Evidence for hydrogen bonds in a ribonucleoprotein

In view of evidence that the ribonucleoproteins represent a stage in the process of protein biosynthesis[1], the nature of the protein–ribonucleic acid (RNA) linkage is of considerable biological interest. While the bonding between nucleic acid and protein in the deoxyribonucleoproteins is considered to be electrostatic[2], it has been noted that certain properties of the ribonucleoproteins are not those of pure electrostatic complexes, and it has been suggested that hydrogen bonds may be involved[3,4,5].

In a preliminary investigation of this problem, ribonucleoproteins, before and after treatment with urea, have been subjected to zone electrophoresis in starch over the pH range 3.5–10.6 at ionic strength 0.05–0.1. The initial findings indicate that hydrogen bonds play an important role in linking the RNA and protein.

The nucleoprotein was prepared from *Escherichia coli* at 0–3° in 0.005 *M* neutral phosphate buffer containing 0.00075 *M* $CaCl_2$[6]. Logarithmic phase cells were ground with glass powder and centrifuged for 1 h at 20,000 × *g*. The supernatant was treated with 1.25% sodium desoxycholate, and one volume of ethanol was added. The material was then precipitated twice with barium acetate (being redissolved with buffer containing 0.01 *M* Na_2SO_4) and 3 or 4 times with $(NH_4)_2SO_4$, ending with the fraction sedimenting between 0.3 and 0.5 saturation.

The final preparations were dialyzed against either buffer or water. They were free of deoxyribonucleic acid, were stable in the cold for at least a week, and were attacked by crystalline pancreatic ribonuclease at 29° and at 0–3°. They contained 60–70% RNA by weight (RNA as per cent of RNA plus protein), corresponding to a molar ratio of ribonucleotide to amino acid residues of about 0.5–0.7. Thus, the RNA content was equivalent to the highest values previously reported for ribonucleoproteins from other sources[4,7,8]. There was present, however, a minor protein component which was electrophoretically distinct from the nucleoprotein (Figs. 1A and 2A).

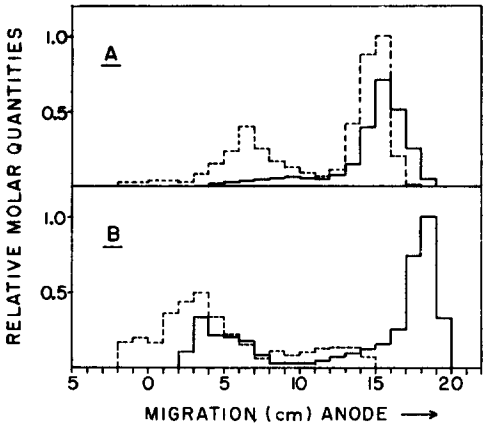

Fig. 1. Zone electrophoresis in starch at 0–3°. Sodium phosphate, pH 9.0, ionic strength 0.1; 2.75 h at 435–395 volts followed by 3 h at 350–340 volts. Starch block cut into 1 cm segments and extracted. Protein (broken line) and RNA (solid line) contents of extracts given as relative molar quantities of monomer residues, based on residue weights of 116 and 345, respectively. A, Intact nucleoprotein; B, Nucleoprotein in 7.7 *M* urea.

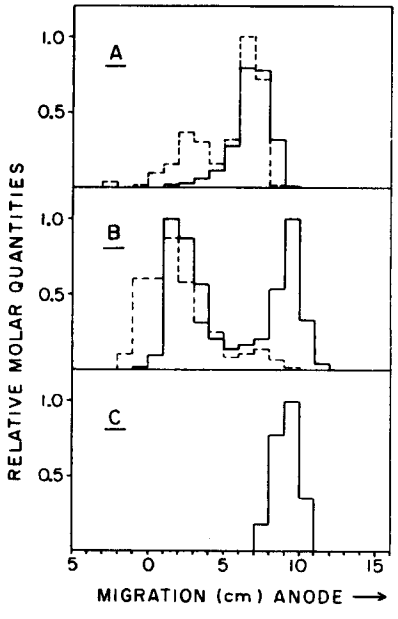

Fig. 2.

Fig. 2. As in Fig. 1. Sodium phosphate, pH 8.2, ionic strength 0.08; 12 h at 150 volts. A, Intact nucleoprotein; B, Nucleoprotein in 4 *M* urea; C, RNA (purchased; deproteinized and dialyzed).

