The Effect of pH and Some Selected Chemicals on the Temperature-Reversible Aggregation of Carnation Ringspot Virus

J. H. Tremaine and W. P. Ronald

Research scientist and technician, respectively, Research Station, Agriculture Canada, 6660 N.W. Marine Drive, Vancouver, B.C., Canada, V6T 1X2.

The authors wish to thank E. M. McGauley for technical assistance and S. W. MacDiarmid for photographs.
Accepted for publication 6 October 1984.

ABSTRACT

Tremaine, J. H., and Ronald, W. P. 1985. The effect of pH and some selected chemicals on the temperature-reversible aggregation of carnation ringspot virus. Phytopathology 75:467-471.

Two strains of carnation ringspot virus, CRSV-R and CRSV-N aggregate and disaggregate in a temperature-reversible manner. The temperature of solutions of these viruses (0.8 mg/ml) was raised from 25 C to 60–80 C at a constant rate of 0.25 C/min while the absorbance at 340 nm was recorded. Then the temperature was lowered to 25 C and denaturation was monitored by observing precipitation and testing serological activity by gel diffusion. The aggregation temperature in 0.1 M tris acetate buffers in the pH 4.0 to 8.0 range was lowest at pH 5.0 for CRSV-R (50 C) and at pH 6.0 for CRSV-N (51 C). The effects of increasing concentrations of chemicals were studied with CRSV-R at pH 5.0 and 7.0. The greatest reductions in aggregation temperature without denaturation occurred with 100 mM EDTA (12 C at pH 5.0 and 24 C at pH 7.0) and with 100 mM ethyleneglycol-*bis*-(β-amino-ethyl ether)-*N, N', N'*-tetraacetic acid (EGTA) (8 C at pH 5.0 and 16 C at pH 7.0). Sucrose, at 1,000 mM, raised the aggregation temperature into the 70–80 C range, but virus denaturation occurred at these temperatures in the presence or absence of sucrose. Denaturation occurred at 50–60 C with KCNS, acetamide, thiourea, and guanidine at pH 7.0 and with guanidine and uranyl acetate at pH 5.0. Small decreases in aggregation temperature without denaturation occurred with increasing concentrations of NaCl, acetamide, urea, Triton X-100, Tween-20, and alginic acid. SDS at concentrations ranging from 0.1 to 4 mM at pH 5 had little effect on the aggregation temperature and virus dissociation occurred at higher SDS concentrations.

Tremaine et al (13) isolated three strains of carnation ringspot virus (CRSV) with unusual aggregation properties. One strain, CRSV-A, formed stable aggregates of 12 virus particles and linked aggregates. Two other strains, CRSV-R and CRSV-N, aggregated and disaggregated in a temperature-reversible manner. The temperature of solutions of these viruses at varying concentrations at pH 5.0 was raised from 25 C to 60–70 C at a constant rate of 0.25 C/min while the absorbance at 340 nm was recorded (12). The maximum absorbance increase and the maximum rate of absorbance increase were proportional to the logarithm of virus concentration. The temperature at one half the maximum absorbance increase was inversely proportional to the logarithm of virus concentration. At high concentrations (>0.4 mg/ml) the N strain required higher temperatures for aggregation than the R strain but at lower concentrations (<0.4 mg/ml) the R strain required higher temperatures than the N strain. The thermal aggregation of these strains is a useful model system for studying virion-virion interactions which may be important in virus purification and characterization.

The purpose of the experiments reported here was to measure the effect of pH and some selected chemicals on the temperature-dependent aggregation-disaggregation of carnation ringspot virus.

