The Phosphorylation of Sendai Virus Proteins by a Virus Particle-associated Protein Kinase

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SUMMARY

Highly purified Sendai virus contained a protein kinase activity which catalysed the phosphorylation of endogenous polypeptides or exogenous protamine sulphate. The virus contained very low levels of phosphoprotein phosphatase activity. Polyacrylamide gel analysis of the reaction product indicated that the phosphorylation was specific for certain polypeptides and varied according to whether the virus was grown in eggs or in tissue culture. This variation was partially associated with the difference in the polypeptide pattern that occurred when the virus was grown in eggs or in tissue culture. Characterization of these phosphoproteins demonstrated that the phosphate was incorporated predominantly in a phosphoester linkage with threonine residues. Using a detergent and high salt solubilization procedure, the protein kinase activity was found associated within glycoprotein free virus particles but not with the nucleocapsid-associated polypeptides. In vivo phosphorylation occurred when Sendai virus was grown in eggs or in tissue culture with [32P] and the phosphorylated polypeptides were similar to those of the protein kinase reaction product. Phosphorylation could also be detected in the infected cell and could occur once the virus particle polypeptides were being synthesized. The non-structural polypeptides were not phosphorylated.

INTRODUCTION

Protein kinases are widely distributed in nature. The enzymes transfer the γ-phosphoryl group of ATP to O-seryl and O-threonyl linkages in proteins. They have been implicated in the control of several metabolic pathways involving cyclic AMP (cAMP) (Krebs, 1972). The best characterized process is the degradation of glycogen to glucose, catalysed by phosphorylase; two protein kinases act in a cascade reaction that depends on Ca²⁺ ions and cAMP (Hale, 1967).

Protein kinase activity has been detected in a number of enveloped viruses (Strand & August, 1971; Gravell & Cromeans, 1972, Hatanaka, Twiddy & Gilden, 1972; Randall et al. 1972, Rubenstein, Gravell & Darlington, 1972; Tan & Sokol, 1972; Silberstein & August, 1973). Among the negative strand RNA viruses, protein kinases have been reported for vesicular stomatitis virus (Sokol & Clark, 1973; Imblum & Wagner, 1974), rabies virus (Sokol & Clark, 1974) and Sendai virus (Roux & Kolakofsky, 1974). Unlike cell protein kinases, the virus particle-associated enzymes do not require cAMP (Krebs 1972).

This paper presents the characteristics of Sendai virus protein kinase, and extends the observations of Roux & Kolakofsky (1974).

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METHODS

Cells and virus. Chick embryo lung (CEL) and fibroblast (CEF) cells were prepared by methods described by Darlington, Portner & Kingsbury (1970) and Borland & Mahy (1968), and grown in medium 199 supplemented with 10% calf serum on 150 mm Petri dishes to form monolayers. Maden-Derby bovine kidney (MDBK) cells were grown in minimal essential medium (MEM) supplemented with 15% calf serum.

Sendai virus (obtained from Dr D. W. Kingsbury) was inoculated into 11-day embryonated eggs and harvested after 72 h incubation at 32°C. The allantoic fluid containing virus was clarified by low-speed sedimentation (2000 g for 10 min), snap frozen in ampoules and stored at −70°C. Sendai virus was grown in cell cultures as follows. After discarding the growth medium, cells were washed with phosphate buffered saline and exposed to virus (25 to 50 EID₅₀/cell) for 30 min at 20°C. After removal of the virus, each dish received 30 ml medium 199 containing 1% calf serum and was incubated at 32°C for 72 h. Medium containing virus was clarified by low-speed sedimentation and purified further.

Preparation of [³²P]-labelled Sendai virus. Confluent CEL cells received 10 ml phosphate-free 199 medium containing 50 μCi of carrier free [³²P]-orthophosphate/ml 12 h prior to infection. After 30 min virus adsorption, the medium containing [³²P] was added back to the cells. Virus was harvested and purified after incubation at 32°C for 72 h.

Egg grown, [³²P]-labelled Sendai virus was obtained from embryonated eggs that had received 200 μCi of [³²P]-orthophosphate 1 h prior to infection.

