Protein Kinase Associated with Sendai Virions

LAURENT ROUX AND DANIEL KOLAKOFSKY

Département de Biologie Moléculaire, Université de Genève, Genève, Switzerland

Received for publication 27 September 1973

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%
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 $^{32}$P has been 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 nmoles 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, 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.

We thank Pierre-François Spahr for generous advice, encouragement, and support.

This work was supported by Research Grant no. 3.816.72 from the Fonds National Suisse de la Recherche Scientifique.

LITERATURE CITED