MATERIALS AND METHODS

After their isolation in April 1973, CRSV-R and CRSV-N were maintained by drying infected cowpea leaves in an Edwards freeze dryer, sealing them in glass tubes under nitrogen, and storing them at 4 C. The dried cultures were renewed at 2-yr intervals. The viruses were purified by the pH 5.0 method (13) and dissolved in 0.01 M sodium acetate buffer, pH 5.0, containing 0.1 M NaCl. Virus concentration was estimated spectrophotometrically by using the extinction coefficient of 6.5 cm^2/mg at 260 nm (5). Concentrated virus preparations (20–40 mg/ml) were diluted to 0.8 mg/ml in 0.1 M tris acetate buffers at the desired pH and containing appropriate concentrations of selected chemicals. The pH of the chemical solutions was adjusted to pH 5.0 or pH 7.0 with 0.1 N HCl or 0.1 N NaOH before addition to the virus. The absorbance of each of three virus solutions was measured at 340 nm in 0.3-ml thermocuvettes (1-cm path) in a Gilford recording spectrophotometer equipped with a model 2535 automatic reference compensator and a model 2527 thermoprogrammer. In most experiments, the temperature was raised at a constant rate of 0.25 C/min. When the absorbance stopped increasing, the temperature was decreased rapidly to 25 C and the absorbance was recorded. The thermocuvettes were removed, shaken, and replaced for an additional absorbance measurement. The samples were removed from the cuvettes and stored at 4 C overnight. A series of twofold serial dilutions prepared from each sample in 0.1 M tris acetate buffer, pH 7.0, were used in gel diffusion tests. The rate of temperature increase of 0.25 C/min was most satisfactory for reproduction of results (12). The temperature at which one half the maximum absorbance increase was attained was defined as the aggregation temperature (12).

Gel diffusion tests were done on Formvar-coated glass slides with wells cut by a template (11). Antigen (40 μl) in a range of twofold serial dilutions (1/2 to 1/256) was pipetted into eight outer wells. Forty microtiters of CRSV-R antiserum (12) at a dilution of 1/4 was pipetted into two inner wells. Each test was replicated three times. Variation in antigen titer among replicates was never greater than one twofold dilution higher or lower. Precipitin bands were in a position typical of whole virus antigen. The antiserum was not reactive with virus subunit dissociated by 1% SDS.

RESULTS

Temperature dependent aggregation-disaggregation. A solution of CRSV-R at 0.8 mg/ml in 0.1 M tris acetate buffer, pH 5.0, was heated from 25 to 69 C at a rate of 2 C/min and cooled at the same rate (Fig. 1A). The absorbance at 340 nm increased rapidly between 50 and 55 C and on cooling decreased at a slower rate to a value slightly higher than the original. If the reaction proceeded in a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

completely reversible manner, the absorbance at a temperature on the rise would be the same at that temperature on the decline. In a series of experiments we attempted to decrease the temperature manually when the absorbance decreased to the value attained at that temperature on the rise. However, the slow rate of absorbance decrease necessitated holding the same temperature for periods exceeding 15 min. When the temperature was maintained above 50 C for extended periods, irreversible precipitation occurred.

Effect of pH on temperature-dependent aggregation-disaggregation. Strains R and N at 0.8 mg/ml and various pH values between 4.0 and 8.0 were heated to 65 C at 0.25 C/min. The aggregation temperature was lowest at approximately pH 5 for the R strain or pH 6 for the N strain (Table 1). The maximum absorbance values were similar with both strains at all pH values. There was an irreversible precipitation with strain N at pH 4.0 and 8.0 and with strain R at pH 8.0. These differences between strains R and N confirm differences found in their serology, amino acid composition, and aggregation properties (12).

TABLE 1. Effect of pH on the aggregation-disaggregation of carnation ringspot virus strains R and N

Strain	pH	Temperature[a]	A_m[b]	A_r[c]	Precipitate[d]
R	4.00	54	1.77	0.03	−
	4.25	52	1.77	0	−
	4.50	51	1.86	0.03	−
	4.75	51	1.83	0	−
	5.00	50	1.89	0.06	−
	5.00	50	1.89	0.06	−
	5.25	50	1.86	0	−
	5.50	51	1.86	−0.03	−
	6.00	53	1.83	−0.03	−
	6.50	54	1.77	0.03	−
	7.00	54	1.77	0.03	−
	8.00	57	1.68	0.21	+
N	4.00	61	2.07	1.11	+++
	5.00	53	1.98	0.12	−
	6.00	51	2.01	0.06	−
	6.50	51	1.86	0.06	−
	7.00	54	1.89	0.21	+
	8.00	57	1.96	1.38	+++

[a] The temperature at which one half the maximum absorbance increase was attained on raising temperature at 0.25 C/min.
[b] The maximum absorbance increase at 340 nm attained on heating.
[c] The absorbance of the solution after cooling to 25 C.
[d] A visual estimation of precipitate formed in cuvette: −, no precipitate; +, ++, and +++, small, moderate, and large amounts of precipitate, respectively.