Virus purification. Clarified allantoic fluid or tissue culture medium was centrifuged for 45 min at 70000 g in an IEC-International B60 centrifuge, using the A 54 rotor. Pellets were resuspended in NTE buffer (0.01 M-tris-hydrochloride, 0.001 M-EDTA, 0.1 M-NaCl, pH 7.4) and layered on to linear gradients of 15 to 60% (w/v) sucrose/NTE and centrifuged in a Spinco SW27 rotor (17 ml buckets) for 1 h at 20000 rev/min. The broad visible band of virus was collected with a bent needle and syringe, diluted 20 times with NTE, pelleted and resuspended in a small volume of NTE and layered on to linear gradients of 15 to 65% (w/v) sucrose/D₂O containing NTE. After centrifuging for 16 h at 20000 rev/min in a Spinco SW27 rotor, the narrow visible band of virus was collected and pelleted. The virus was resuspended in PBS and showed no loss of protein kinase activity after storing at 4°C for 2 months.

Protein kinase assay. The reaction mixtures (0.2 ml total vol.) contained 5 μmol of tris-hydrochloride (pH 8.5), 2 μmol dithiothreitol (DTT), 4 μmol of MgCl₂, 20 μl of 0.2% Nonidet P40 (NP40) (unless where stated), 10 nmol of γ-[³²P]-ATP and up to a total of 50 μl of enzyme and acceptor proteins. Unless otherwise stated, assays were performed on egg-grown Sendai virus. The reaction mixtures were incubated at 32°C for 30 min after which 1 ml of 0.125 M-Na₂P₂O₅ containing 0.2% bovine serum albumin (BSA), was added to terminate the reaction. Acid insoluble radioactivity was determined as described by Mahy & Bromley (1970).

Reaction mixture products for polyacrylamide gel electrophoresis analysis were precipitated with 10% trichloracetic acid (TCA) directly after incubation. After 30 min at 0°C, the precipitate was collected by centrifuging at 20000 g for 10 min. The precipitate was washed with n-butanol at −20°C, centrifuged at 3000 g for 20 min and then washed with absolute ethanol at 0°C and centrifuged at 3000 g for 20 min. The samples were then dried in vacuo.

Polyacrylamide gel electrophoresis. High-resolution polyacrylamide gel electrophoresis, using tris-glycine buffer and sodium dodecyl sulphate (SDS) (Laemmli, 1970) was used for analysis of virus polypeptides.
Protein kinase of Sendai virus

Slab gels were prepared between two glass plates, separated by 2 mm spacers and electrophoresed in an apparatus supplied by Raven Scientific Ltd., Haverhill, Suffolk, England. Ten % acrylamide gels were used throughout and the 3:2 % stacking gel was modified to contain 5 % (v/v) glycerol. Templates were used to provide nine slots per slab gel. Samples were prepared in 0:0625 M-tris-hydrochloride, pH 7-2, and 20 % (v/v) glycerol. Prior to analysis, SDS, DTT and bromophenol blue were added to the samples to final concentrations of 2 %, 2 % and 0:005 % respectively. The slab gels were electrophoresed for 16 h at a constant voltage of 40 V. After electrophoresis the gels were soaked in 50 % TCA for 5 h and then immersed in a solution containing 50 % TCA and 0:1 % Coomassie brilliant blue for 1 h at 37 °C. Gels were destained in 7 % glacial acetic acid at 65 °C. The gels were dried under vacuum on to Whatman 3M chromatography paper and autoradiographed with Kodak AP 54 X-ray film. Exposure times varied from 1 h to 24 h. Films were processed in a Kodak 8 min X-omat.

Acid hydrolysis of phosphorylated product of Sendai virus. A tenfold reaction mixture was incubated for 30 min and prepared as for electrophoresis on polyacrylamide gels. The sample was then suspended in 0:5 ml of 6 N-HCl in a thick-walled glass ampoule which was then evacuated and sealed. The contents were then heated at 110 °C for 1 h. The HCl was removed in vacuo, the residue suspended in H2O and again dried in vacuo. The sample was suspended in 25 μl of electrophoresis buffer consisting of 2:5 % (w/v) formic acid and 7:8 % (w/v) acetic acid, pH 1:9; 200 nmol of unlabelled phosphoserine and phosphothreonine was also dissolved in separate 25 μl samples of electrophoresis buffer. All samples were analysed by electrophoresis for 3 h at 320 V on a thin-layer plate of cellulose. The unlabelled markers were detected by spraying with ninhydrin and their positions marked. The plate was then autoradiographed for 1 h using Kodak AP54 X-ray film and processed in a Kodak 8 min X-omat.

