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Presence of Nucleoside Triphosphate Phosphohydrolase Activity in Purified Virions of Reovirus

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A nucleoside triphosphate phosphohydrolase activity has been discovered in reovirus virions. This activity converts nucleoside triphosphates to nucleoside diphosphates in vitro. Properties of this enzyme are presented, with evidence that this activity is an integral part of the virion core.

Reovirus, an animal virus whose genome consists of double-stranded ribonucleic acid (RNA), has been shown to carry an RNA polymerase (transcriptase) in the core of the virion (2, 11, 13). The polymerase activity can be demonstrated in vitro after the virions have been treated either to remove or to damage the outer protein coat and expose the core to the substrates. During studies on the properties of the reovirus-core RNA polymerase in vitro, the presence of a nucleoside triphosphate phosphohydrolase activity in the purified viral cores was detected. This activity converts the nucleoside triphosphates (NTP) to nucleoside diphosphates (NDP) in vitro. In this communication, some properties of this enzyme are presented with evidence which indicates that it is an integral portion of the virion cores.

MATERIALS AND METHODS

Cells and virus. Monolayers of mouse L cells were grown in Minimal Essential Medium (MEM; Gibco, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (FBS; Flow Laboratories, Inc., Rockville, Md.) at 37 C in 5% CO_2 gas flow incubators. When the cells were just reaching confluence, the medium was poured off, and the monolayers were infected with 10 to 50 plaque-forming units (PFU)/cell of reovirus type 3 (plaque purified stock obtained from A. Graham, Wistar Institute, Philadelphia) in a small volume of lysate. Adsorption was allowed to proceed for approximately 1 to 1.5 hr at room temperature, after which the monolayers were fed by the addition of prewarmed (37 C) MEM plus 1% FBS and incubated at 37 C in a 5% CO_2-gassed incubator. At times ranging from 24 to 48 hr postinfection, the culture fluid was poured off; the cells in the infected monolayers were harvested by trypsinization (0.10% trypsin, 5 min at 37 C), pelleted (1,000 × *g*, 5 min), washed with phosphate buffered saline (PBS), and pelleted once again. The pellet was resuspended in PBS (ca. 5 ml of packed wet cells in a final volume of 15 ml) and was then either frozen and stored at −50 C or used immediately for virus purification.

Virus was released from the infected cells by homogenization with Freon-113 (Dupont of Canada Ltd, Toronto, Ont.) and purified by isopycnic centrifugation on CsCl gradients as described elsewhere (16). In some preparations, the culture fluid from the infected cells was also processed. In such cases, the virus in the culture fluid was pelleted (75 min at 35,000 × g in a Lourdes beta-fuge) and then resuspended in 0.10 M tris(hydroxymethyl)aminomethane(Tris)-hydrochloride (*p*H 8.3) and purified on CsCl along with the virus freed from the cells by the homogenization procedure. The final purified virus band (density = 1.37 to 1.38 g/cm^3) from the CsCl gradient was dialyzed against 0.10 M Tris-hydrochloride (*p*H 8.3) and was stored at 4 C in this same buffer, as was the viral "top" component [density = 1.29 g/cm^3 (8)]. Virus was quantitated spectrophotometrically in terms of the optical density (OD) at 260 nm. One OD_{260} unit is equivalent to approximately 0.18 mg of reovirus (15).

Materials. Unlabeled nucleosides and nucleoside mono-, di-, and triphosphates were purchased from P-L Biochemicals, Inc., Milwaukee, Wis. Labeled NTP samples were purchased from Schwarz BioResearch, Orangeburg, N.Y., and included adenosine triphosphate(ATP)-*8-*^{3}*H* (24.5), deoxyadenosine triphosphate(dATP)-*8-*^{3}*H* (17.7), ^{3}*H*-guanosine triphosphate (GTP) (1.16), cytidine triphosphate(CTP)-*5-*^{3}*H* (20.4), and uridine triphosphate(UTP)-*5-*^{3}*H* (17.1), where the numbers in brackets indicate the specific activity of each compound in curies per mmole. NaCl, LiCl, KCL, $MgCl_2$, $MnCl_2$, $CaCl_2$, Na_2HPO_4, and $Na_4P_2O_7$ were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J. THAM (Tris) was purchased from Fisher Scientific Co., Fairlawn, N.J. Chymotrypsin (CHT) was purchased from Worthington Biochemical Corp., Freehold,

N.J. Ouabain (Strophantin-G) was purchased from Sigma Chemical Co., St. Louis, Mo.