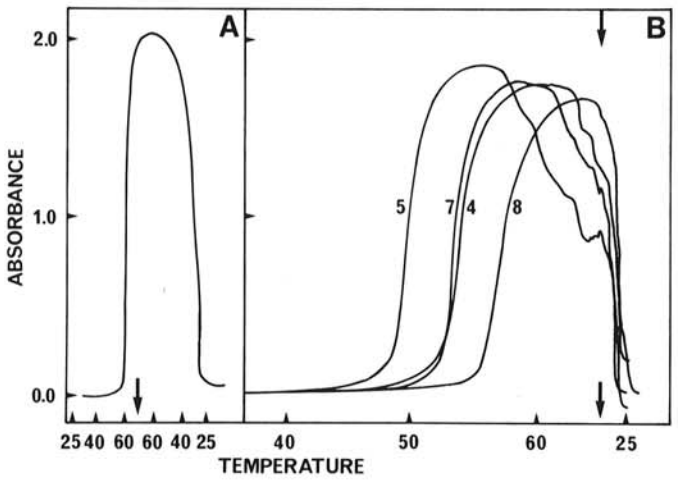

Fig. 1. Effect of temperature on the absorbance at 340 nm of a 0.8 mg/ml solution of carnation ringspot virus strain R. **A,** The temperature of the solution at pH 5.0 was raised at 2 C/min from 25 to 68 C, then cooled to 25 C at the same rate. **B,** The temperature of solutions at pH values of 4.0, 5.0, 7.0, and 8.0 was raised at 0.25 C/min from 25 to 65 C then cooled rapidly to 25 C. Arrows on the graphs indicate when cooling was initiated.

Absorbance-temperature scans for strain R at pH 4.0, 5.0, 7.0, and 8.0 are shown in Fig. 1B. At pH 5.0, the absorbance increased to a maximum, maintained this until 57 C, then decreased to 0.87 at 65 C. On cooling, the absorbance was less than the initial value. Other experiments at pH 5.0 indicated that large aggregates formed at temperatures higher than 57 C. These aggregates sedimented to a position lower than the light path. On lowering to 25 C these aggregates dissociated, leaving a concentration gradient in the cuvette. The solutions at pH 4.0 and 7.0 behaved similarly.

Effect of selected chemicals on temperature-dependent aggregation. Absorbance-temperature scans of CRSV-R in the presence of urea, EDTA, and sucrose are presented in Figs. 2, 3, and 4 and data for all chemicals are in Table 2. At pH 5.0, increasing concentrations of urea increased the aggregation temperature from 50 to 53 C in control tests up to 59 C at 1,000 mM urea. The absorbance on cooling to 25 C was similar to the original absorbance, and gel diffusion serological tests did not detect a loss of virus. At pH 7.0, the absorbance scans at 100 and 500 mM urea showed an aggregation temperature decrease but the absorbance scan at 1,000 mM and the absorbance and appearance of the solution on cooling to 25 C indicated that the virus was denatured. Serological tests showed a decrease in virus concentration at 1,000 mM urea but not at 100 and 500 mM. Therefore, the absorbance scan at 1,000 mM indicated denaturation, but some reversible aggregation may have occurred as well.

At pH 5.0, 100 mM EDTA reduced aggregation temperature by 12 C (Fig. 3) without detectable denaturation or loss of serological activity. Decreases in aggregation temperature were minimal at 1–10 mM EDTA (Table 2). At pH 7.0, 100 mM EDTA reduced aggregation temperature by 24 C with no loss of serological activity. Aggregation temperature decreases at 1–10 mM EDTA were much greater at pH 7.0 than at pH 5.0 (Table 2). The experiment was repeated at pH 5.0 and 7.0 at 100 mM EDTA and the EDTA was removed from aliquots by dialysis in pH 5.0 or pH 7.0 buffer. The removal of the EDTA at pH 5.0 increased the aggregation temperature from 41 to 51 C, but dialysis at pH 7.0 denatured the virus. The effect of ethyleneglycol-*bis*-(β-amino-ethyl ether)-*N*,*N*′,*N*′-tetraacetic acid (EGTA) (Sigma Chemical Co.) was similar to that of EDTA (Table 2).

