A STUDY OF INFLUENZA VIRUS 4S RNA AS PRIMER FOR RNA-DEPENDENT DNA SYNTHESIS

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Experimental data on various forms of persistence of influenza virus and reports indicating that under experimental conditions this virus may have oncogenic potential [1] give new urgency to the search for molecular components facilitating integration of the influenza virus genome (or its parts) with the genome of the host cells. One such component for avian and mammalian oncornaviruses is known to be the low-molecular-weight (lmw)RNA which performs the function of primer during synthesis of DNA-provirus on the virion RNA template [5, 10, 13].

The object of this investigation was to analyze the primer properties of 4S RNA of influenza virus in RNA-dependent DNA synthesis.

EXPERIMENTAL METHOD

Influenza virus strain A (Texas) 1/77 (H3N2) was used. The virus was accumulated by using the ordinary method of infection of 10-11-day chick embryos into the allantoic cavity. The virus was purified by ultracentrifugation (K-2 centrifuge, continuous-flow K-3 rotor, from "Electronucleonics," USA) in a concentration gradient of 0-60% sucrose. The purified virus was sedimented to the bottom of the tube (1,000,000g, 1-2 h, 4°C). The residue was suspended in HTE buffer (0.15M NaCl; 0.05 M Tris-HCl, pH 7.4; 1 mM EDTA), containing 0.5% diethylpyrocarbonate (from Sigma, USA); Na dodecylsulfate was added to a concentration of 1%, and the contents were shaken at 20°C for 2 min. An equal volume of 80% phenol saturated with HTE buffer was then added. RNA was extracted three times with phenol and three times with chloroform at 20°C. RNA was sedimented from the aqueous phase by the addition of 2.5 volumes of ethanol containing 2% CH_3COONa. RNA was fractionated by electrophoresis in 8% polyacrylamide gel as described previously [2]. After electrophoresis, pieces were cut out of the gels corresponding to 4S RNA and to high-molecular-weight RNA (hmwRNA). RNA was extracted from the gels by the method suggested previously [3]. To isolate poly(A)-containing RNA, total chick embryonic RNA obtained by the hot phenolic method [4, 8], was chromatographed on unmodified cellulose of the Sigma cell-38 brand (from Sigma, USA) by the method of Shutz et al. [12], with slight modification: The application buffer contained 1 mM EDTA instead of Mg^{++} and all operations were carried out at 0-4°C.

Poly(A)-containing material was adsorbed and RNA precipitated with ethanol (−20°C, 12 h). After centrifugation, the RNA was dissolved in the application buffer and again chromatographed. All the operations were then repeated again.

RNA-dependent DNA synthesis was carried out in an incubation system described in [9]. Before the reaction, the flasks, together with the primers, were incubated in a reaction mixture containing all the components except RNA-dependent DNA polymerase of avian myeloblastosis virus at 37°C for 10 min.

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TABLE 1. Results of RNA-Dependent DNA Synthesis (M ± m)

Variant of incubation	Incubation time	Template	Primer	Incorporation into DNA-product, cpm
1	0	—	—	390±7
2	70	—	—	700±71
3	70	Poly(C)	Oligo(dG)	163250±1610
4	0	Poly(A)+ RNA, 6 μg	—	700±150
5	70	The same	—	870±140
6	70	" "	Oligo(dT)$_{12-18}$, 0.6 μg	2000±250
7	0	" "	—	557±172
8	60	Poly(A)+ RNA, 6 μg	—	920
9	60	The same	Oligo(dT)$_{12-18}$, 0.6 μg	1443±128
10	60	" "	4S RNA IV, 1 μg	1123±140
11	60	" "	4S RNA IV, 2 μg	1182±10
12	60	" "	4S nuclear RNA, 2 μG	1863±360
13	60	" "	Poly(U), 1 ug	915±116
14	60	hmwRNA, IV, 3 μg	Oligo(dT)$_{12-18}$, 0.6 μg	347±51
15	60	The same	4S RNA IV, 1 μg	246±73
16	60	" "	—	197±39
17	60	—	4S RNA IV, 1 μg	378±97

