sodium salt, 0.00225*M* $MgCl_2$, 0.00551*M* Na_2HPO_4, and 0.0890 m*M* citric acid at a final *p*H of 7.4. Duration of electrophore is was 135 minutes at a voltage gradient of 18 volt/cm.

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Assembly of Protein and Nucleoprotein Particles from Extracted Tobacco Rattle Virus Protein and RNA

Abstract. *Protein extracted from tobacco rattle virus by treatment with formic or acetic acid reassociated in vitro to form rings and tubular structures of indeterminate lengths in 0.25 molar glycine at* p*H 7.5 to 9.5. When tobacco rattle virus RNA that had been extracted with phenol was incubated with the protein at 9°C, particles with more regular lengths formed; these particles correspond in size and sedimentation properties to the short particles that normally accompany infection by tobacco rattle virus. The biological activity of the reconstituted short particles was identical to that of native short particles.*

Reconstitution of plant viruses characterized by helical symmetry was first accomplished with tobacco mosaic virus (*1*) and with barley stripe mosaic virus (*2*). Tobacco rattle virus (TRV) has a similar cylindrical construction; however, repolymerization of extracted protein resulted in only extremely short particles (*3*).

An unusual property of infection by TRV is the recovery of nucleoprotein particles of two distinct lengths, both of which are necessary for production of complete progeny particles (*4*). The longer rod (190 nm) presumably regulates production of the viral nucleic acid while the shorter rod, 50 to 115 nm (depending on the isolate), codes for production of the protein coat. Infection with only long rods results in the production of infectious RNA, or the unstable form of TRV, whereas typical nucleoprotein particles, or the stable form of TRV, are recovered only after infection by both long and short particles. We report the extraction of protein from purified TRV and the reassociation of protein subunits into elongated tubular structures and biologically active nucleoprotein particles, 80 to 90 nm, with the addition of TRV-RNA.

The "C" isolate of TRV used in these studies was purified as reported (*5*). This isolate is composed of particles of three lengths, 50, 85, and 190 nm (Fig. 1, a and b) which sediment as distinct populations in sucrose density gradients (Fig. 2a). A 1 to 2 percent virus suspension was treated with 1 to 2 volumes of formic or acetic acid (*6*) in an ice bath for 30 minutes, cleared by low-speed centrifugation, and dialyzed (1 to 2 days) at 4°C against several changes of water. After centrifugation at 100,000*g* for 1 hour, the supernatant was brought to 0.5 saturation with $(NH_4)_2SO_4$. The resulting precipitate was recovered by centrifugation, resuspended, and dialyzed against water adjusted to *p*H 4 to 4.5 with 10 percent acetic acid.