Sucrose induced the greatest increases in aggregation temperature (i.e., 25 C at pH 5.0 and 20 C at pH 7.0) (Fig. 4, Table 2). Temperatures above 80 C denatured the virus and destroyed serological activity even in the absence of sucrose. In separate tests, the solutions were cooled after heating to 65 C and denaturation and loss of serological activity did not occur.

Results with the other chemicals (Table 2) showed KCNS and acetamide reduced the aggregation temperature at pH 5.0, but denaturation and a decrease in serological activity occurred at pH

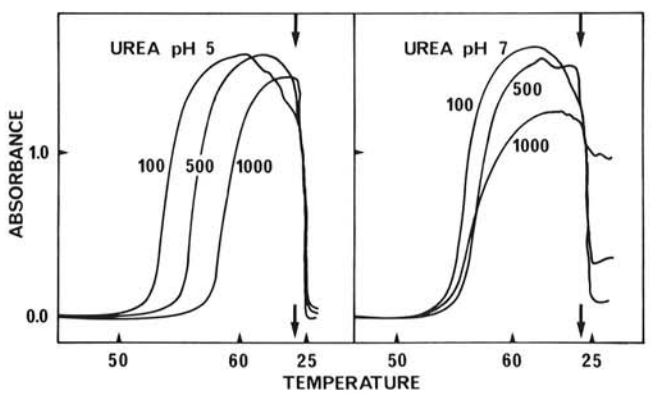

Fig. 2. Effect of temperature on the absorbance at 340 nm of 0.8 mg/ml solutions of carnation ringspot virus strain R in 100, 500, and 1,000 mM urea at pH 5.0 and 7.0. Temperature was raised at 0.25 C/min from 25 to 64 C at pH 5.0 or to 66 C at pH 7.0, then cooled rapidly to 25 C. Arrows on the graphs indicate when cooling was initiated.

7.0. Increasing concentrations of NaCl increased the aggregation temperature at pH 5.0 and 7.0 without denaturation. Thiourea had little effect at pH 5.0 and denaturation occurred at pH 7.0. Denaturation occurred at pH 5.0 with uranyl acetate and at pH 5.0 and 7.0 with guanidine. Small aggregation temperature decreases were observed with Triton X-100 at pH 5.0 and 7.0, with Tween-20 at pH 5.0 (but not at pH 7.0) and with alginic acid at pH 5.0. These reagents did not affect serological activity or induce denaturation.

At pH 5.0, SDS at 0.1–4 mM had little effect on the aggregation temperature and decreased the virus antigen titer. At concentrations greater than 4 mM the virus was serologically inactive (Table 3). At pH 7.0, the virus is dissociated by 0.1 mM SDS (9).

DISCUSSION

The absorbance increase of CRSV-R on heating in chemicals (Table 2) was induced by aggregation or by denaturation or by a combination of the two. Denaturation was detected by the absorbance of the solutions on redispersing precipitates and by serological tests. KCNS, acetamide, urea, thiourea, guanidine, and uranyl acetate at pH 7 and guanidine at pH 5 denatured CRSV at 50–60 C. NaCl, acetamide, urea, Triton X-100, Tween-20 and alginic acid induced small changes in aggregation temperature without denaturation. However, the divalent ion chelators EDTA and EGTA lowered the aggregation temperature greatly without denaturation. The effect with EDTA was reversed by dialysis at pH

