CIRCULAR DICHROISM AND ASSOCIATION–DISSOCIATION OF RIBOSOMES

G.N. KABASHEVA, L.S. SANDAKHCHIEV and A.P. SEVASTYANOV
Institute of Organic Chemistry, Siberian Division of the Academy of Sciences, Novosibirsk, U.S.S.R.

Received 21 January 1971

1. Introduction

The association–dissociation of ribosomes is caused by changes in the concentration of magnesium ions; this transformation does not involve profound conformational changes as revealed by absence of hyperchromic effect [1], of any alterations of the ORD spectra [2, 3] or of a difference in susceptibility to attack of SH-reagents [4]. Nevertheless, a remarkable difference of the conformations of RNA in associated and dissociated subunits of *E. coli* ribosomes has been found recently with the use of circular dichroism in the UV-region [1]. However, the latter studies did not attempt to evaluate the contribution of conformational changes with the separated subunits. The increase of dichroic absorption upon formation of 70 S particles was regarded as a direct consequence of the interaction of the contact areas of the subunits.

The present communication is concerned with the circular dichroism (CD) spectra of ribosomes fixed with glutaraldehyde [5]. It was found that changes in the concentration of magnesium ions result in a change of CD spectra of fixed ribosomes similar to that observed with intact ribosomes although the former are incapable of association–dissociation. Hence, change of the CD spectrum reflects some changes of the conformation of rRNA caused by change of environment and is not a direct consequence of the association–dissociation.

2. Materials and methods

Ribosomes of *E. coli* MRE-600 were isolated as described earlier [5] and transferred into 0.05 M triethanolamine-HCl buffer, pH 7.8 (TEA-buffer) containing 10^{-3} M Mg^{2+} or 1.4×10^{-2} M Mg^{2+} by 10 hr dialysis at 0–5°. At the same concentrations of Mg^{2+}, the ribosomes were fixed with glutaraldehyde (0.14%, 2 hr, 0°) [5]. The product of each reaction was divided into two parts: one was dialysed for 6 hr against TEA-buffer–10^{-3} M Mg^{2+} and the other against TEA-buffer–1.4×10^{-2} M Mg^{2+}. The native and the fixed ribosomes were characterised by sedimentation in a G-110 analytical ultracentrifuge (MOM, Hungary; schlieren optics) [5] and by quantitative analysis in sucrose gradients [6].

The subunit ratios as revealed by centrifugation of native ribosome preparations were: in 10^{-3} M Mg^{2+}, 78% 50 S subunits and 22% 30 S subunits; in 1.4×10^{-2} M Mg^{2+}, 67% 70 S subunits, 30% 50 S subunits and 3% 30 S subunits. These ratios were evaluated by absorbancy at 260 nm.

Treatment with glutaraldehyde in 10^{-3} M Mg^{2+} does not lead to any alteration of the subunit ratio, whereas in 1.4×10^{-2} M Mg^{2+} some change does occur (77% of 70 S, 18% of 50 S and 5% of 30 S).

The concentration of ribosomes is expressed as moles of phosphate groups; the amount of phosphate was determined in each of the experiments from the known molar extinction coefficients of 70 S, 50 S and 30 S particles [7] and from the ratio of the components determined by centrifugation in sucrose gradients. It was assumed that the extinction coefficients do not change on modification with glutaraldehyde.

The CD spectra of the ribosomes were measured using a Roussel-Jouan dichrograph D-185 (France)

Table 1
Characteristics of the CD spectra of native and fixed ribosomes.

No.	Type of ribosomes	Concentration of Mg^{2+} and Na^{+} during measurement of the spectra	λ_{max}	E_{max}	λ_{min}	E_{min}
1	Native ribosomes	10^{-3} M Mg^{2+}	265	6.40 ± 0.15	300	−0.46
2	Native ribosomes	1.4×10^{-2} M Mg^{2+}	265	7.36 ± 0.15	300	−0.30
3	Native ribosomes	1.4×10^{-2} M Mg^{2+} and 0.5 M Na^{+}	265	7.25 ± 0.19	300	−0.30
4	Ribosomes fixed in 10^{-3} M Mg^{2+}	10^{-3} M Mg^{2+}	265	6.56 ± 0.19	300	−0.38
5	Ribosomes fixed in 10^{-3} M Mg^{2+}	1.4×10^{-2} M Mg^{2+}	265	8.25 ± 0.15	300	−0.30
6	Ribosomes fixed in 1.4×10^{-2} M Mg^{2+}	1.4×10^{-2} M Mg^{2+}	265	8.36 ± 0.19	300	−0.47
7	Ribosomes fixed in 1.4×10^{-2} M Mg^{2+}	10^{-3} M Mg^{2+}	265	7.03 ± 0.18	300	−0.39
8	Ribosomes fixed in 1.4×10^{-2} M Mg^{2+}	1.4×10^{-2} M Mg^{2+} and 0.5 M Na^{+}	265	9.03 ± 0.14	300	−0.31

in 1.0 cm cells at 20° at ribosome concentrations of about 1 absorbancy unit per ml. The spectra are shown in figs. 1–3, and the amplitude values in table 1.