Phosphoprotein phosphatase activity. Phosphorylated Sendai virus was obtained using 380 μg of protein in a 30-fold protein kinase assay. The reaction was terminated by adding unlabelled ATP to a final concentration of 20 mM and chilling the sample on ice. The virus was purified to remove soluble radioactivity as described by Silberstein & August (1973).

Phosphoprotein phosphatase activity was measured by two methods in reaction mixtures containing a total vol. of 1-4 ml: (A) as for protein kinase assay; (B) 10 μmol tris-hydrochloride (pH 7-6), 200 nmol of MnCl2, 40 μmol DTT, 200 μmol of NaCl and 0-02 % (v/v) NP40. Both reaction mixtures contained 96 μg of Sendai virus protein and 85 000 cpm/min of [32P]-labelled Sendai virus. Incubation was at 32 °C. At various times 0-2 ml samples were removed and acid-insoluble radioactivity determined as described above except that half volumes of all solutions were used and all supernatant fluids from each assay were collected and pooled. The supernatant fluids were counted for acid soluble radioactivity by Cerenkov radiation.

Protein measurement. Protein content of virus preparations and virus fractions were measured by the method of Lowry et al. (1951) using BSA as a standard.

Reagents. Salmon sperm protamine sulphate, histone fraction 3, BSA (lyophilised) DTT, tris base, cAMP, phosphoserine and phosphothreonine were obtained from Sigma Chemical Co. SDS (specially pure) and glycine (chromatographically pure) were obtained from British Drug Houses Ltd. Triton X-100 scintillation grade was obtained from Koch-Light Ltd. RNases A and T1 were obtained from Worthington Biochemical Ltd and Pronase (grade B) from Calbiochemicals. MEM, 199 medium, phosphate free 199 medium and calf serum were obtained from Bio-Cult, Paisley, Scotland. γ-[32P]-ATP and [32P]-sodium phosphate were obtained from the Radiochemical Centre, Amersham, Bucks.
Fig. 1. Distribution of haemagglutinin activity (HA) and protein kinase activities in sucrose gradients of Sendai virus. Virus was grown in eggs and purified as described in Methods. 0.5 ml of purified virus was layered on a 17 ml linear gradient of sucrose (15 to 60 % (w/v) in NTE) and centrifuged for 1 h at 20000 rev/min in a Spinco SW27 rotor. Twenty four fractions were collected from the bottom of the tube. Protein kinase was measured as described in Methods, using 10 µl duplicates of each fraction without any exogenous acceptor protein. Fifty µl duplicate samples of each fraction were titrated for HA. ○--○, H.A.U./ml; ●--●, ct/min.

RESULTS

Properties of the protein kinase of Sendai virus

Protein kinase activity could be detected in detergent disrupted preparations of purified Sendai virus. A preparation of egg-grown virus was purified by rate zonal and isopycnic sedimentation, followed by a second cycle of rate zonal sedimentation. The distribution of protein kinase activity coincides with that of the major haemagglutinating component in the final gradient (Fig. 1). The small amount of HA at the top of the gradient (probably derived from disrupted particles) did not contain enzyme activity. In these experiments, it has been found that low concentrations (0.02 %) of NP40 are essential for phosphorylation of endogenous proteins. Higher concentrations of NP40 (0.1 %) slightly increased the incorporation of TCA insoluble counts. In the absence of NP40, virtually no activity could be detected (Table 1). These results differ from those of Roux & Kolakofsky (1974), who reported that detergent is not required for the detection of protein kinase activity. The kinetics of the protein kinase reaction are shown in Fig. 2. At the lowest virus protein concentration, the kinetics were linear for at least 1 h. At higher protein concentrations (× 4 and × 12.5), the reactions were linear for shorter times, although activity could be re-stimulated
Protein kinase of Sendai virus