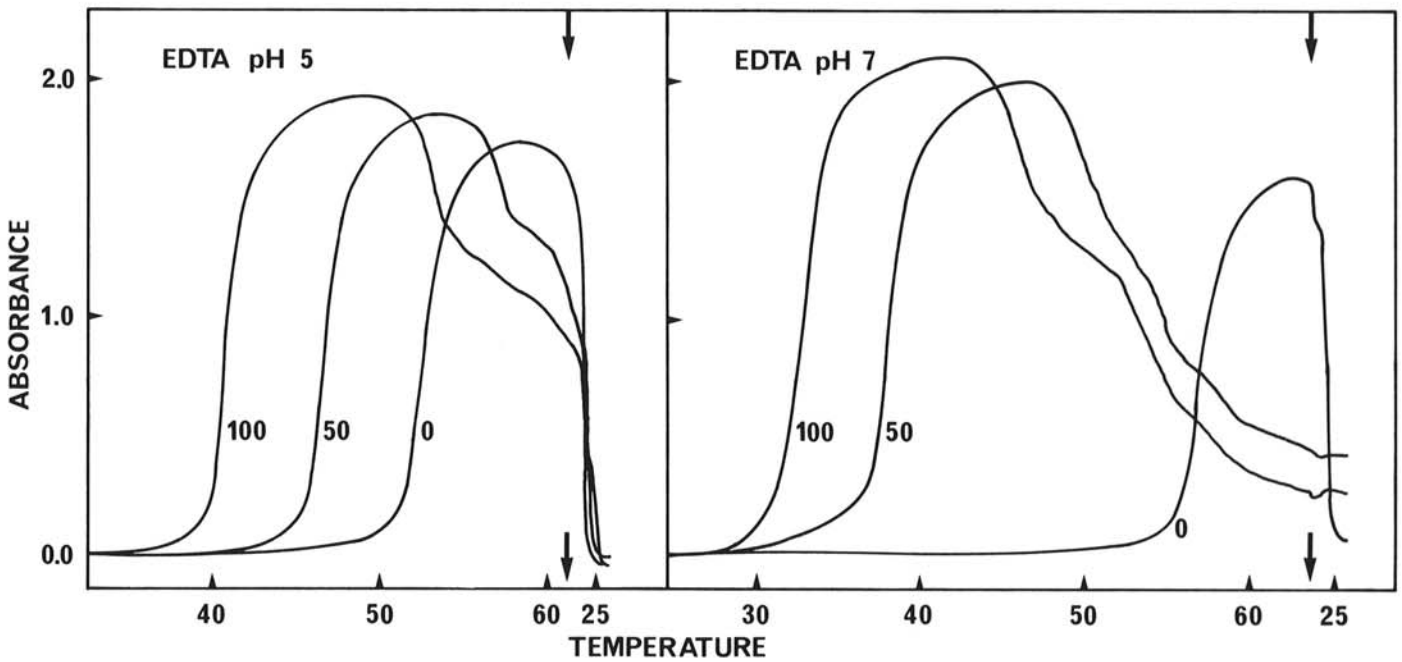

Fig. 3. Effect of temperature on the absorbance at 340 nm of 0.8 mg/ml solutions of carnation ringspot virus strain R in 0, 50, and 100 mM EDTA at pH 5.0 and 7.0. Temperature was raised at 0.25 C/min from 25 to 61 C at pH 5.0 or 63 C at pH 7.0, then cooled rapidly to 25 C. Arrows on the graphs indicate when cooling was initiated.

