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Enzymes and Nucleotides in Virions of Rous Sarcoma Virus

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In addition to the previously described deoxyribonucleic acid (DNA) polymerase, DNA ligase, DNA exonuclease, and DNA endonuclease activities, purified virions of Schmidt-Ruppin strain of Rous sarcoma virus (SRV) have nucleotides and nucleotide kinase, phosphatase, hexokinase, and lactate dehydrogenase activities. The SRV virions have no glucose-6-phosphate dehydrogenase activity. All enzyme activities, but glucose-6-phosphate dehydrogenase and adenosine triphosphatase, were increased by disruption of the virions. The DNA polymerase, DNA ligase, and hexokinase activities had a higher specific activity in purified virion cores. It is suggested that during assembly virions of SRV may pick up cytoplasmic components which bind to virion proteins. The role of these components in viral replication is not known at present.

Purified virions of Schmidt-Ruppin strain of Rous sarcoma virus (SRV) contain several enzyme activities: ribonucleic acid (RNA)-, deoxyribonucleic acid (DNA)-RNA hybrid, and DNA-directed DNA polymerase activities (21, 35), DNA endo- and exonuclease activities (21, 22), and DNA ligase activity (22). In our earlier studies of these activities, some discrepant phenomena were observed. For example, the incorporation of deoxythymidine monophosphate (dTMP) into trichloroacetic acid-insoluble material by the SRV endogenous DNA polymerase system in the absence of deoxyguanosine triphosphate (dGTP) or deoxycytidine triphosphate (dCTP) was at 40% of the rate of incorporation of dTMP in the presence of all four deoxyribonucleoside triphosphates (35); the endogenous DNA polymerase activity of SRV was slightly stimulated by the addition of adenosine triphosphate (ATP) (Table 1); and no clear-cut requirement for exogenous ATP for the SRV virion DNA ligase reaction could be demonstrated (22). To explain these results, we suggested that there were nucleotides present in the virion of SRV.

In the present paper, we demonstrate the presence of nucleotides and of nucleotide kinase (nucleoside diphosphate phosphotransferase, nucleoside monophosphate phosphotransferase) activities in purified virions of SRV and in isolated cores of SRV. Further, because of the large number of enzyme activities in the purified virions, we looked in virions and cores for the presence of several enzymes which might represent cytoplasmic contamination of the virus.

MATERIALS AND METHODS

Radiochemicals. ^{3}H-ATP, ^{3}H-guanosine triphosphate (GTP), ^{3}H-cytidine triphosphate (CTP), ^{3}H-uridine triphosphate, ^{3}H-dGTP, ^{3}H-dCTP, ^{3}H-thymidine triphosphate, and ^{14}C-glucose were purchased from Schwartz BioResearch Inc. (Orangeburg, N.Y.). Carrier-free ^{32}P-orthophosphate and [γ-^{32}P] ATP were purchased from New England Nuclear Corp. (Boston, Mass.). ^{3}H-deoxyadenosine triphosphate, ^{3}H-deoxyadenosine diphosphate (dADP), and ^{14}C-deoxycytidine diphosphate (dCDP) were kind gifts of R. D. Wells.

Nucleotides. All nucleotides were purchased from Calbiochem (Los Angeles, Calif.).

Other materials. Polyethylene-imide (PEI) cellulose thin-layer plates were products of Baker Chemical Co. (Phillipsburg, N.J.). Nonidet P-40 was a product of Shell Chemical Co. (New York, N.Y.).

Sources of virus, methods of growth of virus (33, 34) and virus purification (21), and preparation of cores from virions (10) were described previously.

Fractionation of chicken embryo fibroblast cells and SRV-infected cells. Tissue culture cells were washed twice with cold saline. The cells were homogenized in 0.25 M sucrose in 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride containing 0.025 M KCl, 0.005 M $MgCl_2$, and 0.001 M 2-mercaptoethanol by the method described by Baril et al. (3) and then fractionated by centrifugation at 600 $\times$ g for 10 min. The supernatant fluid was used for most enzyme assays.