Table 1. Requirements of Sendai virus associated protein kinase

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Average ct/min</th>
</tr>
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<tbody>
<tr>
<td>1. Complete (0.02 % NP40)</td>
<td>31 719</td>
</tr>
<tr>
<td>2. -DTT</td>
<td>23 794</td>
</tr>
<tr>
<td>3. +NP40 (0.1 %)</td>
<td>36 142</td>
</tr>
<tr>
<td>4. -NP40</td>
<td>5 028</td>
</tr>
<tr>
<td>5. -NP40 - DTT</td>
<td>4 342</td>
</tr>
<tr>
<td>6. +Ca²⁺ (5 mM)</td>
<td>15 032</td>
</tr>
<tr>
<td>7. -Mg²⁺ + Mn²⁺ (10 mM)</td>
<td>1 017</td>
</tr>
<tr>
<td>8. +Pronase (200 µg/ml)</td>
<td>346</td>
</tr>
<tr>
<td>9. +RNase (200 µg/ml)</td>
<td>33 452</td>
</tr>
<tr>
<td>10. +5 nmol cAMP</td>
<td>30 608</td>
</tr>
<tr>
<td>11. +0.5 nmol cAMP</td>
<td>28 419</td>
</tr>
<tr>
<td>12. +Ca²⁺ + 5 nmol cAMP</td>
<td>14 142</td>
</tr>
<tr>
<td>13. +100 µg histone</td>
<td>30 542</td>
</tr>
<tr>
<td>14. +100 µg protamine sulphate</td>
<td>54 117</td>
</tr>
</tbody>
</table>

A typical reaction mixture incorporated 410 pmoles/mg protein/h. Duplicate reaction mixtures (0.2 ml) with omissions or additions indicated, were incubated for 30 min at 32 °C. Acid-insoluble radioactivity was determined as described in Methods.

Fig. 2. Kinetics and effect of Sendai virus concentration on endogenous protein kinase reaction. At indicated times, 0.2 ml duplicate samples were removed from 14-fold reaction mixtures containing 8 (○—○), 32 (▲—▲) or 100 (□—□) µg of Sendai virus protein. Samples were analysed for TCA-precipitable radioactivity as described in Methods.

Fig. 2. Kinetics and effect of Sendai virus concentration on endogenous protein kinase reaction.

by addition of further γ-[³²P]-ATP and was proportional to the amount of Sendai virus present in the reaction mixture.

The phosphorylation reaction of Sendai virus associated protein kinase had similar requirements to that of frog virus 3 (Silberstein & August, 1973). The effect of temperature on the protein kinase activity of Sendai virus is shown in Fig. 3(a), while the effect of pH is shown in Fig. 3(b). The temperature optimum of the reaction was 32 °C, and declined rapidly above 37 °C; there was little activity at 0 °C. The kinase shows a broad range of pH specificity with considerable activity both at pH 6.5 and pH 9.5. The pH optimum of the reaction was 8.5.
Further requirements of the Sendai protein kinase are summarized in Table 1. Enzyme activity was totally dependent on magnesium ions with an optimum at 20 mM; higher concentrations were slightly inhibitory. Manganese could not replace magnesium. The addition of calcium ions alone or together with 5 nmol cAMP inhibited the virus particle-associated enzyme. The reaction was unaffected by ribonucleases A and T1 at 200 µg/ml and 50 units/ml respectively, a concentration of nucleases that digested 30 µg of ribosomal RNA in less than 3 min. Pronase completely destroyed the protein kinase activity, as did boiling the virus preparation for 5 min before the assay. While histone fraction 3 did not stimulate the reaction, protamine sulphate considerably enhanced the activity, presumably acting as an exogenous substrate. In this last respect, the Sendai protein kinase differs from that of frog virus 3.

Product of the protein kinase reaction

The endogenous phosphate acceptor molecules of Sendai virus were analysed by tris-glycine buffered SDS-polyacrylamide gel electrophoresis. A 10-fold reaction mixture incubated for 30 min, was precipitated with TCA and run on 10% slab gels. After electrophoresis the gel was stained with Coomassie brilliant blue, dried and autoradiographed.