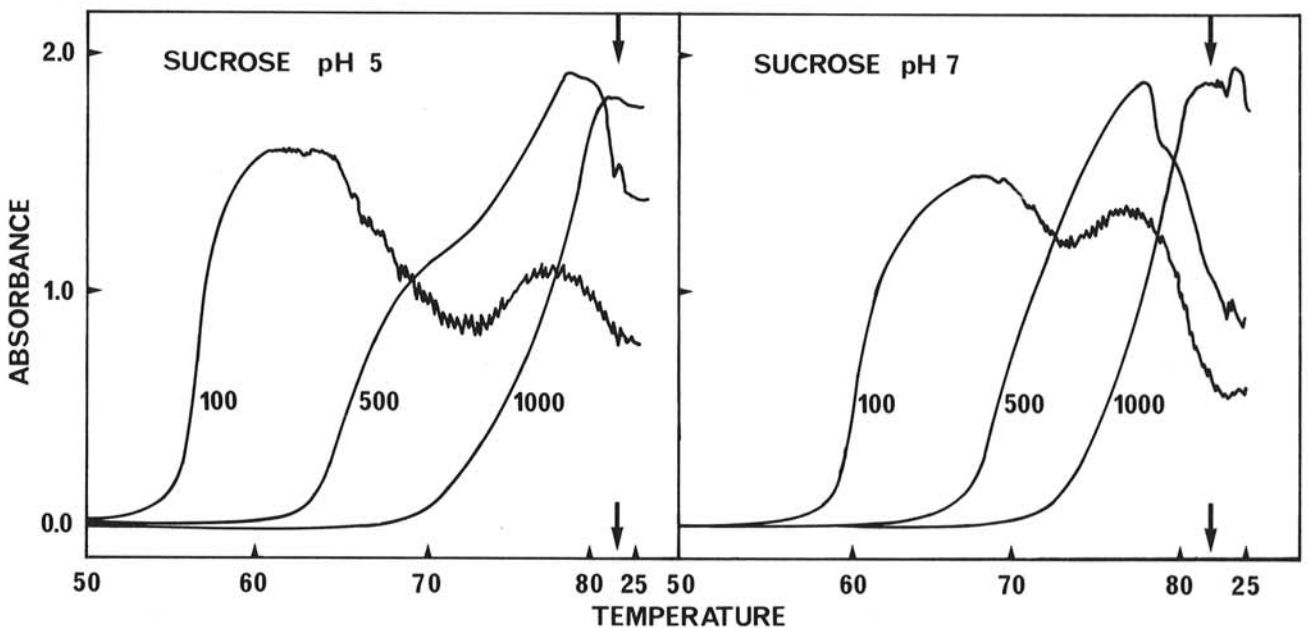

Fig. 4. Effect of temperature on the absorbance at 340 nm of 0.8 mg/ml solutions of carnation ringspot virus strain R in 100, 500, and 1,000 mM sucrose at pH 5.0 and 7.0. Temperature was raised at 0.25 C/min from 25 to 82 C, then cooled rapidly to 25 C. Arrows on the graphs indicate when cooling was initiated.

5.0. The effect was probably not caused by increased ionic strength because NaCl did not induce a similar effect (Table 2). EDTA at 100 mM increased the dissociation of CRSV-R into RNA and protein at pH 7.5 in 1 M NaCl (*unpublished*). However, there is no evidence that CRSV is stabilized by divalent ions as are the sobemoviruses (3) and tombusviruses (2). Sucrose raised the aggregation temperature into the 70 C range where denaturation occurred without added sucrose. The increased viscosity of sucrose solutions would reduce the movement of virus monomer but alginic acid had very little effect on aggregation. Sucrose may inhibit aggregation of some viruses during their purification. The denaturing effect of uranyl acetate was unexpected; it was used as a negative stain for CRSV aggregates in electron microscopy (12). The non-ionic detergents, Triton X-100 and Tween-20, were expected to favor disaggregation, but they reduced the aggregation temperature. Hull et al (4) used Triton X-100 to dissociate viroplasm in the purification of cauliflower mosaic virus. Tween-20 prevents protein absorption to plastic plates in enzyme-linked immunosorbent assays (1).

The aggregation-disaggregation system of CRSV-R and CRSV-N resembles the polymerization-depolymerization system of tobacco mosaic virus protein (TMV-P) (6,12). Both are

TABLE 2. Effect of selected chemicals at two pHs on the aggregation-disaggregation of carnation ringspot virus[a]

