SALT STABILIZATION OF A 5 S RNA–PROTEIN COMPLEX FROM AN EXTREME HALOPHILE, *HALOBACTERIUM CUTIRUBRUM*

Gordon F. WILLICK, Ross E. WILLIAMS, Alastair T. MATHESON* and Withold SENDECKI**

Division of Biological Sciences, National Research Council, Ottawa, Ontario K1A OR6, Canada

Received 12 June 1978

1. Introduction

We have recently isolated and characterized a 5 S RNA–protein complex (RNP) from the ribosomes of an extreme halophile *Halobacterium cutirubrum* [1]. This complex contains two proteins, HL-13 and HL-19, which have been shown to be equivalent to *Escherichia coli* proteins EL-18 and EL-5, respectively [1]. The organism has a high internal salt concentration [2], and accordingly the complex is, as expected, optimally stable in high salt concentrations (greater than 3 M KCl). However, the complex is not stable in salt concentrations less than about 1 M. We shall show here that the breakdown of the complex that is observed as the salt concentration is lowered is correlated with a loss of secondary structure in the binding proteins, rather than a simple charge repulsion between the nucleic acid phosphates and carboxylates of these highly acidic proteins.

2. Materials and methods

The RNP and binding proteins HL-13 and HL-19 were prepared as described previously [1]. Gel sieve chromatography was performed with a 2.5 × 85 cm column of Bio Gel 0.5 M. The flow rate was 15–20 ml/h, and 5–6-ml fractions were collected. Sedimentation equilibrium experiments were carried out with a Beckmann Model E analytical ultracentrifuge. Circular dichroism spectra were measured with a Cary 61 circular dichrograph. Concentrations of proteins were determined both with a fringe count in the analytical ultracentrifuge and an amino acid analysis. The concentration of RNA was determined spectroscopically, using an extinction coefficient of 23.4 l/g. Spectra are presented in units of deg·cm^2 (decimean residue weight)$^{-1}$. The mean residue weight of both HL-13 and HL-19 was taken as 110 g/l, that of the RNA as 320 g/l.

3. Results and discussion

Figure 1 shows the gel chromatography results of runs with *H. cutirubrum* RNP in the presence of increasing concentrations of KCl, using ^{32}P-labelled chick embryo 5 S RNA as a standard. In 0.34 M KCl (fig.1A), the standard runs almost coincidentally with the *H. cutirubrum* 5 S RNA; there is no complex formation. At 1.0 M KCl, there is a separation between the standard and the *H. cutirubrum* 5 S RNA (fig.1B) which increases to a maximum in 3.4 M KCl, either in low Mg^{2+} (fig.1C) or high Mg^{2+} (fig.1D).

Sedimentation equilibrium data also was consistent with the agarose gel results. The distribution curves for the RNP became identical to that for free RNA as the salt concentration was lowered to 0.34 M KCl. At higher salt concentrations, the data was used to give an estimate of the overall equilibrium constant for the binding reaction, using the theory for heterogeneous reaction systems in the ultracentrifuge [6].

* Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., V8W 2Y2, Canada

** Present address: Department of Biochemistry, Warsaw Medical School, u1 Banacha 1, 02-097 Warsaw, Poland

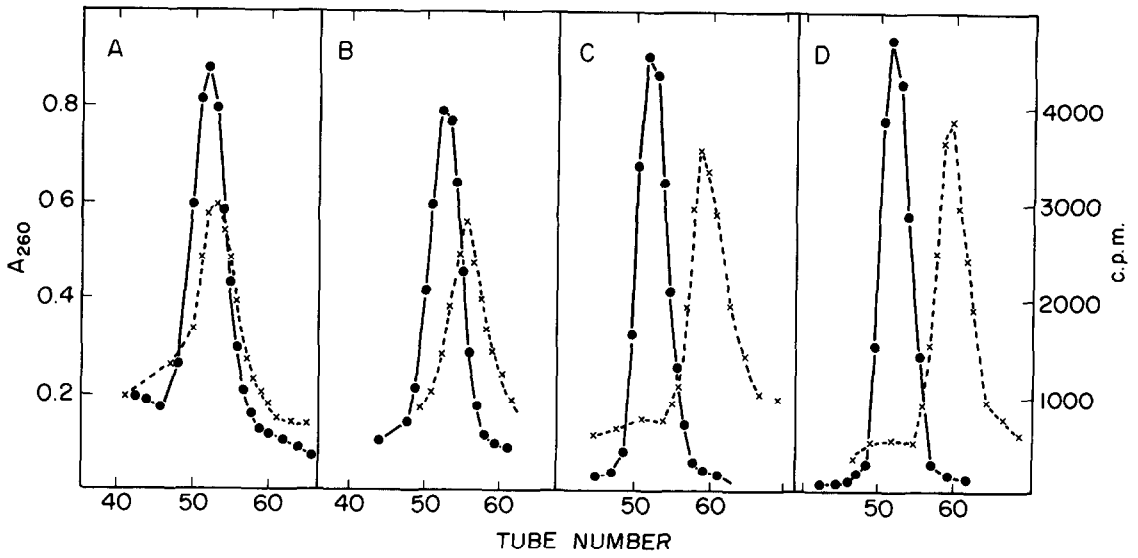

Fig.1. Separation of RNP from chick embryo ^{32}P 5 S RNA in 0.01 M Tris, 0.01 M BME, pH 7.6 plus 0.34 M KCl, 0.01 M $MgAc_2$ (A); 1.0 M KCl, 0.01 M $MgAc_2$ (B); 3.4 M KCl, 0.003 M $MgAc_2$ (C); 3.4 M KCl, 0.10 M $MgAc_2$ (D).

Figure 2 presents the data for the complex in 1.5 M KCl, 10 mM Mg^{2+}, along with a calculated value for the distribution of free RNA under these conditions. The estimation of equilibria distribution for the complex was made using a value of 0.71 ml/mg as the calculated value for the partial specific volumes ($\bar{v}$) of HL-13 and HL-19. An uncertainty exists because of the likely difference between the true thermodynamic and calculated values for $\bar{v}$. However, the calculation was consistent with a high overall equilibrium constant, approximately 10^{12} M^{-2}. The constant increases to about 10^{14} M^{-2} in 3.4 M KCl.

In an attempt to establish the chemical basis of the destabilization of the complex as the salt concentration was lowered, the secondary structures of the two proteins were studied. The circular dichroism spectra of HL-13 and HL-19 as a function of KCl concen-

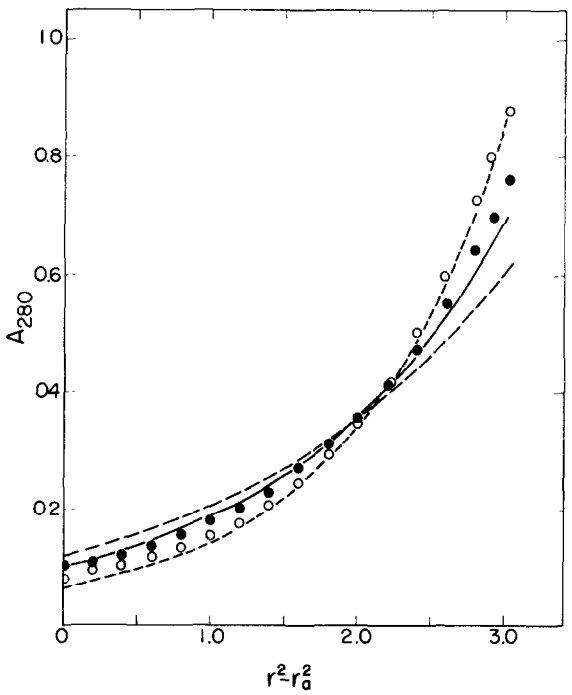

Fig.2. Sedimentation equilibrium distribution of RNP in 1.5 M KCl, 0.01 M $MgAc_2$, 0.01 M Tris, 0.01 M BME, pH 7.6 at 20°C. Total initial absorbance was A_{280} = 0.30. Data shown was taken at 12 000 rpm (•) and at 14 000 rpm (0). Calculated curves shown for an equilibrium constant of 10^{12} M^{-2} at 12 000 rpm (———) and at 14 000 rpm (– – – – –). Theoretical distribution of free 5 S RNA under the same conditions shown for 12 000 rpm (– –).

