EVIDENCE FOR URACIL NUCLEOTIDE-RICH SEGMENTS IN THE RNA MOLECULE OF THE VIRUS BAI STRAIN A (AVIAN MYELOBLASTOSIS)

J. Říman

Laboratory for Biochemical Investigation of Cancer, Department of Molecular Biology, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

Received October 20, 1966

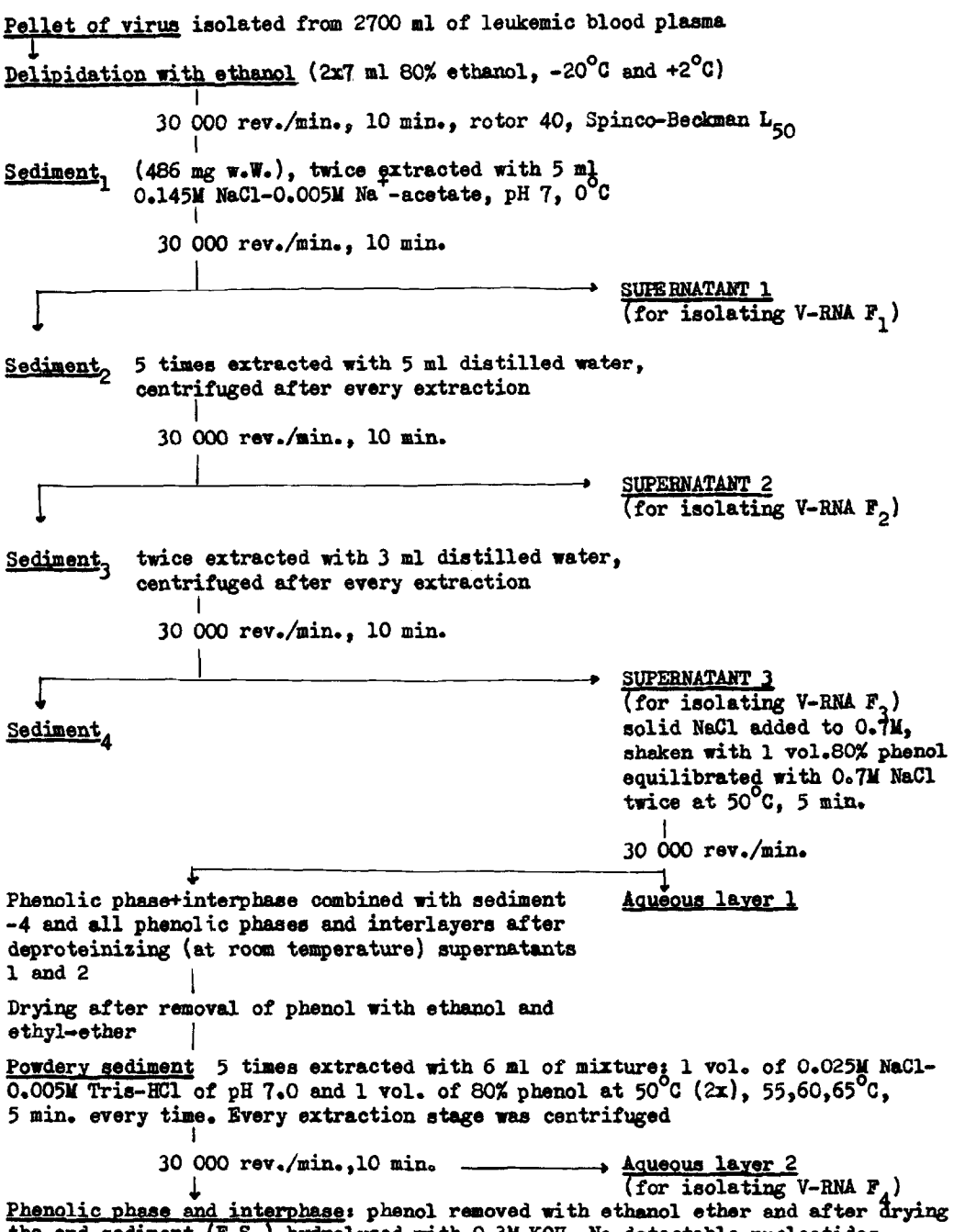
Isolation of an intact template-active RNA from the BAI strain A virus (Říman, et al., in press) brought forth the need for more detailed knowledge of the combination of a large RNA molecule (V-RNA) with the structure of the virus which is complicated morphologically (Bonar, et al., 1963a) and of characteristic chemical composition (Bonar and Beard, 1959). To elucidate these problems, we have investigated the quality of the nucleotide components of V-RNA extracted and isolated from ethanol-lipidated virus under various conditions of ionic strength of the medium. It resulted in the finding that the V-RNA molecule contains sections extractable from the delipidated structure at very low ionic strength, containing as much as 70 moles per cent of uracil nucleotide. These sections of V-RNA could be isolated as 4 S and larger fragments.