Enzyme assays. Unless otherwise indicated, the reaction mixture for nucleotide kinase (nucleoside diphosphate phosphotransferase and nucleoside monophosphate phosphotransferase, 0.050 ml) contained Nonidet P-40-disrupted virions (47 μg of viral protein), 34.6 pmoles [γ-^{32}P]ATP (specific activity is indicated

TABLE 1. *Stimulation of Schmidt-Ruppin strain of Rous sarcoma virus (SRV) endogenous deoxyribonucleic acid polymerase activity by adenosine triphosphate (ATP)*

ATP concn (nmoles)	^{3}H-TMP incorporated[a] (counts per min)
0	3,290
6.25	4,690
25	4,230
50	4,160
100	4,520

[a] One hour of incubation under the conditions described previously (35). SRV viral protein (4.5 μg) was used for the reaction. TMP, thymidine monophosphate.

in each figure legend), 10 μg of acceptor (indicated in each experiment), and 3 mM $MgCl_2$ in 0.01 M Tris-hydrochloride, *p*H 7.5. The reaction was carried out at 40 C and stopped at desired times by adding ethylenediaminetetraacetate (EDTA) to a final concentration of 0.01 M and chilling the reaction mixture in an ice bath. The reaction mixture was analyzed by paper chromatography or PEI cellulose thin-layer chromatography.

Hexokinase (ATP:D-glucose-6-phosphotransferase) was assayed by using ^{14}C-glucose as acceptor in a method using the principles of the assay described by Ives et al. (16) for assay of nucleoside kinase and nucleotidase activities (V. R. Potter and J. Goodman, *personal communication*).

The method described by Bergmeyer et al. (4) was employed for lactate dehydrogenase [L-lactate:nicotinamide adenine dinucleotide (NAD) oxidoreductase].

For glucose-6-phosphate dehydrogenase [D-glucose-6-phosphate NAD phosphate oxidoreductase], the method described by Löhr and Waller (20) was employed.

Preparation of ^{32}P-labeled SRV. The previously described general methods and techniques (1) for infection of chicken embryo fibroblast cells with SRV were followed. Chicken embryo fibroblast cells in 100-mm petri dishes were exposed to SRV and were incubated for 4 to 5 days until all of the cells appeared to be converted. After removal of the medium, the cells were washed once with Eagle's medium modified by Temin (33) containing 0.1 of the ordinary amount of phosphate (reduced phosphate EM), and 5 ml of reduced phosphate EM containing 5% dialyzed calf serum was added to each plate. The cells were incubated for another 12 hr at 38 C in a CO_2 incubator. The medium was replaced by 5 ml of fresh reduced phosphate EM containing 5% calf serum and 50 μCi of carrier-free ^{32}P-orthophosphate per ml. The medium was harvested at 4-hr intervals and pooled. The pooled medium was centrifuged at 10,000 rev/min for 10 min in a Sorvall SS34 rotor to remove cell debris. The supernatant fluid was further centrifuged at 25,000 rev/min for 1 hr in a Spinco type 30 rotor. The sedimented virus was collected by scraping the centrifuge tubes with a rubber policeman and then further purified as described before (21).

Extraction of nucleotides from ^{32}P-SRV. Cold trichloroacetic acid-soluble materials were extracted with 5% trichloroacetic acid in an ice bath. Trichloroacetic acid-insoluble materials (ca. 80% of total ^{32}P counts) were removed by centrifugation. The supernatant fluid was extracted with 1 volume of chloroform-methanol (2:1, reference 12). After removal of the chloroform-methanol layer, the aqueous layer was washed three times with 1 volume of ethyl ether. Trace amounts of ether were removed by evaporating in vacuo. The *p*H of the extracts was adjusted to approximately 8. These extracts (ca. 10% of starting ^{32}P) were analyzed by two-dimensional PEI cellulose thin-layer chromatography.