Pre-coated plates (CEL 300 PEI; polyethyleneimine-impregnated cellulose) for thin layer chromatography (TLC) were purchased from Mondray Ltd., Montreal, P.Q.

Liquid scintillator consisted of Permaflour (Packard Instrument Co., Downers Grove, Ill.) diluted 25× in reagent grade toluene (Anachemia Chemicals Ltd., Montreal, P.Q.).

Nucleoside triphosphate phosphohydrolase assay. Reaction mixtures containing labeled NTP in addition to purified virus and other reagents as indicated were incubated at 37 C for specified lengths of time. Samples were then analyzed on TLC plates to obtain the distribution of radioactivity among the nucleoside mono-, di-, and triphosphates. Comparison of the distributions before and after incubation permitted determination of the amount of NTP hydrolyzed. Each reaction mix contained in a final volume of 50 μliters: 0.03 to 0.10 OD_{260} units of "activated" purified reovirus; 5 μmoles of Tris-hydrochloride, *p*H 7.8; divalent cation as specified; 1.0 μCi (0.02 μmole) of labeled NTP as specified; and various salts and other additives where indicated. Purified reovirus was activated either by heat shocking (61 C, 45 seconds, 25 μliter volume of 0.1 M Tris, *p*H 7.8) or by digesting with CHT (50 μg/ml, 0.1 M Tris, *p*H 7.8, 37 C, 30 min).

TLC plates were developed (ca. 12 cm) in one of two solvents. Solvent I consisted of NaCl in distilled water (2.0 M for adenosine, guanosine, and cytidine phosphorylated derivatives; 1.4 M for uridine phosphorylated derivatives). Solvent II consisted of 1.0 N HCOOH and 0.75 M LiCl (for all four series of di- and triphosphates). After development, each track was cut into 0.5-cm or 1.0-cm wide slices, and the slices were counted in 10 ml of liquid scintillator in a Nuclear-Chicago Mark I liquid scintillation counter. Activity profiles along the track were plotted, and peaks of activity were identified by comparison with standards (absorbing spots under ultraviolet illumination) run either in the same track or in an adjacent track on the TLC plate.

RNA polymerase assay. RNA polymerase activity was determined by measuring conversion of UTP-*5-*^3H into acid-insoluble form. Each reaction mix contained, in a total volume of 0.250 ml: sample of activated reovirus from a CsCl gradient fraction; 25 μmoles of Tris-hydrochloride, *p*H 8.3; 1.0 μmole of ATP; 0.04 μmole of GTP; 0.04 μmole of CTP; 0.04 μmole of UTP-*5-*^3H (1.0 μCi); 0.8 μmoles of $MgCl_2$; and 10 μmoles of LiCl. Incubation was for 60 min at 37 C. Cold 5% trichloroacetic acid (2 ml) and 0.02 M sodium pyrophosphate (NaPP) were added to each reaction mix at the end of the incubation, with ca. 50 μg of yeast RNA as carrier. Samples were filtered on 0.45-μm pore size membrane filters (Millipore Filter Corp., Bedford, Mass.), washed with ~30 ml of cold 5% trichloroacetic acid plus 0.02 M NaPP, and then dried. The radioactivity on the dried filters was counted in 10 ml of liquid scintillator as above.

CsCl gradient analysis of nucleoside triphosphate phosphohydrolase and RNA polymerase activity. Virus which had been activated by CHT digestion was layered on a preformed CsCl gradient (1.33 to 1.46 g/cm^3; 0.10 M Tris-hydrochloride, *p*H 8.3; 1 mM Mg^{2+}). This was spun for 3 hr at 150,000 × *g* in an SW50.1 head in a Beckman L2-65B ultracentrifuge. At the end of this spin, the active bands were in their equilibrium positions along the gradient, as longer spins did not alter the densities at which activity was found. Fractions were collected by dripping from the bottom of the tubes (usually 30 to 33 fractions per tube) and dialyzed to remove CsCl. Each fraction was then assayed for nucleoside triphosphate phosphohydrolase activity and RNA polymerase activity as described above.

Considerable loss of enzymatic activity accompanied the analysis on the CsCl gradient. This loss could be significantly reduced by including 1 mM Mg^{2+} in the gradient. Typically, between 20 and 50% of the nucleoside triphosphate phosphohydrolase and polymerase activity loaded on the gradient was recovered in the fractions.