MATERIAL AND METHODS

Isolation and purification of the BAI strain A virus (Eckert, et al., 1951) from heparin-treated, enzymatically defined (Mommaerts, et al., 1954) blood plasma of leukemic chicks (Říman, 1964) was carried out principally according to Bonar et al. (1963b) (Říman, in press). Differential extraction of ethanol-delipidated virus material with media at various ionic strengths was conducted as shown in Fig. 1. RNA fragments present in the centrifuged extracts (Fig. 1) were deproteinized with phenol (Stansley and Seese, 1965). The V-RNA fragments were precipitated with ethanol (-20°C) from the aqueous

Fig. 1

Flow sheet for differencial extraction of the fragments of RNA of the virus BAI strain A



layers separated by centrifugation, extracted with ethyl-ether to remove phenol, dialyzed against 0.01M NaCl (+2°C, 18 h) and concentrated to 1/10 the original volume on a rotary evaporator. V-RNA fractions (F_1-F_3) were further defined by sedimentation in a linear (20-5%) sucrose gradient (Britten and Roberts, 1960). This sedimentation was also used for subfrantionating V-RNA fractions F_2 and F_3. The V-RNA subfractions (2a,b, 3a,b) were isolated from the contents of combined tubes (Fig. 2) eluted from the sucrose gradient. After dialysis against H_2O (+2°C) and volume reduction the subfractions, as well as the F_1 fractions, were subjected to alkaline hydrolysis (Davison and Smellie, 1952). The same treatment was given to V-RNA F_4 as well as to the material that had gone through all the extraction steps (E.S.- Fig.1). Nucleotides were separated by paper (Whatman 3MM) electrophoresis (Markham and Smith, 1952) in an ammonium formate buffer (pH 3.5, 2.1 V/cm, 1.7 mA/cm, 190 min). The concentration of nucleotides detected in UV light (Mineralight) was determined after descending elution with water by spectrophotometry (Beckman DU Model) by using published E_{mol} (Cohn 1957).

RESULTS AND DISCUSSION

The extraction of ethanol-delipidated BAI strain A virus (Fig. 1) resulted, in agreement with the existing knowledge of the effect of polar solvents on the lipid-nucleoprotein complex of animal viruses (Kates, et al., 1962), in a release of nucleoprotein fragments, the isolated RNA component of which contained (Fig. 2) mostly V-RNA fragments of 4 S size, i.e. V-RNA size attained under extraction conditions not excluding the action of endonuclease Rosenberg, et al., 1965, Trávníček and Říman, 1966).

The V-RNA fragments released by extraction differed in dependence on the ionic strength of the extraction medium in their nucleotide composition (Table I) which, together with the regularity of changes taking place in the nucleotide composition (Plate 1), indicate a differential release of specific parts of the V-RNA molecule associated with the structural component of specific extraction properties. A part of V-RNA (50%) which cannot be

extracted from the virus structure at the ionic strength of physiological saline (0.145M) can be separated only at a very low ionic strength (Fig. 1). Such extraction properties are characteristic for the nucleoprotein complex of DNA with histones or protamines (Chargaff 1955) or for the structural protein, muscle actin (Straub, 1942).

V-RNA fragments released from the structure of the BAI strain A virus at very low ionic strength (Fig. 1) were clearly enriched with the uracil nucleotide. Subfractination of this V-RNA fraction in a sucrose gradient

TABLE 1

Nucleotide Composition of V-RNA Fractions Separated by Differential Extraction from the Structure of an Ethanol-Defatted BAI virus of the A strain

V-RNA Fraction	Moles per cent				% of Total Extracted V-RNA
	A	U	G	C	
F_1	23.3	14.0	38.5	24.3	29.5
$(F_2)^x$	16.0	33.5	26.0	23.0	$(25.8)^x$
F_{2a}	18.0	26.7	28.3	25.7	19.8
F_{2b}	10.0	56.4	15.0	18.6	6.0
$(F_3)^x$	12.2	29.5	42.8	15.8	$(24.3)^x$
F_{3a}	7.4	69.5	10.0	13.6	8.2
F_{3b}	14.6	9.0	59.5	16.8	16.0
F_4	22.1	-	47.0	30.5	20.4
$(F_1\text{-}F_4)^x$	18.65	19.8	38.0	23.4	100.0