Separation of nucleotides. With paper chromatography, Whatman no. 1 paper was used in solvent systems of isobutyric acid-ammonium hydroxide-water (66:1:33) and of 0.1 M phosphate buffer (*p*H 6.8)-ammonium sulfate-*n*-propanol (100:60:2) (25). With PEI cellulose thin-layer chromatography, nucleoside monophosphates were separated by the method of Randerath and Randerath (29). Nucleoside diphosphates were separated by the method of Neuhard (24). Nucleoside triphosphates were separated by the method of Cashel et al (9). The positions of nucleotides were detected with the aid of Chromato-Vue (Ultraviolet Products, Inc.). When measurements of radioactivity were necessary, the spots were cut out and placed in vials containing 10 ml of toluene-Liquifluor (New England Nuclear Corp).

RESULTS

Nucleotide kinase. To determine whether virions of SRV had nucleotide kinase activity, ^{32}P-ATP was incubated with disrupted SRV virions in the presence of Mg^{2+}, with or without an added acceptor. Incubation was carried out at 40 C for 5 min. The reaction was stopped by the addition of EDTA. The ^{32}P-labeled substances were analyzed by paper chromatography in the two solvent systems described above and by two-dimensional PEI cellulose thin-layer chromatography.

^{32}P was transferred from [γ-^{32}*P*]ATP to at least three substances in the virion (Fig. 1, top). Two of them were identified by two-dimensional PEI cellulose chromatography as dGTP and dCTP. The other ^{32}P-labeled substance(s) remained at the origin of a paper chromatogram or a thin-layer chromatogram. The ^{32}P label in the substance(s) at the origin was trichloroacetic acid insoluble. Treatment with Pronase or ribonuclease A caused about 0.5 or 0.1, respectively, of the ^{32}P to become soluble in trichloroacetic acid.

When deoxyguanosine diphosphate (dGDP) or dCDP was added to the reaction mixture, the ^{32}P-peaks in a paper chromatogram corresponding to dGTP or dCTP, respectively, were increased 10- to 20-fold (Fig. 1, middle and bottom). These

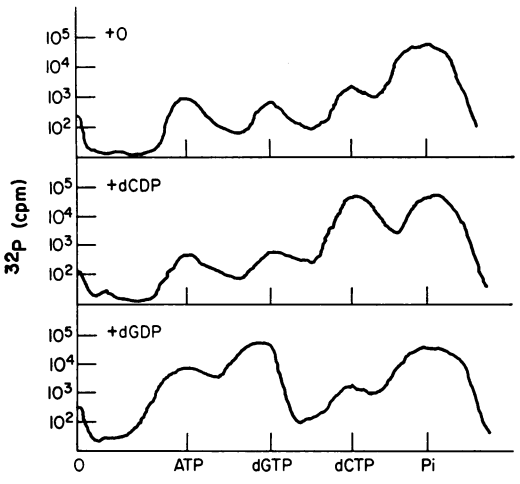

FIG. 1. *Nucleoside diphosphate phosphotransferase activity in SRV virions. The reaction mixture (0.050 ml) contained Nonidet-disrupted SRV virions (47 μg of viral protein), 35 pmoles of [γ-^{32}P]ATP (7.2 Ci/mmole), and 10 μg of dCDP (middle) or 10 μg of dGDP (bottom) in 0.02 M Tris-hydrochloride (pH 7.5) containing 0.003 M $MgCl_2$. The mixtures were incubated at 40 C for 5 min, and the reaction was terminated by the addition of ethylenediaminetetraacetic acid (20 mM). A sample (0.010 ml) was analyzed by paper chromatography in the solvent system: 0.1 M phosphate buffer-ammonium sulfate-n-propanol (100:60:2). The paper chromatograms were scanned with the aid of a Radiochromatogram Scanner (Packard Instrument Co., Inc.).*

^{32}P-labeled substances were eluted with water from a paper chromatogram and identified as dGTP and dCTP, respectively, by two-dimensional PEI cellulose thin-layer chromatography using the solvent systems described in Materials and Methods.

These data indicated the presence of nucleotide kinase activity in the virions of SRV. Some requirements for this activity are given in Table 2. Disruption of virions was required. Heating the disrupted virions at 100 C for 2 min inactivated the kinase activity. A divalent cation such as Mg^{2+}, Mn^{2+}, Ca^{2+}, or Fe^{2+} was essential for the kinase reaction.