RESULTS

Demonstration of nucleoside triphosphate phosphohydrolase activity with ATP as substrate. A reaction mix consisting of 0.10 OD_{260} units of heat activated reovirus, 0.02 μmole of ATP-*8-*^3H (1 μCi), 0.10 μmole of $MgCl_2$, and 5 μmoles of Tris-hydrochloride (*p*H 7.8) in a total volume of 50 μliters was incubated at 37 C. At specified times during the incubation, three-μliter samples were removed with a micropipette and spotted on TLC plates. These were then developed in solvent I, and radioactivity profiles were plotted as described above. Figure 1 (a) shows such a profile, demonstrating the resolution between ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Panel (b) shows the analysis of the labeled nucleoside derivatives in the reaction mix as a function of time at 37 C. This demonstrates that ATP is converted to ADP and that there is no further conversion to AMP.

Minimum conditions for nucleoside triphosphate phosphohydrolase activity. To define the minimum requirements for the phosphohydrolase activity, a series of modified reaction mixes was set up as specified in Table 1. These were incubated for 60 min at 37 C (unless otherwise noted) and were then analyzed as above. The complete reaction mix was as described in the preceding section. From the data, it can be seen that (i) the reaction proceeds at 37 C but not at 0 C; (ii) virus must be activated for the activity to manifest itself; (iii) activity is directly proportional to the amount of activated virus present; (iv) a divalent cation is required, with Mg^{2+} being slightly preferred to Mn^{2+} and greatly preferred to Ca^{2+}; (v) the enzyme can utilize GTP and dATP as substrate, so it is not specific for ATP; and (vi) viral "top" component from CsCl gradients appears to be

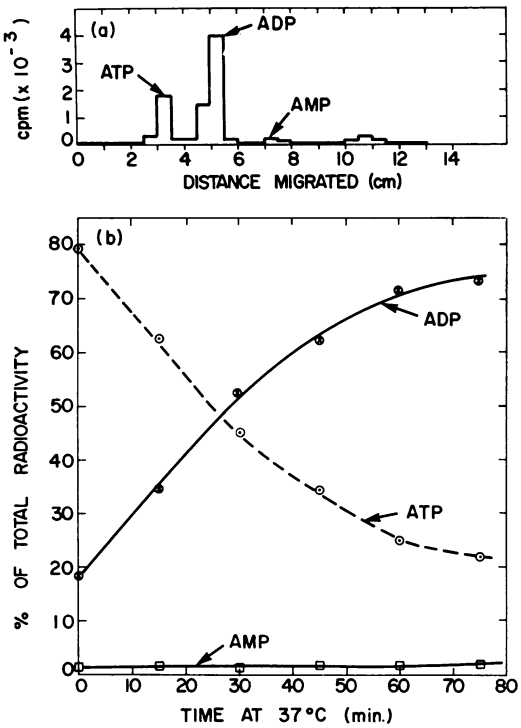

FIG. 1. *(a) TLC separation of ATP, ADP, and AMP. Developed 13 cm in solvent 1. (b) Kinetics of conversion of ATP-8-³H to ADP-8-³H.*

devoid of this nucleoside triphosphate phosphohydrolase activity.

Variation of pH and Mg^{2+} concentration. Activity of the nucleoside triphosphate phosphohydrolase, with ATP-*8-³H* as substrate, was measured over a range of *p*H and of Mg^{2+} concentrations. Figure 2a shows the dependence on *p*H. There is a rather sharp optimum centered between *p*H 7.5 and 8.0 with a rapid decrease in activity to either side of this range. The dependence on Mg^{2+} concentration is shown in Fig. 2b. Activity is seen to increase rapidly with increasing concentration of Mg^{2+}, reaching a maximum by approximately 2 mM and decreasing only slightly at 8 mM. On the basis of these data, standard assay conditions were chosen at 2 mM Mg^{2+} and *p*H 7.8.

