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Phosphorylated Proteins of Sindbis Virus

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The capsid and two membrane proteins of Sindbis virus, grown in chicken cells, contain 0.03 to 0.1 mol of phosphate per mol of protein.

Several viruses have phosphorylated structural proteins (12, 14, 18, 20), but the role of the phosphate is obscure. Its presence cannot be correlated with that of virion-associated protein kinases, because the latter have as yet been found only in enveloped viruses (2, 4, 5, 10, 11, 13, 18-20). Simian virus 40 and adenoviruses are neither enveloped, nor do they exhibit kinase activity (19, 20), yet both have phosphorylated proteins (14, 20). Furthermore, phosphorylation of the proteins in the enveloped rhabdovirus group differs among the several members (18). To learn more about viral phosphoproteins, we examined the structural proteins of Sindbis virus, an enveloped group A arbovirus, and measured the amount of protein-associated phosphate.

Sindbis virus labeled with $^{32}PO_4$ and ^{3}H-leucine was purified, disrupted, and analyzed by acrylamide gel electrophoresis (15). We expected that the only phosphorylated molecules in the gel would be polypeptides, because the virion phospholipids migrate faster than the dye front (M. R. F. Waite, unpublished data), and the high molecular weight of the virion RNA (about 3.6×10^6) (3) should have prevented it from entering the gel. However, a small amount of RNA was always degraded (0.5 to 2%), and the resulting background of ^{32}P partially obscured the protein-associated peaks. To reduce the background level of radioactivity, the disrupted virus preparations were treated with RNase A prior to electrophoresis on discontinuous Tris gels (9). The viral protein was resolved into three polypeptides: two envelope glycoproteins (E1 and E2) and the capsid (C) protein (16). The phosphate was associated primarily with the faster migrating envelope protein (E2), although there was a shoulder in the region of E1, and the capsid protein was phosphorylated (Fig. 1). The RNase treatment did not appear to change the pattern of phosphorylation of the protein. The ^{32}P at the bottom of the gel was presumably in poly(A), arising from the RNase-resistant adenylate-rich region of the Sindbis genome (6, 7).

To determine the molar ratio of phosphate to viral protein, Sindbis was grown in monolayer cultures of primary chicken cells which had been labeled to constant specific activity by growth for 4 days in medium containing $^{32}PO_4$ and ^{3}H-leucine. The virus produced in these cells was analyzed by acrylamide gel electrophoresis (Fig. 1). Assuming that Sindbis proteins have the same leucine composition as those of the closely related Semliki Forest virus, (8, 17), and knowing the specific activity of the medium, we calculated the number of phosphate molecules per molecule of protein (Table 1). Because the membrane glycoprotein peaks were not fully resolved and the ^{32}P and ^{3}H-leucine peaks did not coincide completely, exact determinations of the $^{32}PO_4$/protein ratio were not possible. Accordingly, we estimated the highest and the lowest values for each protein. Fewer than 10% of the protein molecules in the mature virus were phosphorylated.

The phosphate label in peak E2 and in the capsid peak usually had slightly lower electrophoretic mobilities than did the leucine-labeled polypeptides. A similar result was noted by Sokol and Clark (18) with Kern Canyon virus. This may mean that the ^{3}H-leucine peaks are comprised of several species of protein, not all of which are phosphorylated, or that a small fraction of an otherwise homogeneous population of protein molecules is heavily phosphorylated and hence of larger molecular weight.

We cannot propose a role for the phosphorylation of Sindbis proteins. The small amount of phosphate associated with the structural proteins may be the result of two enzymes with opposite effects, a phosphatase and a kinase. If this is true, the kinase must be within the cell, because protein kinase activity is absent from the purified Sindbis virion (K. B. Tan, F. Sokol,

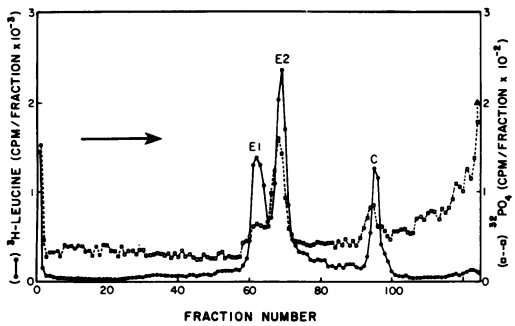

FIG. 1. *Discontinuous Tris-SDS gel electrophoresis of RNase-treated Sindbis virus. Chicken cells were plated in low-phosphate Eagle medium containing 10 Ci of ^{3}H-leucine (0.4 mM) per mol and 172 Ci of $^{32}PO_4$ (0.25 mM) per mol. This radioactive medium was renewed daily until the cells had grown to both constant specific activity (2 days) and confluence (4 days). They were then infected with Sindbis virus, in the constant presence of radioactive medium. The virus was purified directly from the medium by centrifugation on a discontinuous sucrose gradient (15). The fractions containing radioactive virus were pooled, and the virus was sedimented by high-speed centrifugation and dissolved in 0.1 M phosphate buffer (pH 6.9) containing 1% sodium dodecyl sulfate (SDS). After dialysis at room temperature against low-ionic-strength buffer (15 mM NaCl) and 1.5 mM sodium citrate), the preparation was treated with RNase A (Worthington RASE, 250 μg/ml, 4,490 U/mg) for 2 h at 37 C, and then was dialyzed against 0.1 M phosphate buffer (pH 6.9) containing 0.1% SDS. Mercaptoethanol was added to 5%, the preparation was placed in a boiling water bath for 5 min, and analyzed on a discontinuous Tris-SDS gel. The gel was sliced, and the 1-mm segments were incubated overnight at 37 C in NCS-toluene scintillation fluid, and then counted. The arrow indicates the direction of migration. E1 and E2 are the two envelope glycoproteins, and C indicates the location of the capsid protein (16).*

and M. R. F. Waite, manuscript in preparation). Perhaps the phosphate is necessary only early in virus maturation and is subsequently partially removed. Alternatively, a few phosphorylated polypeptides may be necessary for the proper assembly of the protein subunits of the virion, or the phosphorylated molecules may have some other special function.

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TABLE 1. *Molar ratio of phosphate to proteins of Sindbis virus grown in chicken cell cultures*

Protein type	Moles of phosphate per mole of protein[a]	
	Prep 1[b]	Prep 2[c]
E1	—[d]	0.03–0.06[e]
E2	0.08–0.12	0.09–0.11
C	—[d]	0.03–0.05

[a] Sindbis virus was grown, purified, and treated with RNase (Fig. 1) and was analyzed by acrylamide gel electrophoresis on a discontinuous Tris gel. The moles of protein in each peak were calculated from both the known specific activities of the medium and the counts in each peak (Fig. 1). Correction was made for quenching, as well as for the ^{32}P counts appearing in the ^3H channel.

[b] The medium contained 8 Ci of ^{3}H-leucine (0.4 mM) per mol and 30 Ci of $^{32}PO_4$ (1.1 mM) per mol and was changed as described (Fig. 1).

[c] The medium contained 10 Ci of ^{3}H-leucine (0.4 mM) per mol and 173 Ci of $^{32}PO_4$ (0.25 mM) per mol and was changed as described (Fig. 1).

[d] The $^{32}PO_4$ counts in these peaks were too low to allow a significant determination.

[e] Independent determinations from the same preparation agreed within 10%.

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