The kinetics of hydrolysis of ^{32}P-ATP and the formation of ^{32}P-dGTP and inorganic ^{32}P were studied in the presence of dGDP as a ^{32}P-acceptor (Fig. 2). There was a rapid disappearance of ^{32}P-ATP with appearance of ^{32}P-dGTP and inorganic ^{32}P. The recovery of ^{32}P-dGTP and inorganic ^{32}P was only approximately 60% of the amount of ^{32}P-ATP hydrolyzed because there are other acceptors within the virion, for example, nucleoside mono- and diphosphates (see later), as well as large molecules which do not migrate in a paper chromatogram or a thin-layer chromatogram.

TABLE 2. *Conditions for kinase reaction*[a]

Complete system (+dCDP, 10 μg)	^{32}P-distribution in nucleotides (%)			
	Origin	ATP	dCTP	dGTP
Without virions	0.2	98.5	0.2	0.2
With nondisrupted virions	4.0	72.0	10.0	14.0
With disrupted virions	0.7	8.7	89.0	1.6
With disrupted, heated virions	0.5	96	1.5	2.0
With disrupted virions				
$-Mg^{2+}$	0.6	95.0	2.3	2.1
$-Mg^{2+}$, $+Mn^{2+}$	0.8	11.2	86.0	2.0
$-Mg^{2+}$, $+Ca^{2+}$	1.3	22.0	71.0	5.7
$-Mg^{2+}$, $+Fe^{2+}$	0.7	18.0	79.0	2.3

[a] Abbreviations: dCDP, deoxycytidine diphosphate; ATP, adenosine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate.

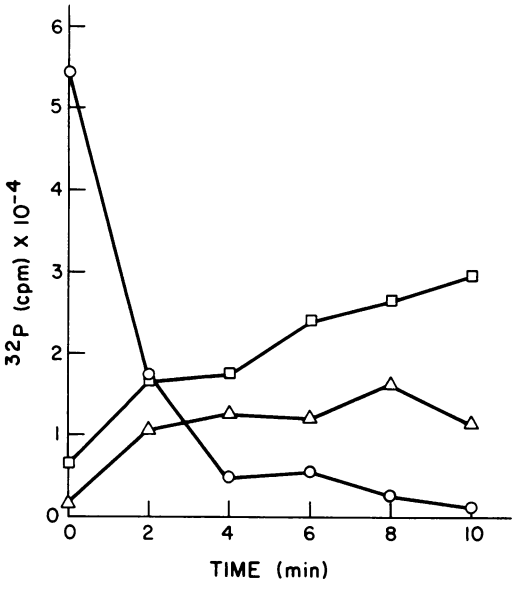

FIG. 2. *Kinetics of nucleoside diphosphate phosphotransferase reaction. The reaction mixture (0.10 ml) contained [γ-^{32}P]ATP (70 pmoles: 1.5 × 10^4 disintegrations per min per pmole) and Nonidet P-40-disrupted SRV virions, 95 μg of viral protein, in 0.02 M Tris-hydrochloride (pH 7.5) containing 0.003 M $MgCl_2$. At the times indicated, a sample (0.010 ml) was withdrawn and chilled, and ethylenediaminetetraacetic acid was added. The reaction mixtures were then analyzed by paper chromatography (see legend of Fig. 1). Symbols: (○), ATP; (□), Pi; (△), dGTP.*

Formation of inorganic ^{32}P is probably the result of the action of a hydrolytic enzyme such as nucleoside triphosphatase or phosphatase (see below).

Properties of the nucleotide kinase in virions of SRV. To investigate the specificity of the acceptors for the kinase(s), separate mixtures of [γ-^{32}P]-ATP with disrupted virions were incubated at 40 C for 5 min with each of the common deoxyribonucleoside mono- or diphosphates or the common ribonucleoside mono- or diphosphates. The reaction mixtures were mixed with known standard nucleotides and analyzed by two-dimensional PEI cellulose thin-layer chromatography as described above.

