Protein Kinase Associated with Sendai Virions

LAURENT ROUX AND DANIEL KOLAKOFSKY

Departement de Biologie Moléculaire, Université de Genève, Genève, Switzerland

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A protein kinase activity has been found associated with purified Sendai virions. Most of the virion proteins and exogenous protamine served as substrates for this enzyme.

A protein kinase activity has been found associated with several enveloped RNA- and DNA-containing animal viruses (1, 2, 5, 6, 8-11). This communication reports that Sendai virus (parainfluenza type I) also contains protein kinase activity.

When purified Sendai virus was centrifuged to equilibrium in sucrose gradients, the virus, as detected by optical absorbance at 275 nm or hemagglutinating activity, was found as a broad band with a peak at a density of 1.181 gm/cm³ (Fig. 1). When each fraction of the gradient was assayed for its ability to incorporate ³²P-γ-ATP into trichloroacetic acid-insoluble material, kinase activity was found to roughly follow the absorbance and the hemagglutinating activity profiles, with the peak activity found at a density of 1.183 gm/cm³ (Fig. 1).

Figure 2 shows that the kinase activity of the viral preparation is proportional to the amount of viral protein added. Boiling the viral preparation in TNE buffer (3) for 10 min completely destroyed the kinase activity. The products of a similar reaction mixture were characterized as phosphoproteins by their susceptibility to nucleolytic and proteolytic degradation: more than 80% of the radioactivity was rendered trichloroacetic acid-soluble after treatment with Pronase (440 μg/ml, 30 min at 37 C) in 0.1% sodium dodecyl sulfate (SDS), whereas all the product remained trichloroacetic acid-insoluble after treatment with RNase A (73 μg/ml, 30 min at 37 C) in 0.1% SDS. Under the latter conditions, cellular rRNA is completely degraded.

The virion-associated kinase was also found to phosphorylate added protamine. Although Nonidet P-40 (NP40) was not required for the phosphorylation of endogenous viral proteins, the presence of NP40 (0.4%) was found to stimulate the phosphorylation of exogenous protamine fourfold (data not shown). This suggests that the virion-associated kinase is

unavailable to exogenous protein but is freed by the solubilizing effect of the nonionic detergent.

To determine which viral proteins were phosphorylated, labeled virus was recovered from a 60-min reaction by centrifugation, solubilized in buffer containing SDS and 2-mercaptoethanol, and subjected to electrophoresis on a 10%

FIG. 1. *Equilibrium sedimentation of Sendai virus in sucrose gradients.* Sendai virus (Harris strain) was grown in embryonated chicken eggs and purified as previously described (3). Approximately 2 mg of purified virus was layered onto a 25 to 65% linear sucrose gradient in TNE buffer (3) and centrifuged for 5 h at 39,000 rpm (4 C) in an SW41 Spinco rotor. Fractions were collected by puncturing the bottom of the tube. The density of selected fractions was determined by weighing 100-μliter samples in a Pedersen micropipette, and the absorbance at 275 nm of each fraction was determined after diluting 10-fold with water. Kinase activity was assayed on 30-μliter samples as described in the legend of Fig. 2, except that the final volume was 110 μliters. The hemagglutinating activity of 10-μliter samples was determined after incubation for 30 min at 25 C with an equal volume of receptor destroying enzyme (Microbiological Associates, Bethesda, Md.) diluted 50-fold in phosphate-buffered saline, using chicken erythrocytes.

FIG. 2. Protein kinase activity as a function of viral protein concentration. Reaction mixtures in a final volume of 50 μ liters contained: 50 mM Tris-hydrochloride (pH 8.0), 10 mM $MgCl_2$, 3 mM dithiothreitol, 0.27 mM ^{32}P - γ -ATP (57 counts per min per pmol), and varying amounts of purified virus as indicated. The reaction mixture was incubated at 37 C for 60 min, and the reaction was stopped by adding 1 ml of cold 10% trichloroacetic acid containing 50 mM sodium pyrophosphate. After 10 min in ice, the acid-insoluble material was collected on Whatman GF/C filters and washed three times with 3 ml of 5% trichloroacetic acid containing 50 mM sodium pyrophosphate. The filters were then dried and counted by liquid scintillation. Reaction mixtures without added virus yielded blank values of 15 pmol.

polyacrylamide gel. The gel was first stained with Coomassie brilliant blue, scanned in a Gilford spectrophotometer, and then sectioned and counted by liquid scintillation to localize radioactivity. The results (Fig. 3) show that most, but not all, of the virion proteins serve as substrates for protein kinase and are phosphorylated to different extents. Virus grown in embryonated eggs in the presence of $^{32}PO_4$ and subjected to similar analysis in polyacrylamide gels showed similar results; i.e., the same viral proteins were phosphorylated to the same relative extent in vivo as well (data not shown). Hence, the in vitro phosphorylation observed might be due to incomplete phosphorylation in vivo or to regeneration of sites through the action of a phosphatase which may also be associated with mature virions.