Analysis of nucleoside triphosphate phosphohydrolase activity on CsCl density gradients. To eliminate the possibility that the observed nucleoside triphosphate phosphohydrolase activity was the result of contamination of the purified reovirus preparations with cellular constituents, activated reovirus (50 mg of CHT per ml, 37 C, 30 min) was analyzed on a preformed CsCl gradient as described above. Fractions were collected, dialyzed, and assayed for nucleoside triphosphate phosphohydrolase activity with ATP-*8-³H* as substrate and for RNA polymerase activity as described above. The latter activity was used as a marker for viral cores (13). Figure 3 shows the results. It can be seen that there are two main peaks of nucleoside triphosphate phosphohydrolase activity, banding at a density of 1.44 and 1.42 g/cm³, respectively, which are coincident with the RNA polymerase activity These two peaks of activity probably are a reflection of the presence of infectious and defective virions in the virus stock, which give rise to heavy and light cores, respectively (10*a*). The significance of the small amount of nucleoside triphosphate phosphohydrolase activity at a density of 1.34 g/cm³ is unknown. These data are interpreted as indicating that the nucleoside triphosphate phosphohydrolase activity is associated with the virus core. This is consistent with the observation (Table 1) that no nucleoside triphosphate phosphohydrolase activity manifests itself until the virion has been disrupted, either by heat shock or by CHT digestion. Taken together, these observations make it highly improbable that the observed nucleoside triphosphate phosphohydrolase activity represents a cellular contaminant.

TABLE 1. *Minimum conditions for reovirus nucleoside triphosphate phosphohydrolase activity*

Condition	Relative activity
Complete (60 min, 37 C)	1.00
Complete (0 min, 37 C)	0.00
Complete (60 min, 0 C)	0.01
Complete, but virus not heat-activated	0.05
Complete, no heat activation of virus but CHT treatment (37 C, 30 min, 50 μg/ml)	1.21
Minus virus	0.00
Minus virus, plus 50 μg of CHT per ml	0.00
Half the amount of virus	0.53
Minus Mg^{2+}	0.31
Minus Mg^{2+}, plus ethylenediaminetetraacetic acid (0.10 μmole)	0.01
Minus Mg^{2+}, plus Mn^{2+} (0.10 μmole)	0.81
Minus Mg^{2+}, plus Ca^{2+} (0.10 μmole)	0.36
Minus ³H-ATP, plus ³H-GTP (1.0 μCi, 0.02 μmole)	0.66
Minus ³H-ATP, plus ³H-dATP (1.0 μCi, 0.02 μmole)	1.44
Minus virus, plus "top" component (0.10 OD_{260} unit)	0.01
Minus virus, plus heat-activated "top" (0.10 OD_{260} unit)	0.00

Effects of Na^+, K^+, and Li^+ ions. Na^+ and K^+ are known to exert a strong influence on microsomal adenosine triphosphate phosphohydrolase

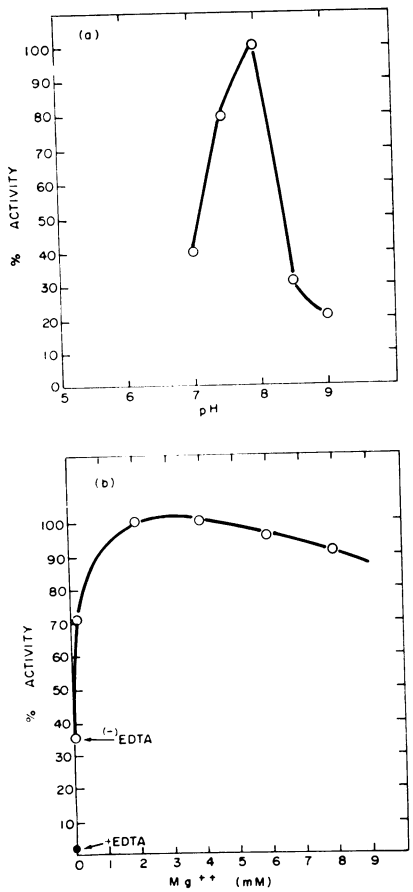

FIG. 2. *(a) pH dependence of reovirus nucleoside triphosphate phosphohydrolase activity with ATP-8-^3H as substrate. Conditions same as for Fig. 1, except that each reaction mix was buffered at the indicated pH. (b) Mg^{2+} concentration dependence of reovirus nucleoside triphosphate phosphohydrolase activity. Conditions same as for Fig. 1 except Mg^{2+} concentration varied.*

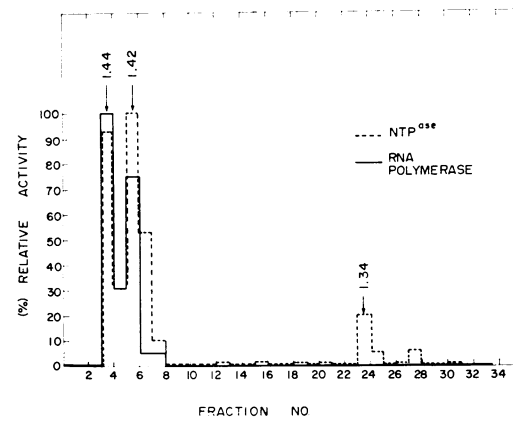

FIG. 3. *Analysis of reovirus nucleoside triphosphate phosphohydrolase (with ATP-8-^3H as substrate) and RNA polymerase on a CsCl gradient to show coincidence of the two activities.*