		pH 5.0				pH 7.0			
Chemical	Conc[b]	Temp[c]	A_m[d]	A_r[e]	A_p[f]	Temp[c]	A_m[d]	A_r[e]	A_p[f]
NaCl	50	49	1.81	0.03	0.04	52	1.78	0.03	0.03
	100	50	1.86	0.03	0.05	53	1.83	0.11	0.12
	200	51	1.86	0.06	0.07	55	1.77	0.14	0.11
NaCl[g]	50	53	1.94	−0.06	0.01	53	1.83	0.03	0.03
	100	54	1.99	−0.06	0.01	55	1.78	0.21	0.30
	200	55	2.01	−0.06	0.02	58	1.88	0.45	0.50
KCNS	10	51	1.98	−0.03	−0.03	56	1.62	0.11	0.48
	50	53	1.76	−0.03	−0.03	57	1.63	0.33	0.57
	100	56	1.68	0	0	59	1.49	0.90	0.75
Acetamide	100	51	1.82	−0.06	−0.06	56	1.61	0.45	1.53
	500	55	1.68	0	0	57	1.59	0.87	1.89
	1,000	58	1.52	0.24	0.24	59	1.59	0.99	2.16
Sucrose	100	57	1.61	0.70	2.16	61	1.48	0.55	1.66
	500	69	1.93	1.05	2.27	71	1.90	0.88	2.11
	1,000	78	1.83	1.78	2.79	77	1.87	1.84	1.96
Urea	100	54	1.60	0.01	0.07	56	1.56	0.10	0.50
	500	57	1.60	0.02	0.14	57	1.59	0.34	0.74
	1,000	59	1.48	0.07	0.26	57	1.25	0.98	1.25
Thiourea	75	51	1.93	0	0.01	55	1.81	0.13	0.72
	100	52	1.67	0	0.01	58	1.58	0.37	0.98
	125	52	1.62	0	0.01	60	1.72	1.10	1.73
EDTA	0	53	1.73	0		57	1.56	0.06	
	1	53	1.65	0.06		50	1.57	0.06	
	5	52	1.77	0.09		49	1.68	0.15	
	10	51	1.77	0.09		48	1.67	0.21	
	50	47	1.84	−0.03		38	1.96	0.36	
	100	41	1.92	−0.03		33	2.08	0.24	
EGTA[h]	0	52	1.73	−0.06		58	1.61	0.06	
	50	49	1.78	−0.06		42	1.80	0.15	
	100	44	1.89	−0.06		42	1.72	0.15	
Guanidine	100	57	1.64	0.02	0.51	56	1.49	0.80	0.90
	500	62	1.64	0.92	1.60	45	1.50	2.05	2.05
	1,000	60	1.64	0.80	1.92	42	1.35	1.27	1.46
Uranyl acetate	5	47	1.97	0.63	2.02				
	10	40	2.42	0.27	2.23				
	50	38	∞	0.02	∞				
Triton X-100	0.01%	51	1.76	0	0.01	56	1.62	0.05	0.06
	0.10%	50	1.78	0	0.01	54	1.67	0.04	0.10
	1.00%	48	1.87	−0.01	0.06	53	1.60	0.02	0.07
Tween-20	0.01%	55	1.54	0	0	55	1.82	0	0.10
	0.10%	51	1.78	0	0.05	56	1.72	0.04	0.08
	1.00%	49	1.75	−0.01	0.02	54	1.81	0.01	0.09
Alginic acid	12.5%	51	1.83	0.01	0.01				
	25 %	50	1.83	0.02	0.02				
	50 %	48	1.80	0.01	0.01				

[a] Experiments were done with the R strain except where noted.
[b] Concentration presented as mM except where specified as percentage (%).
[c] The temperature at which one half the maximum absorbance increase was attained on raising the temperature at 0.25 C/min.
[d] The maximum absorbance increase obtained on heating.
[e] The absorbance of the solution after cooling to 25 C.
[f] The absorbance of the solution measured after removing the cuvettes, shaking, and replacing them in a spectrophotometer.
[g] These results were obtained with the N strain.
[h] Ethyleneglycol-*bis*-(β-amino-ethyl ether)-*N*,*N*',*N*'-tetraacetic acid (Sigma Chemical Co.).

TABLE 3. Effect of sodium dodecyl sulfate (SDS) on the aggregation-disaggregation of carnation ringspot virus, strain R, at pH 5.0

mM SDS	Temperature[a]	A_m[b]	A_r[c]
0.1	51	1.78	0
1	52	1.72	−0.01
2	53	1.12	0
3	52	1.23	0
4	51	1.25	0
5	49	0.07	−0.01
6	52	0.21	−0.02
10	51	0.41	−0.09

[a]The temperature at which one half the maximum absorbance increase was attained on raising temperature on 0.25 C/min.
[b]The maximum absorbance increase at 340 nm attained on heating.
[c]The absorbance of the solution after cooling to 25 C.