3. Results and discussion

The spectra of native ribosomes (fig. 1) are very similar to those published by Miall and Walker [1]. The small difference between the absolute amplitude values may be due to differences in the ribosome preparations and of the conditions of the measurement. The general appearance of the spectra, the presence of characteristic negative extremum at 300 nm and, most important, the increase of positive dichroic band amplitude under the association conditions (cf. curves 1 and 2 in fig. 1) are in complete accord with the data of the above workers. In the case of native ribosomes, we have also measured the CD spectrum in 1.4×10^{-2} M Mg^{2+} with 0.5 M NaCl (curve 3 in fig. 1). It was demonstrated earlier [5] that the addition of NaCl to this concentration causes complete dissociation of ribosomes into stable subunits. Despite this dissociation, no changes of the CD spectrum were observed.

The CD spectra have been measured of ribosomes

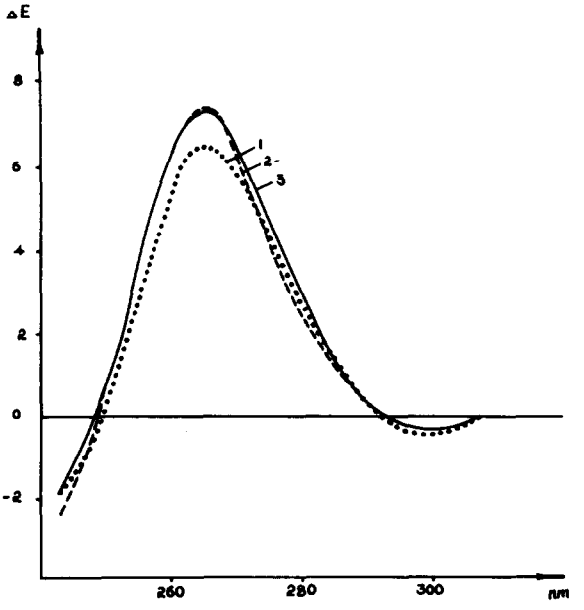

Fig. 1. CD spectra of native ribosomes in 10^{-3} M Mg^{2+} (1), in 1.4×10^{-2} M Mg^{2+} (2) and in 1.4×10^{-2} M Mg^{2+}–0.5 M NaCl (3).

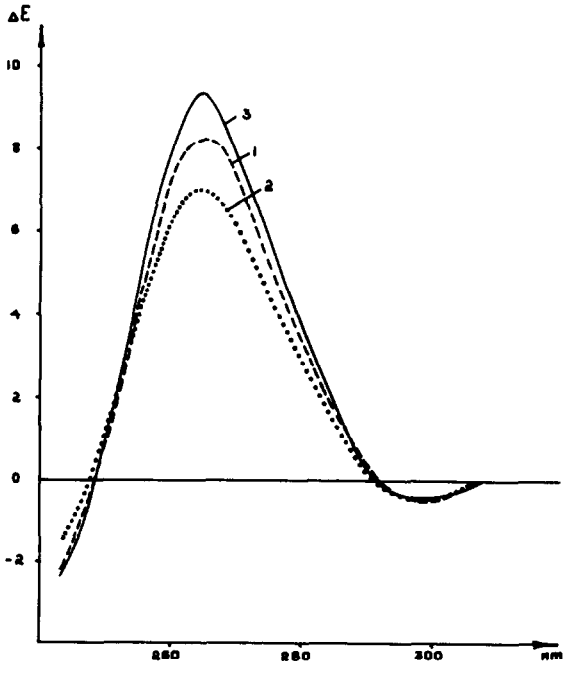

Fig. 3. CD spectra of ribosomes fixed in 1.4×10^{-2} M Mg^{2+}, measured in 1.4×10^{-2} M Mg^{2+} (1), in 10^{-3} M Mg^{2+} (2) and in 1.4×10^{-2} M Mg^{2+}–0.5 M NaCl (3).