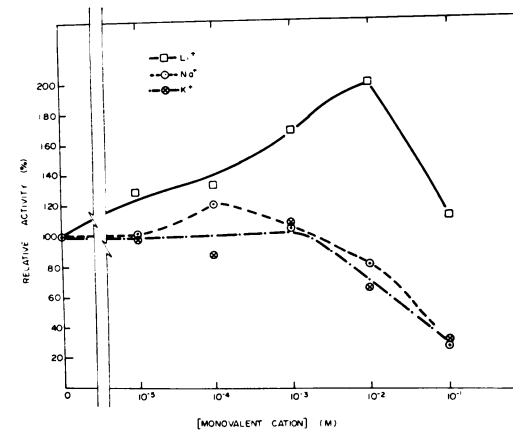

FIG. 4. *Effects of Na^+, K^+, and Li^+ on reovirus nucleoside triphosphate phosphohydrolase activity with ATP-8-^3H as substrate. A series of reaction mixtures identical to that described for Fig. 1 was set up. Individual reaction mixtures were adjusted to the indicated concentration of monovalent cation, incubated at 37 C for 30 min, and analyzed as described in Methods.*

(14). Hence, the response of the reovirus nucleoside triphosphate phosphohydrolase to Na^+, K^+, and Li^+, with ATP-8-^3H as substrate, was investigated. Figure 4 shows the results. The data indicate that Na^+ and K^+ inhibit activity at concentrations above 10^{-3} M, whereas Li^+ markedly stimulates the enzyme in this concentration range.

Effect of ouabain. Ouabain is known to inhibit cellular adenosine triphosphate phosphohydrolases (10, 14). Hence, the reovirus nucleoside triphosphate phosphohydrolase activity with ATP-8-^3H as substrate was tested for susceptibility to inhibition by this compound. At the tested concentrations of 10^{-5} M, 10^{-4} M, 3×10^{-4} M, and 10^{-3} M, the per cent of inhibition was 1, 0, 6, and 2, respectively. This indicates that the reovirus nucleotide triphosphate phosphohydrolase activity is unaffected by this drug.

Effects of inorganic orthophosphate (P_i) and pyrophosphate (PP_i). Figure 5 shows nucleoside triphosphate phosphohydrolase activity as a function of P_i and PP_i concentration in the reaction mix, with ATP-8-^3H as substrate. At $\sim 10^{-5}$ M, both P_i and PP_i appear to stimulate the enzyme to a slight extent, whereas higher concentrations of either are inhibitory, with PP_i being more so than P_i at a given concentration.

Relative reaction rates with different substrates. It has already been shown (Table 1) that the nu-

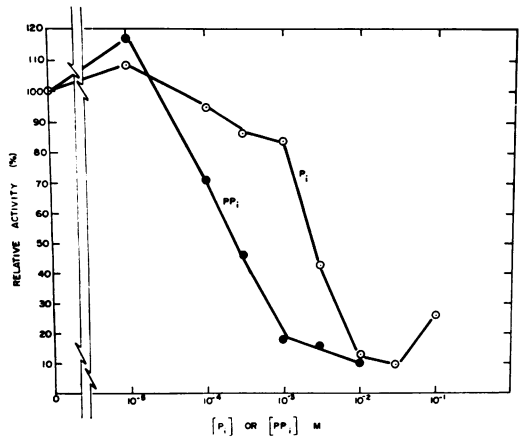

FIG. 5. *Effects of inorganic ortho- and pyrophosphate on reovirus nucleoside triphosphate phosphohydrolase activity with ATP-8-^3H as substrate. Details same as for Fig. 1, except that individual reaction mixes were adjusted to the indicated concentration of P_i (by using Na_2HPO_4) or PP_i (by using $Na_4P_2O_7$)*