The water-clear solution had a typi-

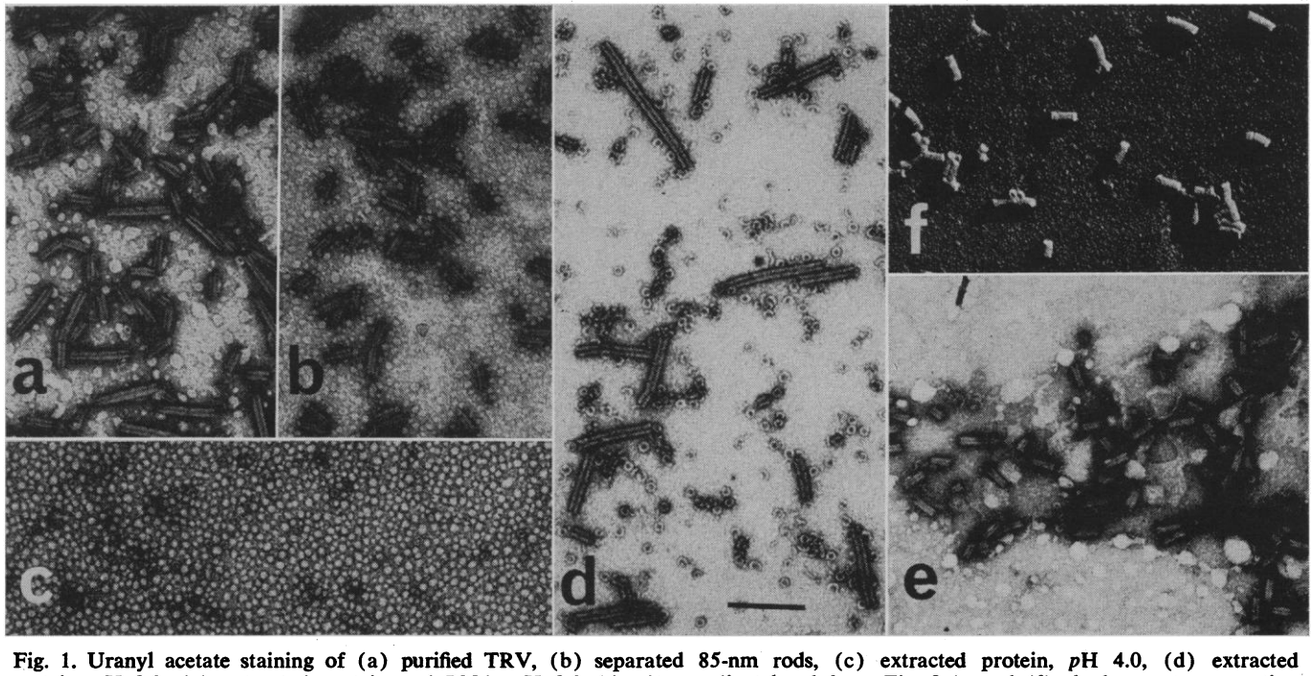

Fig. 1. Uranyl acetate staining of (a) purified TRV, (b) separated 85-nm rods, (c) extracted protein, *p*H 4.0, (d) extracted protein, *p*H 8.0, (e) extracted protein and RNA, *p*H 8.0 (density gradient band from Fig. 2c), and (f) shadow-cast preparation of (e). Scale is 0.25 μ.

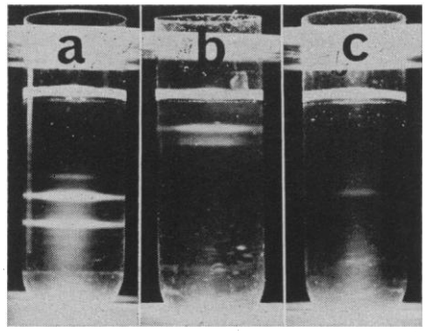

Fig. 2. Rate sedimentation in 10 to 40 percent sucrose density gradients for 1.75 hours at 24,000 rev/min in a Spinco SW-25.1 rotor. (a) Purified TRV in 0.1*M* phosphate, *p*H 7.0; (b) extracted protein in 0.2*M* glycine, *p*H 8.0; (c) extracted protein and RNA in 0.1*M* phosphate, *p*H 7.0.

cal protein ultraviolet spectrum with a maximum at 284 nm and a minimum at 254 nm, the ratio of the absorption at 260 and 280 nm being 0.50 to 0.60. Electron micrographs of preparations stained with uranyl acetate indicated clumps of protein aggregates and a few ring structures (Fig. 1c). With dialysis at 30°C in 0.05 to 0.5*M* glycine adjusted to *p*H 7.5 to 9.5, the solution became opalescent, and long tubular structures as well as large numbers of rings were observed in electron micrographs (Fig. 1d). The tubular and ring structures banded near the meniscus after sedimentation in 10 to 40 percent sucrose density gradients in 0.25*M* glycine at *p*H 8.0 (Fig. 2b). Purified TRV was partially degraded by similar conditions of sedimentation. When dialyzed against 0.1*M* phosphate, *p*H 7.0, and sedimented in gradients of the same medium, the reassembled tubular forms dissociated.