Qualitatively, all 15 of the nucleotides tested, that is deoxyadenosine monophosphate, deoxyguanosine monophosphate (dGMP), deoxycytidine monophosphate, dTMP, dADP, dGDP, dCDP, deoxythymidine diphosphate (dTDP), adenosine monophosphate (AMP), guanosine monophosphate, cytidine monophosphate (CMP), uridine monophosphate (UMP), guanosine diphosphate, cytidine diphosphate, and uridine diphosphate (UDP) [adenosine diphosphate (ADP) was not tested], acted as acceptors of ^{32}P from [γ-^{32}P]ATP to form a corresponding nucleoside diphosphate or nucleoside triphosphate. Lower labeling with ^{32}P was observed with the ribonucleoside mono- and diphosphates as acceptors (*data not shown*). This lower transfer to ribonucleotides seems to be the result of the presence in the disrupted virions of SRV of hydrolytic activities (e.g., phosphatase or nucleotidase, or both) which are more active toward ribonucleotides than deoxyribonucleotides (see below).

Donor specificity was determined by incubating each of the eight nucleoside triphosphates (Table 3) in a kinase assay with disrupted virions and ^{14}C-dCDP as an acceptor. The reaction mixtures were analyzed by one-dimensional PEI cellulose thin-layer chromatography in the solvent system of 0.85 M KH_2PO_4, *p*H 3.4 (Table 3). GTP and dGTP were significantly poorer donors (one-third to one-fourth) than the others. With ^{3}H-dADP as an acceptor in the kinase reaction, GTP and dGTP were also found much less (ca. one-half) active as phosphate donors than the other (*data not shown*).

Phosphatase. The incubation of [γ-^{32}P]ATP with disrupted virions of SRV led to the formation of inorganic ^{32}P, as well as to the transfer of ^{32}P to nucleotide acceptors (Fig. 1 and 2). To characterize further this phosphatase activity, ^{3}H-ATP was incubated with disrupted virions of SRV, and the reaction mixture was analyzed by paper chromatography. ^{3}H-ATP was hydrolyzed to ADP, AMP, and adenosine (Fig. 3). This hydrolysis of ATP was dependent on the presence of a divalent cation such as Mg^{2+}, Ca^{2+}, or Fe^{2+}. Heating the disrupted virions at 100 C for 2 min destroyed the enzyme activity. ^{3}H-CTP and ^{3}H UTP were also hydrolyzed by disrupted virions to

TABLE 3. *Donor specificity of nucleotide kinase in the virion of Schmidt-Ruppin strain of Rous sarcoma virus*[a]

Donor	^{14}C distribution (%)	
	dCDP	dCTP
ATP	67	33
GTP	90	10
CTP	63	37
UTP	64	36
dATP	62	38
dGTP	89	11
dCTP	56	44
dTTP	68	32

[a] Abbreviations: dCDP, deoxycytidine diphosphate; dCTP, deoxycytidine triphosphate; ATP, adenosine triophosphate; GTP, guanosine triphosphate; CTP, cytidine triphosphate; UTP, uridine triphosphate; dATP, deoxyadenosine triphosphate, dGTP, deoxyguanosine triphosphate; dTTP, deoxythymidine triphosphate.

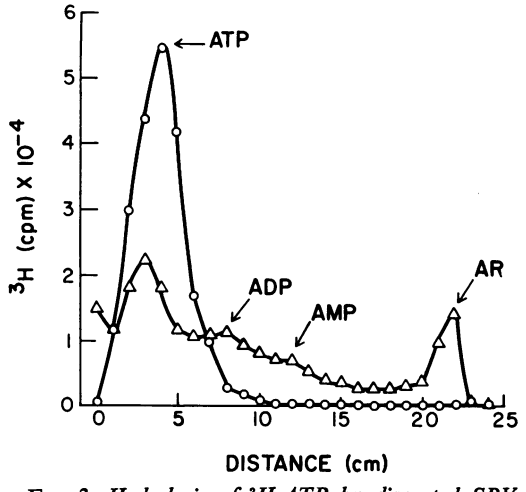

FIG. 3. *Hydrolysis of ^{3}H-ATP by disrupted SRV virions. The reaction mixture (0.05 ml) contained ^{3}H-ATP (25 pmoles; 9,000 counts per min per pmole), and Nonidet P-40-disrupted SRV virions (54 µg of viral protein) in 0.02 M Tris-hydrochloride (pH 7.5) containing 0.003 M $MgCl_2$. The mixtures were incubated at 40 C for 15 min. The reaction was terminated by heating at 100 C for 2 min. The reaction mixture was analyzed by paper chromatography by using the solvent system isobutyric acid-ammonium hydroxide-water (66:1:33). Symbols: (○), disrupted and heated virions; (△), disrupted virions.*