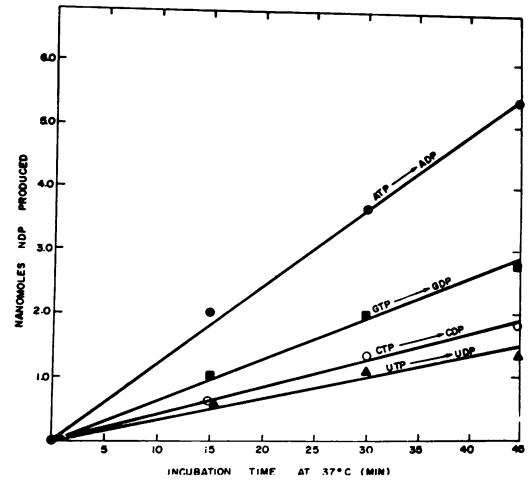

FIG. 6. *Kinetics of nucleoside triphosphate phosphohydrolase activity with different substrates. Four reaction mixes were set up which were identical, except that each contained a different ^3H-NTP as substrate (0.02 μmole per reaction in each case). Other conditions same as for Fig. 1, except 0.06 OD_{260} of purified reovirus was used.*

cleoside triphosphate phosphohydrolase activity is not specific for ATP. Hence, the activity of the enzyme was measured with ATP, GTP, CTP, and UTP individually, at equal molar concentrations, as substrate. Figure 6 shows the kinetics of the reaction with each substrate. From this it can be seen that the enzyme can utilize all of the NTP species tested as substrate, with the relative rates of hydrolysis being ATP:GTP:CTP:UTP$\approx$ 1:0.53:0.34:0.27.

Inhibition of activity by NDP. One of the products of the nucleoside triphosphate phosphohydrolase activity is the NDP. To check for product inhibition of this enzyme, its activity with a fixed amount of substrate was measured in the presence of different NDP forms. Figure 7 shows the results when a fixed amount of ATP-8-^3H was incubated in a standard reaction mix (as per text for Fig. 1) together with specified amounts of ADP, guanosine diphosphate (CDP), cytidine diphosphate (GDP) or uridine diphosphate (UDP). The data indicate that all of the nucleoside diphosphates tested inhibit the reaction. These observations are consistent with the interpretation that (i) activity of this enzyme is inhibited by its product (the above results with P_i indicate this as well, since P_i is also a product of the reaction); and (ii) the same catalytic site may be involved with all four NTP species tested as substrate, since any NDP inhibits activity with ATP. If separate sites were involved for each of the NTP forms, then it might be expected that the product of any one would not inhibit the activity of any of the others. The mixed substrate experiments described below bear on this same point.

Experiments with mixed substrates. The data of Fig. 6 clearly indicate that all four of the NTP forms tested are converted to the corresponding NDP by the nucleoside triphosphate phosphohydrolase activity present in reovirus virions. The question then arises as to whether separate enzymes (or at least catalytic sites) exist, each specific for one of the NTP types, or whether a single enzyme exists which can utilize all of the NTP forms as substrate at various efficiencies. To answer this question, a series of experiments was carried out in which one labeled NTP was incubated in a standard reaction mix with an excess of unlabeled triphosphate of a different nucleoside. Table 2 shows the results. The data clearly demonstrate that the NTP forms interfere with the utilization of each other in the phosphohydrolase reaction. Other interesting aspects of the data in Table 2 are referred to below.

Competitive nature of GTP inhibition of ATP hydrolysis. It has been demonstrated that the various NTP forms interfere with the utilization of each other in the phosphohydrolase reaction. In this section, one of the interactions, that involving GTP inhibition of ATP hydrolysis, is discussed in more detail. Reaction mixtures were set up in which the concentration of ATP was varied in both the presence and absence of a fixed amount of GTP. Reaction rates for the hydrolysis of ATP-8-^3H were determined, and the results were plotted in a Lineweaver-Burk plot (Fig. 8). The solid lines were fitted to the data points by

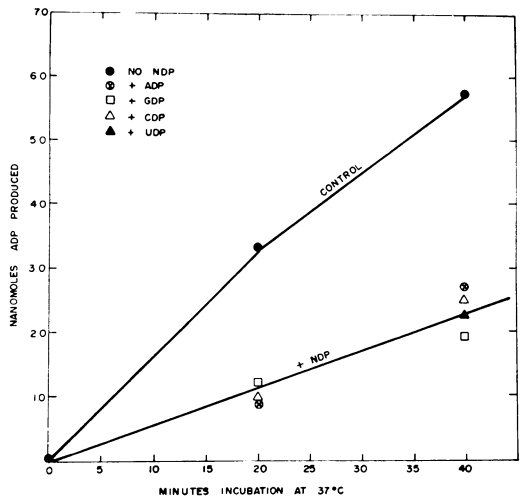

FIG. 7. *Inhibition of reovirus nucleoside triphosphate phosphohydrolase activity (ATP-8-^{3}H as substrate) by NDP. Five identical reaction mixes were set up containing 0.02 μmole ATP-8-^{3}H as substrate. One served as a control, and each of the remaining tubes received 0.07 μmole of one NDP as indicated. Samples (4 to 5 μliters) were removed after 0, 20, and 40 min of incubation at 37 C and analyzed as described in Methods. Other details same as in Fig. 1, except 0.06 OD_{260} of purified reovirus was used.*

