SUBUNITS OF ONCORNAVIRUS HIGH-MOLECULAR-WEIGHT RNA
I. STEPWISE CONVERSION OF 60 S AMV (AVIAN MYELOBLASTOSIS VIRUS) RNA TO SUBUNITS

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SUMMARY: The effect of formamide on the dissociation of aggregate structure of high-molecular-weight RNA of avian myeloblastosis virus, an oncornavirus, was studied. It has been found that the pretreatment with increasing formamide concentration leads to the stepwise conversion of 60 - 70 S RNA molecule to 50 - 54 S and 30 - 40 S components; the 50 - 54 S intermediate is then further converted to 30 - 40 S subunits and smaller heterogenous RNAs. It is suggested that the subunits forming the aggregate RNA molecule of oncornaviruses are held together by not equally stable double stranded regions.

High-molecular-weight RNA of oncornaviruses with the sedimentation velocity of 60 - 70 S (1 - 5) is a highly structured molecule, quite different from RNAs of non-oncogenic viruses. This molecule can be converted by heating or by 90 % dimethylsulfoxide treatment to 30 - 40 S subunits and some smaller heterogenous RNAs (6 - 9). It has been suggested (6, 9) that there are 3 - 4 molecules of 30 - 40 S RNA subunits per 60 - 70 S molecule of oncornavirus RNA, probably linked together by hydrogen bonded regions.

In this study we used the unique property of formamide - to reduce the thermal stability of double stranded DNA (10,11) - in an effort to contribute to our present knowledge of the subunit structure of high molecular weight RNA of one of the representative of oncornavirus group - avian myeloblastosis virus. We report here the conditions of mild and highly controlled conversion of 60 S viral RNA to slowly sedimenting components.

We found a distinct and previously not described intermediate with the sedimentation velocity of 50 - 54 S. This component is successively converted to 30 - 40 S subunits as a main and final product and to smaller minor RNAs.

MATERIALS AND METHODS

Uridine ^{14}C (277 mCi/mmole) was supplied by Institute for Uses Research and Production of Radioisotopes, Prague, Czechoslovakia, carrier free ^{32}P orthophosphate (isotonic, sterile) by ROTOP, Dresden, German Democratic Republic. Formamide was purchased from E. Merck, AG, Darmstadt, German Federal Republic. Diethylpyrocarbonate (DEPC) was product of Serva, Heidelberg, German Federal Republic.

Production of labelled virus and isolation of labelled viral RNA

AMV was used in all experiments. The virus was propagated in chick leukaemic myeloblasts (12) cultivated in suspension (7-8 x 10^7cells/ml) as previously described (13). For all labelling experiments the tissue culture medium was supplemented with dialysed chick serum. When ^{32}P was added to label the viral RNA, phosphate-free medium was used.

Labelling of the virus with either 3 - 4 μCi/ml of ^{14}C uridine or with 70 - 80 μCi/ml of ^{32}P was in 30 ml. Usually the medium was continuously labelled for 24 hrs with two changes at 5-hour and one change at 12-hour intervals. Then the medium without isotope was added and the virus produced after further 5 hours was again labelled and collected. Labelled viruses produced by leukaemic cells after 5-hour labelling periods were purified (14) after supplementing the medium with plasma of leukaemic chicks as the virus density marker and used for isolation of total viral RNA (15).

Following the centrifugation of total viral RNA in 10 - 30 % gradient of glycerol containing 0.01 M TRIS pH 7.5, 0.1 M NaCl, 0.005 M EDTA (TNE) in a SW 39 rotor (Spinco 65 B preparative ultracentrifuge) at 39 000 rpm for 3 hours, approx. 35 fractions were collected. Small aliquots of each fraction were precipitated with trichloroacetic acid to estimate the sedimentation profile of total viral RNA. ^{32}P or ^{14}C radioactivities were collected on Milipore filters and counted on a Packard liquid scintillation counter in the BBOT - toluene scintillation fluid. The fractions containing high molecular weight RNA were then pooled, the RNA was precipitated with ethanol, dissolved in TNE containing 0.1% sodium dodecylsulphate and used for the experiments.

All solutions used for the preparation of viral RNA and for any other manipulation with isolated viral RNA were always freshly shaken with 0.2 % DEPC for 1 hour and then heated to 95°C for 20 min. to destroy residual DEPC.

RESULTS AND DISCUSSION

To date relatively little has been known regarding the structure of the subunits of oncornavirus high-molecular-weight RNA and their degree of homogeneity. It is not known whether 30 - 40 S subunits are identical or whether they bear different genetic messages. At the same time there are no informations available concerning the nature, lenght and degree of homogeneity of double stranded regions which are supposed to hold the subunits together to form the aggregate molecule with the sedimentation velocity of 60 - 70 S.

Since formamide reduces the thermal stability of double stranded DNA (10) and since a linear relationship exists

between the Tm and formamide concentration (11), an attempt has been made to dissociate the aggregate structure of oncornavirus high-molecular-weight RNA under mild conditions.

Were this RNA formed by the combination of the subunits with hydrogen bonded regions which are not equal and have sufficiently differing thermal stability, a controlled formamide treatment might dissociate successively the aggregate RNA molecule to smaller subunits according to the degree of thermal stability of double stranded regions involved.