CDP, CMP, and cytidine, and UDP, UMP, and uridine, respectively. Deoxythymidine triphosphate (dTTP), dATP, dCTP, dGTP, and GTP were not hydrolyzed to their respective nucleosides. Paranitrophenyl phosphate was hydrolyzed.

Virions of avian myeloblastosis virus (AMV) have previously been reported to have an adenosine triphosphatase on their envelope (14). To compare the phosphatase activity of SRV and AMV, disrupted and nondisrupted virions were incubated with ^{3}H-ATP, in the absence or presence of 10 mM phosphate, and the products were analyzed by paper chromatography (Table 4). AMV had a phosphatase which did not require virion disruption. SRV had an adenosine triphosphatase which did not require virion disruption. Upon disruption of SRV virions, further phosphatase activity was revealed. This internal SRV phosphatase was not inhibited by 10 mM phosphate.

Nucleotides in the virion of SRV. The presence in the virion of SRV of acceptors for the nucleotide kinase reaction was demonstrated in the experiment presented in Fig. 1, top. To determine the quantity and type of nucleotides present in virions of SRV, ^{32}P-labeled SRV was extracted with 5% cold trichloroacetic acid, and phospholipids were removed by extraction with chloroform-methanol (2:1) and with ethyl ether. The ^{32}P-labeled substances remaining in the aqueous phase were analyzed by two-dimensional PEI cellulose thin-layer chromatography. To avoid ^{32}P contamination from other substances, in some experiments, the nucleotides were first purified by adsorption to acid-washed activated charcoal (Norite). After washing with 0.1 M KH_2PO_4 and water, the nucleotides were eluted with ethanol-ammonia-water (65:35:0.3) (9). The results were almost exactly the same as those in experiments which did not involve adsorption to charcoal.

TABLE 4. *Hydrolysis of ^{3}H-adenosine triphosphate by purified avian myeloblastosis virus (AMV) and Schmidt-Ruppin strain of Rous sarcoma virus (SRV)*[a]

Virus	Reaction products	
	With nondisrupted virions	With Nonidet P-40 disrupted virions
AMV	Adenosine	Adenosine
+Pi[b]	Adenosine	ADP >> adenosine
SRV	ADP	ADP > adenosine > AMP
+Pi[b]	-	ADP > adenosine > AMP

[a] Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate.
[b] Phosphate buffer at 10 mM, *p*H 7.0.

TABLE 5. *Nucleotides present in Schmidt-Ruppin strain of Rous sarcoma virus virions*[a]

More than 100 molecules per virion	50 to 100 molecules per virion
ADP	GMP
CTP	UTP
dCTP	AMP
GDP	ATP
dATP	dTTP
	UDP
	dGMP

[a] Abbreviations: ADP, adenosine diphosphate; GMP, guanosine monophosphate; CTP, cytidine triphosphate; UTP, uridine triphosphate; dCTP, deoxycytidine triphosphate; AMP, adenosine monophosphate; GDP, guanosine diphosphate; ATP, adenosine triphosphate; dATP, deoxyadenosine triphosphate; dTTP, deoxythymidine triphosphate; UDP, uridine diphosphate; dGMP, deoxyguanosine monophosphate.

The extracts were mixed with known nucleotides before loading on thin-layer plates. Nucleotides were detected with the use of Chromato-Vue. Spots were cut out, and the radioactivity of each spot was measured.