Legend. In variants 1-3 incubation mixtures contained 1 μCi dGTP-^{3}H (12 Ci/mmole, from Radiochemical Center, Amersham, England); in other variants they contained 1 μCi dCGP-^{125}I (greater than 1400 Ci/mmole, from Radiochemical Center). Mixtures of variants 1-6 each contained 33 units RNA-dependent DNA polymerase, remaining mixtures each contained 29 units of "Sigma" (USA) primer; IV) influenza virus.

EXPERIMENTAL RESULTS

As Table 1 shows, the DNA polymerase used gave a high level of incorporation of label into DNA on the synthetic poly(C)–oligo(dG) template-primer complex. The level of incorporation on the natural template was much lower and, moreover, substantial stimulation of synthesis was observed on the addition of the synthetic primer oligo(dT) 12-18. These results indicate that the isolated poly(A) plus RNA is able to control RNA-dependent DNA synthesis, and also that RNA-dependent DNA polymerase can synthesize DNA on it with sufficient intensity to be recorded experimentally.

During investigation of the action of influenza virus 4S RNA and primer it was found that the addition of this lmwRNA stimulates synthesis of complementary DNA, but by a lesser degree than the addition of the synthetic primer oligo (dT)12-18. Polyuridylic acid (from Reanal, Hungary), as might be expected, had no primer activity. This was evidently because this commercial preparation includes polynucleotide chains 200 residues long or more, forming extensive double-stranded regions with poly(A)-blocks which, as we know, cannot be utilized by RNA-dependent DNA polymerase for the initiation of transcription.

Significant stimulation of incorporation of label into DNA was observed when the 4S fraction of nuclear lmwRNA was used as primer. This RNA proved to be a no less effective primer than oligo(dT). It should be noted that 4S nuclear RNA consists largely of tRNA [11], some types of which can successfully carry out priming during DNA synthesis on oncornavirus virion RNA [10, 13]. The phenomenon discovered, in the writers' view, deserves further study. The results given above thus demonstrate the evident potential ability of influenza virus 4S RNA to participate in RNA-dependent DNA synthesis in the role of primer.

The last group of data characterizes the template activity of isolated influenza virus hmwRNA in RNA-dependent DNA synthesis. It will be clear from Table 1 that RNA-dependent DNA polymerase cannot synthe-

size complementary DNA on the template of influenza virus hmwRNA either in the presence of oligo(dT) or if influenza virus 4S RNA is used as primer. The first result is in agreement with data on the absence of poly(A)-blocks in molecules of influenza virus virion RNA [6, 7], and the second result characterizes the template-primer properties of the complex of virion hmwRNA and 4S RNA in vitro. The results suggest that during interaction between influenza virus and cells its genome RNA cannot control synthesis of complementary DNA in the presence of RNA-dependent DNA polymerase of normal cells or of latent oncornaviruses.

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MECHANISMS OF DISTURBANCE OF MITOCHONDRIAL ADENINE-NUCLEOTIDE TRANSPORT IN THE COURSE OF ACUTE LIVER ISCHEMIA

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Disturbance of oxidative phosphorylation (OP) in acute ischemia of an organ is explained by structural injuries to the mitochondria. It has been shown that long-chain acyl-CoA, physiological inhibitors of mitochondrial adenine-nucleotide (AN) translocase (ANT) [6], accumulate in the ischemized myocardium [15]. Meanwhile, Shrago et al. [14] consider that the mechanism of disturbance of OP precedes structural damage to the mitochondria. However, AN transport in the mitochondria can be regulated not only by the acyl-CoA level; there is evidence that this process depends on the metabolic reserves of intramitochondrial AN (AN_i) [10]. It is therefore interesting to make a closer study of relations between disturbances of AN transport and OP in the course of acute liver ischemia.

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