To test this assumption, we have studied the fate of high-molecular-weight AMV RNA in formamide system, following its sedimentation changes after the treatment with increasing formamide concentration. ^{14}C-uridine and ^{32}P-labelled high molecular weight AMV RNA isolated from virions harvested following 5-hour intervals of continuous isotope labelling were used. Approximately 5×10^3 cpm of ^{14}C- and ^{32}P-labelled high--molecular-weight AMV RNA in TNE containing 0.1% SDS was separately treated with formamide at different concentrations (0 - 40%) at 37°C for 10 min in a total volume of 0.04 ml and then rapidly chilled. After dilution of each sample with TNE to 0.4 ml, two differently treated samples, bearing ^{14}C or ^{32}P radionuclides were mixed and cosedimented to insure the safe detection of any differences in sedimentation behaviour of treated samples. The formamide concentration in mixed RNA samples reached maximally 2 % of formamide.

Typical results of this treatment are shown in Fig. 1. Untreated high-molecular-weight AMV RNA sediments at about 60 S in glycerol gradient buffered in TNE. The first change in sedimentation behaviour was observed already after the pre-treatment of 60 S RNA with very low (3 - 5 %) formamide

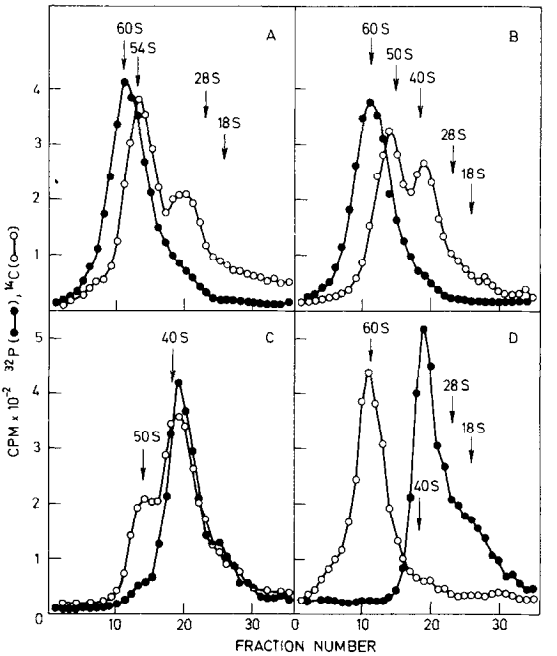

Fig. 1. Cosedimentation of ^{14}C and ^{32}P labelled high-molecular-weight AMV RNA after the treatment with various formamide concentrations. Samples containing approx. 5 x 10^3 cpm of ^{14}C- or ^{32}P- AMV RNA in TNE buffer were separately treated at 37°C for 10 min in the total volume of 0.04 ml with indicated formamide concentrations and then quickly chilled. After the dilution of each sample with TNE to 0.4 ml, two differently treated samples, bearing different isotope labells were always mixed and centrifuged in 10 - 30 % glycerol-TNE gradient at 39,000 RPM for 3 hours at 4°C in a SW 39 rotor. Gradient fractions were precipitated with trichloroacetic acid and collected on Milipore filters. ^{14}C and ^{32}P radioactivities of the filters were counted in BBOT - toluene scintillation fluid. A marker of ribosomal RNA of leukaemic myeloblasts measured by A_{260} was included in some gradients. A) Untreated ^{32}P-RNA, ^{14}C-RNA treated with 3 % formamide; B) untreated ^{32}P-RNA, ^{14}C-RNA treated with 5 % formamide; C) ^{14}C-RNA treated with 10 % formamide, ^{32}P-RNA treated with 20 % formamide; D) untreated ^{14}C-RNA, ^{32}P-RNA treated with 40 % formamide.

concentration (Fig. 1a, b). The 60 S RNA peak has disapeared, and a new peak with the sedimentation velocity about 50 - 54 S has been observed. At the same time, however, further 30 - 40 S

peak is present; it is detectable even when 2 % formamide has been used (not shown). With the increasing formamide concentration the 50 - 54 S peak decreases and the radioactivity is reproducibly transferred to 30 - 40 S region of the gradient (Fig. 1c, d). When the formamide concentration used for 60 S RNA pretreatment exceeds 20 %, 50 - 55 S peak disappears completely. At formamide concentration higher than 10 %, the 30 - 40 S subunits continue to be the prominent and final dissociation products of 60 S RNA (Fig. 1d). Increase of formamide concentration above 25 - 30 % has no further effect. Other minor dissociation RNA products of lower sedimentation velocity are also apparent; they form a broad and rather continuous band extending and decreasing toward the upper region of the gradient. The appearance of this material seems to be concomitant with the later stages of the conversion of 50 - 54 S RNA moiety (Fig. 1c, d). It should be noted that results identical to those shown in Fig. 1 were obtained using high-molecular-weight AMV RNA isolated from five independent tissue culture labelling experiments. The high reproducibility of the results and the prominent nature of both 50 - 54 S and 30 - 40 S main peaks indicate the non-random nature of conversion of 60 S RNA molecule by formamide. For a possible explanation of the behaviour of high-molecular AMV RNA in formamide system we may suppose that this aggregate molecule is composed from subunits linked together by not equally stable double stranded regions. It is assumed that 60 - 70 S oncornavirus RNA with the molecular weight of about 10×10^6 is made up from four 30 - 40 S moieties with the molecular weight within the range of $2 - 3 \times 10^6$ (ref. 6, 9, 16, 17); the appearance of a new 50 - 54 S intermediate (molecular weight $5.6 - 6.7 \times 10^6$) as

a consequence of a mild formamide treatment might include the release of one to two of 30 - 40 S subunits bound in 60 - 70 S aggregate molecule by shorter or more labile double stranded regions. At still higher formamide concentrations more stable double stranded regions would be dissociated, 50 - 54 S intermediate would dissappear, being converted to 30 - 40 S subunits and probably also to different minor RNAs of lower molecular weight.

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