Both the urea treatment and the electrophoretic analyses were carried out at 0–3°. The results were similar at urea concentrations of 4 *M* and 7–8 *M*, and those discussed in this note were obtained from electrophoretic runs begun 0.5 to 3 h after addition of urea to the nucleoprotein solution. The starch electrophoresis technique resembled that described by PARDEE *et al.*[9]. Up to 4 rectangular lucite troughs, 30 × 2 × 1 cm, were employed simultaneously at potentials of 300–450 or 150 volts. After the run, a filter paper strip was pressed against the starch; RNA

was located on the paper by means of an ultraviolet lamp, and protein by a staining procedure. In addition, the blocks were sometimes cut into segments and extracted overnight with cold water, the RNA and protein contents being estimated by the ultraviolet absorption and the Folin reaction, respectively. As employed here, this procedure was at best semi-quantitative, since no attempt was made to achieve complete extraction of RNA and protein from the starch.

Nevertheless, a coherent picture has emerged from these preliminary experiments. The electrophoretic findings may be summarized as follows:

1. *The intact nucleoprotein* (not exposed to urea). The nucleoprotein migrated toward the anode over the entire pH range from 3.5–10.6. At pH 4 and lower, protein and RNA moved together as a single entity. At pH 5.5 and higher, the pattern illustrated in Figs. 1A and 2A was consistently obtained. This pattern shows a minor protein zone, and a faster zone containing both RNA and protein and presumably nucleoprotein in character. In this pH range the distributions and mobilities were apparently independent of pH, since four blocks run simultaneously at pH 6, 7, 8 and 9 gave identical patterns. At pH 7.7 the pattern was essentially unchanged when the ionic strength of the electrophoresis buffer was lowered from 0.1 to 0.05.

2. *The protein moiety alone.* (Prepared by mild alkaline hydrolysis of the nucleoprotein and removal of the RNA hydrolysis products by dialysis.) Migration was toward the cathode at pH 5.5 and toward the anode at pH 6.8.

3. *The nucleoprotein after urea treatment.* Exposure to urea produced two electrophoretically distinct "RNA" components (Figs. 1B and 2B). Evidence presented separately[10] indicates that the slow component did not come directly from the nucleoprotein, but was a product of the secondary degradation of the fast component. Therefore, only the fast RNA component will be considered here. At pH 7.7 and higher (ionic strength 0.1) the fast RNA component migrated more rapidly than the intact nucleoprotein and with a speed equal to that of free RNA (Fig. 2). (A control experiment showed urea not to change the mobility of free RNA.) Under these conditions the separation of RNA and protein was essentially complete. It was incomplete at pH 7.7 when the ionic strength was lowered to 0.05, and at pH 5.5, ionic strength 0.1. At pH 4 and lower, protein and RNA migrated together toward the anode.

In summary, urea treatment produced an electrophoretic behavior similar to that shown by mixtures of solutions of protein and nucleic acid, where the two substances are linked only by electrostatic forces and migrate independently when similarly charged and at high enough ionic strengths[11,12]. When not exposed to urea, however, the protein and RNA of the nucleoprotein moved together under conditions where electrostatic forces would not be expected to hold them together.

It seems reasonable to conclude that these ribonucleoproteins contain RNA and protein linked together by bonds other than electrostatic bonds, that these bonds are dissociated by urea, and that they are, therefore, hydrogen bonds. It cannot be said whether other types of non-electrostatic bonds are present until a more detailed investigation has been made.

DAVID ELSON

Biochemistry Section, The Weizmann Institute of Science, Rehovoth (Israel)

1 J. W. LITTLEFIELD, E. B. KELLER, J. GROSS AND P. C. ZAMECNIK, *J. Biol. Chem.*, 217 (1955) 111.
2 E. CHARGAFF, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, 1955.
3 D. ELSON AND E. CHARGAFF, *Biochim. Biophys. Acta*, 17 (1955) 367.
4 E. CHARGAFF, D. ELSON AND H. T. SHIGEURA, *Nature*, 178 (1956) 682.
5 R. A. COX, A. S. JONES, G. E. MARSH AND A. R. PEACOCKE, *Biochim. Biophys. Acta*, 21 (1956) 576.
6 F.-C. CHAO AND H. K. SCHACHMAN, *Arch. Biochem. Biophys.*, 61 (1956) 220.
7 C. H. PARSONS, JR., *Arch. Biochem. Biophys.*, 47 (1953) 76.
8 K. TAKATA AND S. OSAWA, *Biochim. Biophys. Acta*, 24 (1957) 207.
9 A. B. PARDEE, K. PAIGEN AND L. S. PRESTIDGE, *Biochim. Biophys. Acta*, 23 (1957) 162.
10 D. ELSON, *Biochim. Biophys. Acta*, 27 (1958) 216.
11 L. G. LONGSWORTH AND D. A. MACINNES, *J. Gen. Physiol.*, 25 (1942) 507.
12 E. GOLDWASSER AND F. W. PUTNAM, *J. Phys. & Coll. Chem.*, 54 (1950) 79.

Received September 20th, 1957