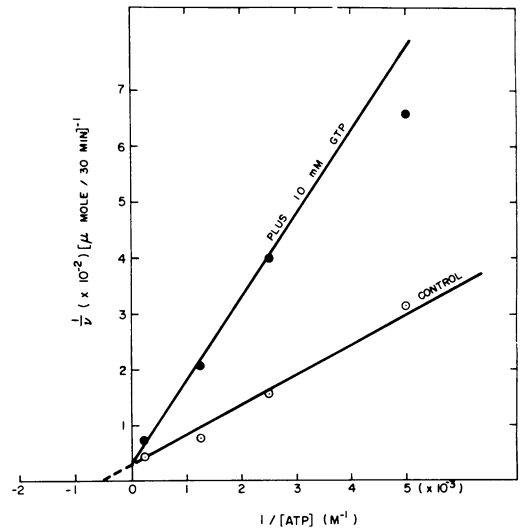

FIG. 8. *Lineweaver-Burke plot of reovirus nucleoside triphosphate phosphohydrolase activity with ATP as substrate in the presence and absence of GTP (0.05 μmole). Each reaction mix contained 0.06 OD_{260} units of CHT activated reovirus, a variable amount of ATP-8-^{3}H, and 0.05 μmole of GTP (when present). Other details same as for Fig. 1. Samples were removed after 0, 15, 30, and 45 min of incubation at 37 C and analyzed as in Methods. Reaction velocities were calculated on the initial slopes of the measured activity curves.*

TABLE 2. *Experiments with pairs of nucleoside triphosphates as substrate*

Labeled NTP	Unlabeled NTP[a]	Relative rate of hydrolysis of labeled NTP
		(%)
ATP-8-^{3}H (0.02 μmole)	None	100
	GTP	9.5
	CTP	21.9
	UTP	28.5
^{3}H-GTP (0.02 μmole)	None	100
	ATP	71.3
	CTP	23.2
	UTP	32.5
CTP-5-^{3}H (0.02 μmole)	None	100
	ATP	64.0
	GTP	21.1
	UTP	21.1
UTP-5-^{3}H (0.02 μmole)	None	100
	ATP	59.3
	GTP	11.4
	CTP	9.8

[a] Where present, 0.2 μmole.

inspection, bearing in mind that the point at the lowest ATP concentration in the presence of GTP was measured at a very low activity level and, hence, was subject to the greatest uncertainty. The data suggest that GTP inhibits ATP hydrolysis in a competitive fashion, since the maximum velocity at infinite substrate concentration in the presence of GTP is the same as in its absence. Fitting the experimental curves to the equation for competitive inhibition of enzyme activity (3) enables one to calculate values for K_m of ATP and $K_{i,slope}$ of GTP: $1/v = 1/V_{max} + K_m/V_{max} \cdot (1 + i/K_{i,slope})\ 1/S$ where v = observed velocity, V_{max} = maximum velocity at infinite substrate concentration, K_m = Michaelis constant for the substrate, S = substrate concentration, $K_{i,slope}$ = inhibition constant, and i = inhibitor concentration. Two such experiments were carried out, giving a mean value of 2.2 mM (actual values 1.7 and 2.7 mM) for K_m (ATP) and 0.7 mM (actual values 0.3 and 1.0 mM) for $K_{i,slope}$ (GTP).

These results support the conclusion that the hydrolysis of both ATP and GTP is catalyzed at a common active site, and, by logical extension from consideration of the data in Table 2, that all four NTP forms tested are hydrolyzed by a single enzyme.