As described previously (Lamb & Mahy, 1974), Sendai virus grown in eggs has a glycoprotein pattern different from that grown in tissue culture. Egg grown virus contains a polypeptide 6 (mol. wt. 47000) and no polypeptide 3 (mol. wt. 74000), whereas tissue culture grown virus contains polypeptide 3 and small amounts of polypeptide 6. It can be shown that polypeptide 3 can be converted to polypeptide 6 by mild trypsin treatment (Homma &
Protein kinase of Sendai virus

Ohuchi, 1973; Lamb & Mahy, 1974; Scheid & Choppin, 1974). This difference in poly-
peptide pattern was partially reflected in the phosphorylated polypeptides. For comparative
purposes the polypeptide composition of Sendai virus grown in tissue culture and labelled
with [35S]-methionine is shown in Fig. 4.

With egg grown virus and 0.02 % NP40, the high mol. wt. 1, 4, 6 and 8 polypeptides were
phosphorylated. At 0.2 % NP40, polypeptide 6 was not phosphorylated. With CEL grown
virus at 0.02 % NP40, the high mol. wt. 1, 4 and 8 polypeptides were phosphorylated and
at 0.2 % NP40, polypeptide 2 was also slightly phosphorylated (Fig. 5). Higher concentrations
of detergent did not necessarily render more polypeptides phosphorylated as, although
it may reveal more active sites to the enzyme, others may also be destroyed.

Identification of covalent linkage

With vaccinia virus particle phosphoprotein (Rosemond & Moss 1973) and frog virus 3
(Silberstein & August, 1973) chemical characterization of the linkage between phosphate
and protein indicated that the phosphate was incorporated as a phosphomonoester of serine.
Analysis of the Sendai product by thin-layer electrophoresis after partial acid hydrolysis
suggests that phosphothreonine was the predominantly phosphorylated amino acid (Fig. 6).
Some [32P] was found after the acid hydrolysis which could be accounted for by the break-
down of phosphoserine or phosphothreonine in the presence of 6 N-HCl. No phosphoserine
could be detected on the thin-layer chromatogram; radioactivity was associated with
additional compounds which are phosphopeptides yielded from the partial acid hydrolysis
of the virus particle proteins (Silberstein & August, 1973). The slight curvature of the indivi-
dual spots from the origin is due to inefficient cooling of the thin-layer plate.

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Fig. 4. Polyacrylamide gel electrophoresis of [35S]-methionine labelled Sendai virus
polypeptides as estimated from a densitometer tracing of an autoradiogram.
Fig. 5. Polyacrylamide gel electrophoresis of the in vitro protein kinase products of Sendai virus. (a) Products of egg grown virus; NP40 concentrations as indicated. (b) Products of CEL grown virus; NP40 concentrations as indicated.

In vivo phosphorylation

When virus was grown in eggs previously inoculated with [32P], purified, and run on SDS polyacrylamide gels, it was found that the high mol. wt. 1, 2, 4 and 8 polypeptides were phosphorylated (Fig. 7a). Sendai virus grown in CEL cells with [32P] produced an identical result (Fig. 7b). These observations are in contrast to the products of an in vitro protein kinase reaction where polypeptide 2 could only be phosphorylated with CEL grown virus at high detergent concentrations.