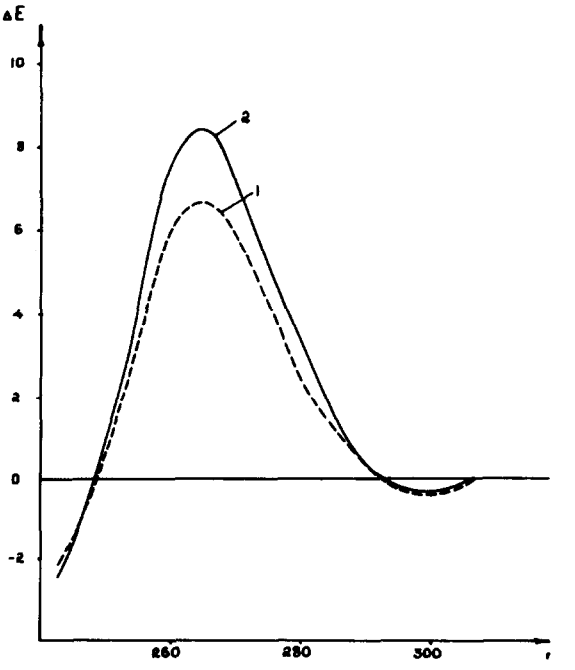

Fig. 2. CD spectra of ribosomes fixed in 10^{-3} M Mg^{2+}, measured in 10^{-3} M Mg^{2+} (1) and in 1.4×10^{-2} M Mg^{2+} (2).

fixed with glutaraldehyde in 10^{-3} M Mg^{2+} (fig. 2 and table 1, experiments 4 and 5). It is seen that the spectrum of fixed ribosomes measured in 10^{-3} M Mg^{2+} is practically identical to that of native ribosomes (table 1, experiment 1). Increase of the concentration of Mg^{2+} to 1.4×10^{-2} M results in an increase of ΔE_{max} somewhat greater than that observed with native ribosomes (of table 1, experiments 2 and 6).

CD spectra of ribosomes fixed in 1.4×10^{-2} M Mg^{2+} have been measured in 10^{-3} M and 1.4×10^{-2} M Mg^{2+}, and also in the presence of NaCl (fig. 3; table 1, experiments 6–8). It is seen that both in 10^{-3} M Mg^{2+} and in 1.4×10^{-2} M Mg^{2+} the ΔE_{max} value is somewhat higher than with native ribosomes. However, the effect of the increase of dichroic absorption at increased concentration of Mg^{2+} is completely retained.

It should be emphasized that fixation with glutaraldehyde prevents both the association and the dissociation of ribosomes [5]. Over a wide range of Mg^{2+} concentrations (10^{-4}–5×10^{-2} M) the ribosomes remain a mixture of 50 S and 30 S subunits when

fixed in 10^{-3} M Mg^{2+}, or a mixture of 70 S, 50 S and 30 S particles of constant composition when fixed in 1.4×10^{-2} M Mg^{2+}.

The above results suggest that the changes of the CD spectra attributed earlier [1] to the association–dissociation phenomenon are not its direct consequence, but reflect a change of the conformation of ribosomal RNA caused by change of the concentration of Mg^{2+} in the solvent.

It should be mentioned that increase of the concentration of Mg^{2+} also results in an increase of the number of protein NH_2-groupings within the ribosomes susceptible to attack by 2,4,6-trinitrobenzensulphonic acid [6]. As in the present case, this increase cannot be attributed directly to the association of subunits.

It may well be that the changes of the conformation of subunits caused by increase of Mg^{2+} concentration are a necessary prerequisite to their association into 70 S particles.

Acknowledgements

We thank Mrs. T.F. Lapina for technical assistance, and Dr. M.A. Grachev for translating this paper into English.

References

[1] S.H. Miall and I.O. Walker, Biochim. Biophys. Acta 166 (1968) 711.
[2] P. Sarkar, J.T. Yang and P. Doty, Biopolymers 5 (1967) 1.
[3] C.A. Bush and H.A. Scheraga, Biochemistry 6 (1967) 3036.
[4] R.R. Traut and A.L. Haenny, European J. Biochem. 2 (1967) 64.
[5] S.A. Nekhoroshev, E.K. Pressman, L.S. Sandakhchiev, A.P. Sevastyanov, Biokhimiya (1971) in press.
[6] E.K. Pressman, L.S. Sandakhchiev and A.P. Sevastyanov, Biokhimiya (1971) in press.
[7] S.H. Miall and I.O. Walker, Biochim. Biophys. Acta 145 (1967) 82.