DISCUSSION

Data presented above clearly demonstrate the existence of a nucleoside triphosphate phospho-

hydrolase activity in the cores of reovirus virions. This enzyme converts NTP to NDP with no further conversion to the level of the monophosphate or nucleoside. The measured apparent K_m for ATP as substrate is 2.2 mM. Divalent cations are required for this activity, with Mg^{2+} being preferred slightly to Mn^{2+} and Ca^{2+} substituting only relatively inefficiently. Na^+ and K^+ ions, which are required by, or stimulate, several known cellular adenosine triphosphate phosphohydrolases, have an inhibitory effect on hydrolysis of ATP by the reovirus enzyme. Li^+ ions stimulate the hydrolysis of ATP by the reovirus enzyme to a marked extent. Ouabain has no effect on the hydrolysis of ATP by the reovirus nucleoside triphosphate phosphohydrolase at concentrations normally found to inhibit cellular adenosine triphosphate phosphohydrolase in vitro. Hence, the reovirus nucleoside triphosphate phosphohydrolase activity, with ATP as substrate, differs qualitatively from several known cellular adenosine triphosphate phosphohydrolases.

An interesting characteristic of the reovirus nucleoside triphosphate phosphohydrolase pertains to the unsymmetrical reciprocal inhibitions of the substrate pairs involving ATP (Table 2). For example ^{3}H-ATP hydrolysis is inhibited 90.5% by 10-fold molar excess of unlabeled GTP, whereas ^{3}H-GTP hydrolysis is inhibited only 28.7% by 10-fold molar excess of unlabeled ATP. This same pattern is also evident in the ATP-CTP and ATP-UTP pairs. In contrast, the pairs involving only GTP, CTP, and UTP show more nearly equal reciprocal inhibitions. Thus, ATP appears to be rather a poor inhibitor of the hydrolysis of GTP, CTP, or UTP, whereas its own hydrolysis is readily susceptible to inhibition by GTP, CTP, or UTP. This may be a required characteristic for operation of the nucleoside triphosphate phosphohydrolase inside the living cell since ATP concentrations intracellularly are probably of the order of 10-fold greater than the concentrations of GTP, CTP, or UTP (4). Under such conditions, if GTP, CTP, and UTP are required to be hydrolyzed, it is obvious that their hydrolysis must be relatively insusceptible to inhibition by the excess ATP. Conversely, this property provides a means of selectively conserving ATP in the presence of active hydrolysis of GTP, CTP, and UTP.

Demonstration of nucleoside triphosphate phosphohydrolase activity in the cores of reovirus virions raises the important question of why it is there. At the present time, one can only speculate on answers to this. One possibility has to do with the question of how the single-stranded poly A associated with reovirus infection (1, 12) is replicated. Perhaps there exists a polymerase in reovirus-infected cells which, like polynucleotide phosphorylase (5, 7), can polymerize NDP species into polynucleotides. The observation that ATP is the preferred substrate, at least with in vitro experiments involving only single substrates, suggests that the nucleoside triphosphate phosphohydrolase could function to provide ADP for such a polymerase. This hypothesis could be tested by searching for such an enzyme in reovirus-infected cells. Another speculation involves the observation (Table 1) that dATP is very efficiently hydrolyzed relative to ATP. This suggests the possibility that the inhibition of cellular deoxyribonuclei acid synthesis late in the viral replication cycle may be a result of depletion of the deoxynucleoside triphosphate pools by the viral nucleoside triphosphate phosphohydrolase. Direct measurements of cellular NTP pools (ribo- and deoxyribo-) during the viral replication cycle should prove useful in assessing this possibility. Reovirions are not unique in possessing a nucleoside triphosphate phosphohydrolase activity, however, since vaccinia virus cores also have been shown to possess such activity (6, 9). The question then is raised about how general this phenomenon is among viruses. Further studies on a variety of purified virions are required to gain some perspective about the generality of this occurrence. This should then indicate whether this property, i.e., possession of a viral nucleoside triphosphate phosphohydrolase, happens only in exceptional cases or whether it is a commonly occurring property which may play some important, as yet unknown, role in virus-cell interactions in general.

ADDENDUM IN PROOF

As this manuscript was in its final stages of preparation, Kapuler et al. (Nature **225**:1209–1213, 1970) reported on their discovery of the nucleoside triphosphate phosphohydrolase activity in reovirus cores. Basically, our work confirms their report but there are some differences in properties. Kapuler et al. indicate that separate sites are involved in the hydrolysis of each of the NTP species, whereas our data indicate that the four NTP species tested are hydrolyzed at a common site. Also, our data indicate a greater sensitivity to inhibition by Na^+, K^+, and P_i than Kapuler et al. found. The reason(s) for these differences is not obvious to us at this time.

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