It was of interest to determine whether phosphorylation occurred at a specific time in the infected cell, e.g. at assembly, or whether it occurred once the specific polypeptide had been synthesized. Infected cultures were labelled for 2 or 4 h periods with [32P] at various times from 10 h post-infection (p.i.) onwards. Analysis of the polypeptides of infected cells by polyacrylamide gel electrophoresis indicated that phosphorylation could occur once synthesis of the virus particle polypeptides began and that the high mol. wt. 1, 2, 4 and 8 polypeptides were phosphorylated. None of the non-structural polypeptides (Lamb & Mahy, 1974) seemed to be phosphorylated (Fig. 8).
It was surprising to find that virus that was phosphorylated in vivo could be further phosphorylated in vitro. It is possible that turnover of the phosphate occurs through the co-ordinated action of a protein kinase and a phosphoprotein phosphatase. This possibility was tested by incubating a mixture of Sendai virus, pre-labelled in vitro with $^{32}$P and unlabelled Sendai virus in the presence of detergent. Using either the reacting conditions for protein kinase or for phosphoprotein phosphatase (Silberstein & August, 1973) only a small
Fig. 7. *In vivo* phosphorylation of egg and CEL grown Sendai virus. Virus was grown either in eggs or CEL cells with $^{32}$P as described in Methods. The virus was purified, run on a polyacrylamide gel and autoradiographed as described in Methods. Autoradiographs were scanned at 540 nm in a Phillips–Pye–Unicam SP 1805 gel scanner. (a) Virus grown on eggs previously inoculated with $^{32}$P. (b) Virus grown in CEL cells inoculated with $^{32}$P.
Protein kinase of Sendai virus


Uninfected

11 h 11 h 11 h 13 h 13 h 15 h 15 h 17 h

Fig. 8. Polyacrylamide gel electrophoresis of polypeptides in Sendai virus-infected cells. At the indicated times after infection [3P] was added to CEL cells for 2 or 4 h. The cells were then washed in ice-cold saline, scraped off the dish, washed again in ice-cold saline and made 5% in SDS and 4% DTT for electrophoresis. DNA was sheared in the samples by syringing through a 22-gauge needle. Samples were prepared and the gel processed as described in Methods.

liberation of acid soluble phosphate was observed (Fig. 9). Under the protein kinase assay conditions used here there was little turnover of phosphoesters and there must be vacant sites for attachment of phosphate in the virus particle.

Fractionation of Sendai virus

The glycoproteins of SV5 (Scheid et al. 1972), NDV (Scheid & Choppin, 1973) and Sendai virus (Lamb & Mahy, 1974; Scheid & Choppin, 1974) can be removed from the virus particle with 2% Triton X-100. The smallest mol. wt. virus particle polypeptide was removed from
Table 2. Fractionation of Sendai virus particles for protein kinase activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ct/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 % Triton + whole virus</td>
<td>14,374</td>
</tr>
<tr>
<td>1 M-KCl + whole virus</td>
<td>5,963</td>
</tr>
<tr>
<td>2 % Triton + 1 M-KCl + whole virus</td>
<td>4,285</td>
</tr>
<tr>
<td>1 % DOC + whole virus</td>
<td>802</td>
</tr>
<tr>
<td>Whole virus</td>
<td>28,419</td>
</tr>
<tr>
<td>2 % Triton pellet</td>
<td>9,025</td>
</tr>
<tr>
<td>2 % Triton supernatant fluid</td>
<td>2,212</td>
</tr>
<tr>
<td>2 % Triton + 1 M-KCl pellet</td>
<td>1,022</td>
</tr>
<tr>
<td>2 % Triton + 1 M-KCl supernatant fluid</td>
<td>10,171</td>
</tr>
<tr>
<td>2 % Triton + 1 M-KCl precipitate</td>
<td>1,573</td>
</tr>
</tbody>
</table>

690 μg of purified Sendai virus was fractionated as described by Scheid & Choppin (1972); either 2 ml 2 % Triton X-100 or 2 ml 2 % Triton X-100 + 1 M-KCl were used as solubilizers. Pellets were resuspended in the original volume of 0.01 M-sodium phosphate buffer pH 7.2. All samples were dialysed against 0.01 M-sodium phosphate buffer pH 7.2, and contained 2 % Triton X-100. Fifty μl samples were taken for protein kinase assay and incubated for 30 min at 32 °C. Acid-insoluble radioactivity was determined as described in Methods.
Protein kinase of Sendai virus

these viruses with the glycoproteins using 2 % Triton and 1 m-KCl. This polypeptide can be isolated from the supernatant fluid as a precipitate after dialysis and it has been suggested that it is the matrix protein (Scheid & Choppin, 1973).