From the data presented in Fig. 2, we calculate that Sendai virion-associated protein kinase incorporates approximately 13 nmol of phosphate per mg of viral protein per h at 37 C. This value is considerably lower than that recently reported by Silberstein and August (9) for the protein kinase of frog virus 3. It is,

FIG. 3. Polyacrylamide gel electrophoresis of in vitro-phosphorylated Sendai proteins. A fourfold kinase reaction was carried out as described in the legend of Fig. 2, except that 0.3 mM ^{32}P - γ -ATP (700 counts per min per pmol) was used. After 60 min of incubation, 1 ml of 0.1 M sodium pyrophosphate was added, and the virus was pelleted by centrifugation (30 min at $15,000 \times g$), resuspended in 1 ml of 0.1 M sodium pyrophosphate, and recentrifuged. The virus pellet was then dissolved in 150 μ liters of 10% glycerol, 5% 2-mercaptoethanol, 3% SDS, and 60 mM Tris-hydrochloride (pH 6.8). A sample containing 65 μ g of protein was boiled for 10 min and then subjected to electrophoresis in a 10% polyacrylamide gel for 12 h at 1 mA per gel as previously described (4). The gels were then first fixed in 50% trichloroacetic acid for 7 h, stained with 0.1% Coomassie brilliant blue, destained in 10% methanol and 7.5% acetic acid, and scanned in a Gilford spectrophotometer at 560 nm. The gels were then sectioned into 1-mm slices, and 0.25 ml of NCS (Amersham/Searle):water (5:1) was added to each slice. After overnight incubation at 37 C, radioactivity was determined by liquid scintillation after the addition of 3 ml of Aquasol (New England Nuclear Corp.).

however, considerably greater than the activity so far reported for other RNA-containing enveloped viruses such as RNA tumor viruses (2, 10, 11).

The data presented here offer no clue as to whether protein kinase is viral coded or host in origin. However, the presence of this enzyme in so many budding viruses suggests that the latter case is more likely to be true.

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LITERATURE CITED

1. Gravell, M., and T. L. Cromeans. 1972. Virion associated protein kinase and its involvement in non-genetic reactivation of frog polyhedral cytoplasmic deoxyribovirus. *Virology* 48:847-851.

2. Hatanaka, M., E. Twiddy, and R. V. Gilden. 1972. Protein kinase associated with RNA tumor viruses and other budding RNA viruses. *Virology* **47**:536-538.
3. Kolakofsky, D. 1972. Transfer ribonucleic acid nucleotidyltransferase and transfer ribonucleic acid in Sendai virions. *J. Virol.* **10**:555-559.
4. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature (London)* **227**:680-685.
5. Paoletti, E., and B. Moos. 1972. Protein kinase and specific phosphate acceptor proteins associated with vaccinia virus cores. *J. Virol.* **10**:417-424.
6. Randall, C. C., H. W. Rogers, D. N. Downer, and G. A. Gentry. 1972. Protein kinase activity in equine herpes-virus. *J. Virol.* **9**:216-222.
7. Rosen, L. 1969. Hemagglutination with animal viruses, p. 276-288. In K. Habel and N. P. Salzman (ed.), *Fundamental techniques in virology*. Academic Press Inc., New York.
8. Rubenstein, A. S., M. Gravell, and R. Darlington. 1972. Protein kinase in enveloped herpes simplex virions. *Virology* **50**:287-290.
9. Silberstein, H., and J. T. August. 1973. Phosphorylation of animal virus proteins by a virion protein kinase. *J. Virol.* **12**:511-522.
10. Strand, M., and J. T. August. 1971. Protein kinase and phosphate acceptor proteins in Rauscher murine leukemia virus. *Nature N. Biol.* **233**:137-171.
11. Tan, K. B., and F. Sokol. 1972. Structural proteins of simian virus 40: phosphoproteins. *J. Virol.* **10**:985-994.