$(\ )^x$ calculated values.

resulted in a separation and isolation of segments of V-RNA molecules consisting of chains containing approximately 10^2 nucleotides (Table 1, Fig. 2, Plate 1) in which the uracil-containing nucleotide separated electrophoretically is represented by as much as 70 mol. % (F_{3a}). Sedimentation components of V-RNA which are present in these fractions (F_2, F_3) are, in comparison with F_1, more heavily represented also by V-RNA components larger than 4 S which could be accounted for by a dilution of the endonuclease present in

the preceding extraction steps. The high representation of uracil nucleotides which is characteristic especially for subfractions F_{2b} and F_{3a}, implies a higher frequency of the uridylic sequences and raises the question of a possible function of such naturally occurring polyuridylic chains in the V-RNA molecule, the template activity of which in the subcellular system of E.coli (Říman, et al., in press) is strictly bound to an intact molecule ($s_{w,20}$ 58.1) or to a structural unit of V-RNA of the size of 30-40 S. The possible participation of uracil-rich segments in the functional properties of V-RNA might be supported by the well-known relationship of polyU to the 30 S ribosomal subunits as well as to rRNA (Watson, 1964).

The concept of the differential binding of specific parts of the V-RNA molecule to the virus structure is in agreement with the nucleotide composition of the F_4 fraction of V-RNA which is most firmly bound to the structure and lacks a uracil nucleotide. A trend toward this behaviour may be seen even in the V-RNA subfraction F_{3b}.

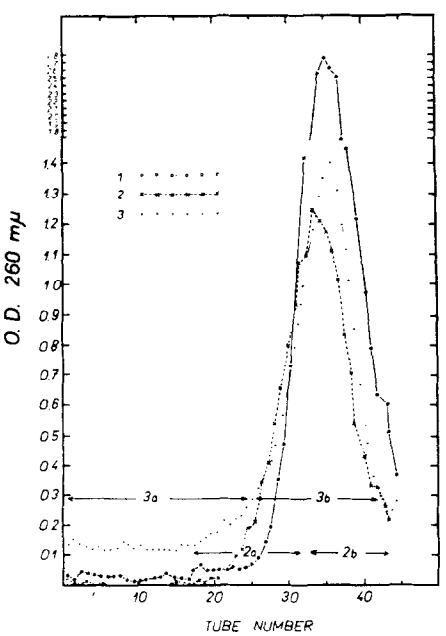


Fig. 2. Sedimentation of V-RNA fractions (F_1, F_2, F_3) on linear (20-5%) sucrose gradient (0.025M NaCl-0.005M Tris-HCl, pH 7), 7 h at 37 000 rpm, SW_{39} rotor, Spinco-Beckman L_{50}. The V-RNA fractions and subfractions were isolated from the contents of combined tubes eluted from the gradient as shown.