The number of nucleotides in a virion was roughly calculated from the total ^{32}P in nucleotides and the ^{32}P in viral RNA, assuming that 70% of the RNA in the virion had a molecular weight of 10^7 daltons (5). ^{32}P recovered in nucleotides was found to average 3.5% of the total ^{32}P in the virion. ^{32}P in RNA was 56%. Approximately 2,000 nucleotides were, therefore, calculated to be present in a virion of SRV. The nucleotides identified are listed in Table 5 in the order of decreasing amounts. ADP was about one-fourth of the total nucleotides.

Other enzyme activities. Detection of this large number of enzyme activities in purified virions of SRV raised the question whether these enzyme activities were merely a random sample of host cell enzymes picked up by the virus during assembly. Therefore, we examined purified virions of SRV and the cytoplasmic fraction of uninfected and SRV-infected chicken cells for hexokinase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase activities. The results (Table 6) indicate that hexokinase and lactate dehydrogenase activities were present in purified virions of SRV and that lactate dehydrogenase activity was increased 10-fold by disruption of the SRV-virion. However, these activities in SRV virions were 10 to 100 times lower per milligram of protein than those in cell extracts. No glucose-6-phosphate

TABLE 6. *Enzyme activities in cells and in Schmidt-Ruppin strain of Rous sarcoma virus (SRV) virions*

Location	Hexokinase (pmole/mg of protein/min)	LDH[a] (nmole/mg of protein/min)	G-6-PDH[b] (nmole/mg of protein/min)
Uninfected cells	370	850	1.6
SRV-infected cells	242	785	18
Nondisrupted SRV	1.3	5	<0.001
Disrupted SRV	2.3	60	<0.001

[a] Lactate dehydrogenase.
[b] Glucose-6-phosphate dehydrogenase.

dehydrogenase activity was found in the purified virions.

Enzyme activities in cores. To determine the location of the enzyme activities in the purified virions, cores were prepared from purified virions. All of viral RNA and 20 to 30% of the protein were in these cores (10). Ribonuclease treatment of these cores solubilized all of the protein (J. Coffin, *personal communication*). The cores still had some of all of the enzyme activities detected in Nonidet P-40-disrupted SRV virions (Table 7), suggesting that these enzymes were not present only in cytoplasmic vesicles. In addition, enzyme activities could be classified into two groups by comparing the specific activity of each enzyme in whole virions with that in cores. One group of enzymes which included DNA polymerase, DNA ligase, and hexokinase activities had an increased specific activity in cores. The other group, nucleosidediphosphate phosphotransferase, lactate dehydrogenase, and adenosine triphosphatase had a decreased specific activity. Glucose-6-phosphate dehydrogenase activity was detected at a high level in SRV-infected cell extracts, but that activity was not detectable in purified SRV virons or in cores.

Nucleotides also seemed to be present in or associated with the core structure, because endogenous acceptors of ^{32}P from [γ-^{32}P]ATP were demonstrated when isolated cores were incubated with [γ-^{32}P]ATP. Actual analysis of nucleotides in cores made from ^{32}P-SRV virions revealed that the amount of nucleotides was about 20% of that in the whole virions. (About 20% of the total protein was also in these cores.)

DISCUSSION

The experiments in this paper were started to explain certain discrepant results in our previous studies of the DNA polymerase and ligase activities of disrupted virions of SRV. These discrepant results included an apparent lack of requirement for all four deoxyribonucleoside triphosphates for the DNA polymerase activity and an apparent lack of requirement for ATP or NAD for the DNA ligase. We have now shown that purified virions of SRV contain various nucleotides and a general nucleotide kinase. Therefore, there were nucleotides present in polymerase and ligase reaction mixtures without addition of nucleotides.

In the course of the experiments of this paper, we also found a phosphatase in purified SRV virions. The presence of all of these enzyme activities, as well as the nucleotides and other species of nucleic acids which have been reported in purified virions of RNA tumor viruses (*see* 37), raises several questions as to their significance. (i) Are they included in the virions or are they contaminants which are not included in the virions? (ii) Are they part of the envelope or core of the virions? (iii) Do they have a role in viral replication? (iv) Are they coded by the virus or the cell?