The phenol-extracted TRV-RNA was added to the protein preparation; the mixture was dialyzed against 0.25*M* glycine, *p*H 8.0, at 9°C and then dialyzed against 0.1*M* phosphate, *p*H 7.0. Particles with a sedimentation rate similar to native 85-nm rods (Fig. 2c) were obtained. Staining and shadow-casting confirmed the presence of typical 80- to 90-nm particles (Fig. 1, e and f). Reduced temperatures enhanced production of the nucleoprotein particles but not the protein tubes. Also, a ratio of protein to RNA in the foregoing mixture less than 10 : 1 (by weight) results in the most homogeneous product, as judged by banding in gradient columns.

Although the phenol extract of the purified virus contains the RNA moieties from the three particle lengths, no evidence of distinct banding could be observed among the 50- to 190-nm particles. No infection resulted when reconstitution mixtures were inoculated to the systemic host, *Nicotiana clevelandii* Grey. Both observations may reflect the differential structural instability of the RNA extracted from the 190-nm particles, as compared to that of the 85-nm particles (*7*), thereby permitting the 85-nm particles to be assembled more readily.

The biological activity of the reconstituted particles was demonstrated by inoculating plants previously inoculated with the unstable form of TRV with the density-gradient banded rods (Fig. 2c). All plants inoculated with the unstable form produced symptoms; however, typical viral particles could be recovered only from plants also inoculated with either reconstituted or native short particles. Extracted protein alone was not effective in changing the unstable to the stable form of infection. In that these reconstituted particles code for the distinct function of coat protein synthesis (*8*), perhaps the nucleic acid might display an affinity for the protein subunit.

On the basis of sedimentation properties, particle morphology, and biological activity, our results indicate that native protein subunits can be extracted from tobacco rattle virus and then polymerized into protein tubes and nucleoprotein particles resembling naturally occurring short rods.

Note added in proof: Reconstitution of long rods (190 nm) has been accomplished under the same condition presented here except with much larger quantities of extracted protein (about 500 μg) and RNA (about 100 μg). The reassembled particles were judged identical to native long rods on the basis of sedimentation rates, particle morphology, and biological activity.

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Aminoacyl Transfer Ribonucleic Acid Synthetases from Cell-Free Extract of Plasmodium berghei

Abstract. *Aminoacyl transfer ribonucleic acid synthetases for leucine, tyrosine, histidine, valine, proline, threonine, and lysine were obtained from cell-free extract of* Plasmodium berghei. *The leucyl-tRNA synthetase can charge tRNA from liver or* Escherichia coli *with leucine-C^{14}, liver tRNA being a better substrate. The amount of aminoacylation increases linearly with respect to the quantity of tRNA added from either source and is dependent on the amount of enzyme added. The rate of aminoacylation is constant for 10 minutes and then decreases. It is enhanced by polyvinylsulfate. One-tenth millimolar pyrimethamine, hydroxystilbamidine, quinacrine, and acriflavine inhibited the formation of C^{14}-valyl-tRNA. Species specificity between tRNA and its charging enzyme with respect to the recognition site is discussed.*

This report is concerned with the study of protein synthesis in extracts from invertebrates (*1–4*). Despite the fact that in all species studied the first reaction leading to protein synthesis is the charging of transfer ribonucleic acid (tRNA) with amino acids and the formation of aminoacyl-tRNA, there are species differences with heterologous interactions. For example, tyrosyl-tRNA from *Escherichia coli* cannot interact with tyrosyl-tRNA synthetase from yeast (*5*). Species differences in charging were observed by using aminoacyl-tRNA synthetase from liver or *E. coli* with heterologous tRNA (*6*). However, once tRNA is charged with an amino acid, it can serve as a precursor for polypeptide

Table 1. Aminoacyl tRNA synthetase activity for different amino acids in cell-free extract of *P. berghei*. Experimental conditions were as described in Fig. 1 except that only liver tRNA (1 mg/ml) was used. Supernatant enzymes were added at a concentration of 3 mg/ml. Each tube contained 0.25 μc of labeled amino acid. Incubation time was 12 minutes.

C^{14}-Amino acid	C^{14}-Aminoacyl-tRNA (count/min)	
	Endogenous	Liver tRNA
Tyrosine	876	1335
Histidine	432	1546
Valine	95	4072
Proline	238	404
Threonine	374	1484
Lysine	582	3578