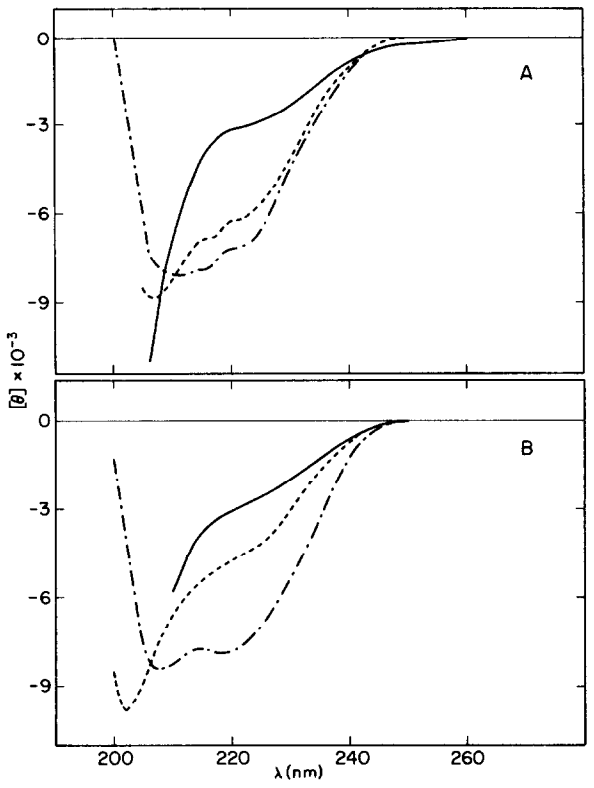

Fig.3. CD spectra of proteins HL-19 (A) and HL-13 (B) in 0.01 M $MgAc_2$, 0.01 M Tris, 0.01 M BME, pH 7.6 plus the following concentrations of KCl: 0.5 M (——), 1.4 M (– – – – –), 2.8 M (A) and 2.4 M (B) (– · – ·).

tration are shown in fig.3. Both proteins undergo a transition to a form with greater secondary structure as the KCl is increased, but the level of the KCl required differs. Based on a two structure model, the mid-point of the HL-19 transition is at 1.5 M KCl. The concentration of KCl required for full secondary structure in HL-13 is slightly higher than that for HL-19. Estimates of the secondary structure based on single wavelength [3] and linear fit [4] analyses are given in table I. The CD changes are seen to be due to increases in the fraction of α-helix as the KCl concentration is increased. For both proteins, the fraction of α-helix approximately doubles to a final level of about 0.25. Although the estimate of β is not so reliable, it is clear that there is probably no change in its amount.

The evidence strongly suggests that the observed

Table 1
Secondary structure of HL-13 and HL-19 from CD spectra

	KCl (M)	$[\theta]_{221}$	Linear fit	
		α	α	β
HL-13	0.5	0.17	0.11	0.14
	1.4	0.21	0.17	0.16
	2.4	0.28	0.27	0.08
HL-19	0.5	0.17	0.13	0.16
	1.4	0.24	0.23	0.16
	2.8	0.26	0.24	0.15

destabilization of the complex is correlated with the effect of the KCl on the secondary structure of the proteins, rather than a salt dependent effect on the direct interactions between protein and nucleic acid groups. On the contrary, these protein–nucleic interactions probably occur at still lower salt concentrations, since the complex appears to be partially stable at slightly lower concentrations of KCl than that at which the protein conformational changes occur. As a consequence, formation of the complex adds some stability to the high salt conformation of the binding proteins.

We speculate that the new α-helix created in HL-13 or HL-19 may be itself required in the binding to the RNA. α-Helical structures have been recently demonstrated to be implicated in the binding of protamine to tRNA [5].

Acknowledgements

The authors thank Dr Ross N. Nazar for the chick embryo ^{32}P 5 S RNA and for helpful discussions.

References

[1] Smith, N., Matheson, A. T., Yaguchi, M., Willick, G. E. and Nazar, R. N. (1978) Eur. J. Biochem. in press.
[2] Matheson, A. T., Sprott, G. D., McDonald, I. J. and Tessier, H. (1976) Can. J. Microbiol. 22, 780–786.
[3] Bewley, T. A., Brovetto-Cruz, J. and Li, C. H. (1969) Biochemistry 8, 4701–4708.
[4] Willick, G. E. and Zuker, M. (1977) Biophys. Chem. 1, 223–227.
[5] Warrant, R. W. and Kim, S.-H. (1978) Nature 271, 130–135.
[6] Roark, D. E. (1976) Biophys. Chem. 5, 185–196.