK buffer at room temperature. The wavelength of maximal emission of proteins S-4 and S-7 is typical of tryptophan-containing proteins while the one of S-8 and S-20 is not. Moreover the number of fluorescent residues (tryptophan and tyrosine) was also determined (table 1).

3.2. *Effect of temperature on fluorescence spectra*

When emission spectra were recorded at various temperatures, no shift of maximal emission wavelength could be detected and a regular decrease of quantum yield was noted between 3°C and 45°C. Therefore, no evidence of structural variations could be obtained this way. This means that no variation of the environment polarity occurred in the vicinity of fluorescent residues. This result was expected, since most tryptophan residues are exposed to solvent [16]. Moreover, all tyrosines are reactive towards tetranitromethane [17], thus most probably being at the surface of the proteins.

Table 1
Tryptophan and tyrosine content of ribosomal proteins S-4 and S-7.

Protein	Mol. wt.	Tryp. (Residue/mole)	Tyr. (Residue/mole)
S-4	26 700	2.2	9.3
S-7	19 600	3.5	3.6

Table 2
Determination of D_r values vs temperature for protein S-4.

t (°C)	$T/\eta \times 10^{-2}$ ($°Kp^{-1}$)	p	τ (ns)	$D_r \times 10^{-8}$ (s^{-1})
3	170	0.130	4.5	067
14	245	0.126	3.9	0.81
18	276	0.125	3.8	0.83
24	326	0.123	3.5	0.94
32	399	0.122	3.2	1.04
37	449	0.104	2.9	1.47
40	479	0.094	2.8	1.75
43	512	0.094	2.7	1.80

$p_0 = 0.34 \pm 0.01$.

Table 3
Determination of D_r values vs temperature for protein S-7.

t (°C)	$T/\eta \times 10^{-2}$ ($°Kp^{-1}$)	p	τ (ns)	$D_r \times 10^{-8}$ (s^{-1})
3	170	0.126	6.0	0.30
6	189	0.133	5.8	0.27
12	210	0.134	5.2	0.30
16	260	0.126	5.0	0.35
22	307	0.130	4.5	0.37
24	326	0.113	4.4	0.50
30	393	0.106	3.8	0.65
33	404	0.108	3.6	0.66
35	427	0.095	3.5	0.85
40	479	0.093	3.0	1.03
41	490	0.094	3.0	1.00

$p_0 = 0.25 \pm 0.01$.

3.3. *Variation of D_r values with temperature*

We determined D_r values at different temperatures for proteins S-4 and S-7, according to eq. 1. The various parameters needed for calculations are presented in table 2 and 3 for proteins S-4 and S-7 respectively. One must be aware that, although we found two terms in decay time determinations for both proteins, we used an average value for calculations, since we were interested only in D_r variation and not by its absolute value.

On the other hand, it is worth noting that the value of p_0 in the case of protein S-4 is unusually high for a tryptophan-containing protein. Further work on this subject enabled us to ascribe this result to an important fluorescent contribution of tyrosines [16].

In fig. 2, the curves of D_r versus temperature for proteins S-4 and S-7 are presented. The slope variation which occurred for both proteins is due to a greater mobility of the fluorescent chromophores. This mobility increase is compatible with a conformational change of protein structure. The breaking point take place at 32°C for S-4 and 23°C for S-7. This means that, upon heating to 42°C in reconstitution solvent PMK, these two independant RNA-binding proteins undergo conformational variations which are possibly related to the mechanism of specific RNA binding activity.

Unfortunately, we could not study this phenomenon at higher temperature because precipitation occurred upon further heating. We found that this effect is very dependant on protein concentration. However, recent work on thermal stability of ribosomal proteins by circular dichroism enabled us to study the complete transition, since this technique requires more dilute solution than does fluorescence measurement. It is also of interest to point out that thermal transitions recorded by circular dichroism occurred in the same range as those studied by fluorescence [2].

Fluorescence techniques therefore are applicable to the study of ribosomal protein thermal transition and open new possibilities to help understand the reconstitution process of the 30 S ribosomal subunit.

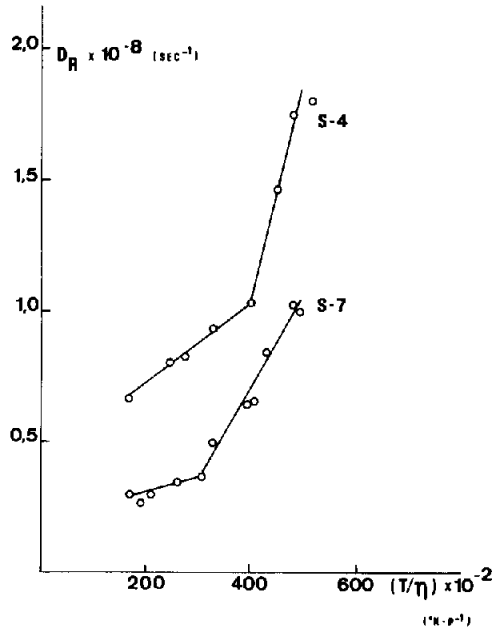

Fig. 2. Variation of D_r for proteins S-4 and S-7 versus T/η.

Acknowledgements

We thank Professors M. Daune and G. Laustriat for their interest in this work. This work was done during the tenure (G.L.) of a scholarship from Ministère de l'Education, Gouvernement du Quebec. This work was supported in part by a grant (Contract No. 73–7–1622) from Délégation Générale de la Recherche Scientifique et Technique.

References

[1] Traub, P. and Nomura, M. (1969) J. Mol. Biol. 40, 391.
[2] Lemieux, G., Lefevre, J.F. and Daune, M. (1973) Submitted to Eur. J. Biochem.
[3] Mizushima, S. and Nomura, M. (1970) Nature 226, 1214.
[4] Shaup, H.W., Green, M. and Kurland, C.G. (1971) Mol. Gen. Genet. 112, 1.
[5] Garrett, R.A., Rak, K.H., Daya, L. and Stöffler, G. (1971) Mol. Gen. Genet. 114, 112.
[6] Shaup, H.W., Green, M. and Kurland, C.G. (1970) Mol. Gen. Genet. 109, 193.
[7] Fdelhoch, H. (1967) Biochemistry 6, 1948.
[8] Craven, G.R., Voynow, P., Hardy, S.J.S. and Kurland, C.G. (1969) Biochemistry 8, 2906.
[9] Traut, R.R., Delius, H., Ahmad-Zadeh, C., Bickle, T.A., Pearson, P. and Tissières, A. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 25.
[10] Gerard, D., Lami, H. and Laustriat, G. (1972) Biochim. Biophys. Acta 263, 482.
[11] Gerard, D., Lami, H. and Laustriat, G. (1972) Biochim. Biophys. Acta 263, 496.
[12] Weber, G. (1956) J. Opt. Soc. Am. 46, 962.
[13] Perrin, F. (1929) Ann. Phys. 12, 169.
[14] Wahl, P. (1963) Ecole d'Eté de Roscoff.
[15] Tanford, C. (1967) Physical Chemistry of Macromolecules, p. 432, John Wiley and Sons, New York.
[16] Gerard, D., Lemieux, G. and Laustriat, G., to be published.
[17] Lemieux, G. (1973). Submitted to Eur. J. Biochem.