This fractionation procedure was applied to egg grown Sendai virus and the fractions obtained were assayed for protein kinase activity. It was found that 2 % Triton and 1 m-KCl considerably reduced the activity of the protein kinase, so all fractions were dialysed to remove KCl and 2 % Triton was added back to the pellets obtained. It was found that 1 % sodium deoxycholate inhibited the reaction to the extent that the nucleocapsids obtained (Mountcastle, Compans & Choppin, 1971) could not be tested. The results shown in Table 2 suggest that much of the protein kinase activity remains in the core after removal of the glycoproteins from the virus particle with 2 % Triton, but that some activity is liberated into the supernatant fluid. Removal of glycoproteins and matrix protein with 2 % Triton and 1 m-KCl liberated 85 % of the total kinase activity. When the matrix protein was recovered as a precipitate after dialysis, it showed little activity as did the ‘core’ particles. It appears that the protein kinase activity was inside the virus particle but was not associated with the nucleocapsid complex. When the 2 % Triton + 1 m-KCl supernatant fluid from this procedure was run on polyacrylamide gels only the two glycoproteins and the matrix protein could be detected (Lamb & Mahy, 1974). This suggests that the protein kinase activity must be present in very small amounts. Similar findings were observed with vesicular stomatitis virus (VSV) (Imblum & Wagner, 1974).

**DISCUSSION**

Sendai virus grown in a variety of cell types contained protein kinase activity that could transfer the γ-P of ATP to virus particle polypeptides and protamine sulphate. This enzyme differs from the well-documented Ca²⁺-dependent protein kinases or cAMP dependent protein kinases found in a wide variety of cells. The reaction required NP₄₀ to disrupt virus; the low level of activity found in the absence of NP₄₀ was probably due to disrupted virus particles. Phosphoprotein phosphatase activity could not be detected under conditions where the protein kinase was active. The protein kinase must therefore be phosphorylating de novo. The extent of phosphorylation of the in vitro product depended both on the concentration of NP₄₀ used to disrupt the virus, and on the type of cell in which the virus was grown. However, in all cases the high mol. wt. 1, 4 and 8 polypeptides were phosphorylated. When the virus was labelled with [³²P] in eggs or CEL cells, phosphorylation of identical polypeptides occurred; namely the high mol. wt. 1, 4 and 8 polypeptides. Labelling infected cells with [³²P], at times after the virus particle polypeptides could be shown to be present (Lamb & Mahy, 1974), suggested that phosphorylation could occur at any time. Examination of the nature of the linkage of phosphorus to the polypeptides using partial acid hydrolysis and thin-layer chromatography indicated that phosphothreonine was the principal product of the reaction. In this respect, the protein kinase activity of Sendai virus differs from the phosphorylating enzymes found in other enveloped viruses.

The polypeptide composition of the protein kinase in Sendai virus still remains to be elucidated. The 2 % Triton + 1 m-KCl fractionation procedure suggests that the enzyme is associated within the matrix protein, but not with the nucleocapsid complex. However, a structural polypeptide cannot be assigned to the kinase activity and it must be present in very small amounts. It is possible that the protein kinase is of cellular origin and that it becomes packaged into the virus. Data to support this hypothesis have been presented for the protein kinase activity of VSV (Imblum & Wagner 1974).
The biological role of the protein kinase remains unknown. Sendai virus grown in eggs was fully infectious for eggs, and some of its polypeptides were phosphorylated. In an in vitro reaction predominantly the same polypeptides were further phosphorylated indicating that some sites remained unphosphorylated in vivo. This may suggest that if phosphorylation is essential to the replication of Sendai virus, its role could be to regulate the uncoating of parental virus particles in the host cell, or the transcription of the virus genome by the virus particle polymerase rather than in the facilitation of polypeptide recognition in the process of virus assembly. It was observed that polypeptides 1 and 4 were more heavily phosphorylated than any others and that these polypeptides have been associated with the virus particle transcriptase activity (Marx, Portner & Kingsbury, 1974). Several models for the control of transcription of virus particle RNA based on the phosphorylation and dephosphorylation of virus core proteins have been proposed (Sokol & Clark, 1973). It would be of great interest to be able to dephosphorylate virus particles prior to using them for the infection of cells and then to monitor the phosphorylation and primary transcription processes.

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