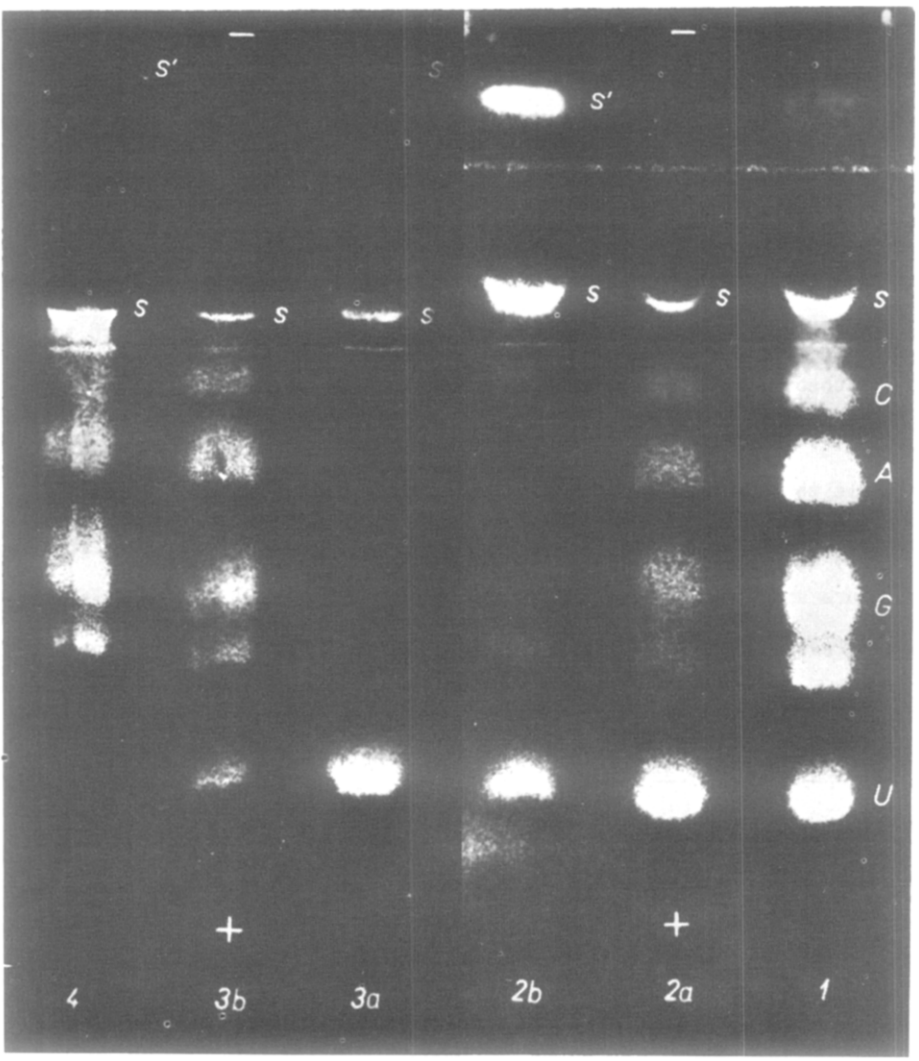


Plate 1. Electrophoreograms of extracted V-RNA fractions and subfractions subjected to alkaline hydrolysis. C,A,G,U (nucleotides of cytosine, adenine, guanine and uracil), S,S´ (nonnucleotide UV-absorbing components, Říman, in press).

REFERENCES

Bonar,R.A. and Beard, J.W., J.Nat.Cancer Inst. 20, 183 (1959).
Bonar,R.A., Heine,U., Beard,D. and Beard,J.W., J.Nat.Cancer Inst. 30, 949 (1963a).
Bonar,R,A., Purcell,R.H., Beard,D. and Beard,J.W., J.Nat.Cancer Inst. 31, 705 (1963b).
Britten,R.J. and Roberts,R.B., Science, 131, 32 (1960).
Chargaff,E. in The Nucleic Acids, vol. I, Chargaff,E., Davidson,J.N., Ed., Acad. Press, New York, 1955, p.307.
Cohn,W., in Methods in Enzymology III, Collowick, S.P. and Kaplan, N.O., Ed., Acad.Press, New York 1957, p. 724.
Davidson,J.N. and Smellie,R.M.S., Biochem.J. 52, 594 (1952).
Eckert,E.A., Beard,D. and Beard,J.W., J.Nat.Cancer Inst. 12, 447 (1951).
Kates,M., Allison,A.C., Tyrell,D.A. and James,A.T., in Cold Spring Harbor Symp. Quant.Biol. XXVII, 293 (1962).
Markham,R. and Smith,J.D., Biochem.J. 52, 552 (1952).
Mommaerts,E.B., Sharp,D.G., Eckert,E.A., Beard,D. and Beard,J.W., J.Nat. Cancer Inst. 14, 1011 (1954).
Rosenberg,M., Lacour,F., Huppert,J., C.R.Acad.Sci. (Paris) 260, 5145 (1965).
Říman,J., Fol. Biol. (Praha) 10, 331 (1964).
Říman,J., Trávníček,M. and Vepřek, L. in press in Fol.Biol. (Praha) 13, No 1, (1967) and this journal.
Říman, J., in press in Fol.Biol. (Praha) 13, No 1 (1967).
Straub,F.B., in Studies from the Institute of Medical Chemistry, Szeged, vol.II, Szent-Györgyi,A., Ed., Karger,S., Basel, New York, 1942, p.1.
Trávníček,M. and Říman,J., Neoplasma 13, 377 (1966).
Watson,J.D., Bull.Soc.Chim.Biol. XLVI, 1399 (1964).