To start to answer these questions, we looked at three enzymes, hexokinase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase, which are present in high concentrations in the cytoplasm of cells producing SRV and would not be expected to be involved in viral replication. No glucose-6-phosphate dehydrogenase activity was found with the purified SRV virions. However, the activities of hexokinase and of lactate

TABLE 7. *Location of enzyme activities in the Schmidt-Ruppin strain of Rous sarcoma virus virion*

Enzyme activity[a]	Nondisrupted virion (pmole/mg of protein/min)	Disrupted virion (pmole/mg of protein/min)	Core (pmole/mg of protein/min)
Endogenous RNA-directed DNA-polymerase	1.5	16	21[b]
DNA ligase	8.4	70	750
Hexokinase[c]	3	5	26
Nucleoside diphosphate kinase[d]	7	73	17
Lactate dehydrogenase	5,000	60,000	200
Adenosine triphosphatase	8	6	4
Glucose-6-phosphate dehydrogenase	<1	<1	<1

[a] RNA, ribonucleic acid; DNA, deoxyribonucleic acid.
[b] Depends upon polymerase and upon RNA concentration in core.
[c] An average of two separate virus and core preparations.
[d] As adenosine triphosphate:deoxycytidine diphosphate phosphotransferase.

dehydrogenase were between 0.5 and 8% of that in the cytoplasms of SRV-producing cells. Therefore, some enzymes apparently unrelated to viral replication were included with purified virions of SRV, whereas others were not. The situation with transfer RNA (tRNA) species is similar. Only some of the tRNA species in myeloblasts are present with purified avian myeloblastosis virus (8-11, 38).

To further evaluate the significance of the components present with purified virions, we studied the effect of disruption of virions on enzyme activity and whether the components were found with purified cores. If these enzyme activities are not present in cytoplasmic vesicles that copurify with virions, several classes of components present with purified SRV can be defined. (i) Components whose activity was not increased by disruption and which had a lower specific activity in purified cores (e.g., adenosine triphosphatase) are probably in the envelope. (ii) Components whose activity was increased by disruption and which had a lower specific activity in purified cores (e.g., lactate dehydrogenase and nucleoside diphosphate kinase) are probably internal to the virion envelope but are not tightly bound to cores. (iii) Components whose activity was only slightly increased by disruption and which had a higher specific activity in purified cores (e.g., glucokinase) may be both in the envelope and bound to cores. (iv) DNA polymerase and ligase are examples of components whose activity has increased by disruption and which had a higher specific activity in purified cores. Nucleotides may be inside virions and only weakly bound to cores.

Other viruses contain enzyme activities in purified virions, as well as some host material, nucleoside triphosphate phosphohydrolase activities have been reported in the virions of AMV (14), vaccinia virus (13, 28), reovirus (7, 17), and frog virus 3 (39). More recently, nucleoside triphosphate phosphohydrolase activity was found in influenza virus, Rauscher leukemia virus, and vesicular stomatitis virus (30). In addition to nucleoside triphosphate phosphohydrolase activity was found in the virions of AMV, Rauscher leukemia virus, vesicular stomatitis virus, and influenza virus (30). Nucleoside diphosphate phosphotransferase activity was also detected in the virion of AMV (R. D. Wells, *personal communication*). DNA-dependent RNA polymerase in the virions of poxvirus group (18, 23); RNA-dependent RNA polymerase activity in the virion of reovirus (6, 32), cytoplasmic polyhedrosis virus (19), vesicular stomatitis virus (2), Newcastle disease virus (15), and influenza virus (9*a*, 26); DNA nuclease activities in the virion of vaccinia virus (27); and DNA polymerase in the virion of Kilham rat virus (31) have also been reported as enzyme activities associated with virions.

The inclusion in purified virions of components not directly related to virus replication may be a general feature of animal viruses. There must be some kind of specific binding relations between virus proteins so they recognize each other and viral nucleic acids. If there is some cross-reaction with cellular components, cellular components could be included in virions. RNA tumor viruses may appear to have more of these cellular components because RNA tumor viruses have been studied more intensively in this way or because RNA tumor viruses may have recently originated from cellular components (36).

Additional experiments will be required to answer questions about the role of these components in viral replication and for those components related to viral replication questions as to whether they are coded by the virus or the cell.

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