endothermic and therefore entropy-driven, and both are reversible. In the polymerization of TMV-P the source of the entropy increase is the accompanying release of water molecules. In studies of the TMV-P system, the temperature was increased gradually and the absorbance was measured on completion of polymerization with each temperature increment. Then the temperature was decreased gradually and measurements of absorbance taken with falling temperature coincided with those taken at the rising temperature. The system is, therefore, reversible in the strict physico-chemical sense. The temperature-absorbance plots were used to calculate T^*, a temperature "characteristic" of the system and with light-scattering theory and linear condensation polymerization equations it was possible to calculate thermodynamic parameters of the polymerization process. The effect of various chemicals, pH, and ionic strength (phosphate buffer) on this "characteristic" temperature and on the thermodynamic parameters was studied (7–9). These tests showed that sucrose and ionic strength favor polymerization, i.e., decreased the "characteristic" temperature. Thiourea, KSCN, acetamide, urea, and EDTA favored depolymerization, i.e., increased the "characteristic" temperature. Shalaby and Lauffer (8) noted that most solutes have predictable effects on polymerization and these are similar to their effects on the structure of water.

The effect of urea, thiourea, and KCNS on the CRSV aggregation system were the same as their effect on the TMV-P system. However, ionic strength (NaCl), EDTA, and sucrose had the opposite effect. These results indicate that these reagents may interact specifically with the CRSV system. However, there are important differences between the TMV and CRSV systems. CRSV disaggregation rate was much slower than the aggregation rate and the reaction was not truly reversible under our conditions. TMV-P polymerizes at much lower temperatures (18 C) than the CRSV system. The product of TMV-P polymerization is much smaller than the CRSV aggregate (12). Irreversible precipitation of TMV-P did not occur but was common with CRSV-R, particularly at pH 7.0. This may reflect stability of the CRSV particle; it swells at pH 7.0 (10) and can be dissociated into RNA and protein at pH 7.5 in 1 M NaCl (*unpublished*).

Aggregation of some viruses during their purification is a serious problem for plant virologists. Our studies on CRSV may be applicable to this problem, particularly relative to the depolymerizing effect of sucrose. However, the possibility of specific interactions of some chemicals with each virus system should be considered.

LITERATURE CITED

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-484.
2. Harrison, S. C. 1983. Virus structure: High-resolution perspectives. Adv. Virus Res. 28:175-240.
3. Hull, R. 1977. The stabilization of the particles of turnip rosette virus and other members of the southern bean mosaic virus group. Virology 79:58-66.
4. Hull, R., Shepherd, R. J., and Harvey, J. D. 1976. Cauliflower mosaic virus: An improved purification procedure and some properties of the virus particles. J. Gen. Virol. 31:93-100.
5. Kalmakoff, J., and Tremaine, J. H. 1967. Some physical and chemical properties of carnation ringspot virus. Virology 33:10-16.
6. Lauffer, M. A. 1975. Entropy-driven processes in biology—Polymerization of tobacco mosaic virus protein and similar reactions. Vol. 20 in: Molecular Biology, Biochemistry and Biophysics. A. Kleinzeller, G. F. Springer, and H. G. Wittman, eds. Springer-Verlag, Berlin. 264 pp.
7. Lauffer, M. A., and Shalaby, R. A. 1980. The effect of ionic strength on the entropy-driven polymerization of tobacco mosaic virus protein. Contributions of electrical work and salting out. Arch. Biochem. Biophys. 201:224-234.
8. Shalaby, R. A. F., and Lauffer, M. A. 1967. Polymerization-depolymerization of tobacco mosaic virus protein. IX. Effects of various chemicals. Biochemistry 6:2465-2473.
9. Smith, C. C., and Lauffer, M. A. 1967. Polymerization-depolymerization of tobacco mosaic virus protein. VIII. Light-scattering studies. Biochemistry 6:2457-2465.
10. Tremaine, J. H., and Ronald, W. P. 1976. Differential effects of sodium dodecyl sulfate on strains of carnation ringspot virus. J. Gen. Virol. 30:299-308.
11. Tremaine, J. H., Ronald, W. P., and Kelly, E. M. 1981. Comparisons of highly basic cyanogen bromide peptides from strains of southern bean mosaic virus. Can. J. Microbiol. 27:654-663.
12. Tremaine, J. H., Ronald, W. P., and McGauley, E. M. 1984. Temperature-reversible aggregation of two strains of carnation ringspot virus. Phytopathology 74:161-165.
13. Tremaine, J. H., Ronald, W. P., and Valcic, A. 1976. Aggregation properties of carnation ringspot virus. Phytopathology 66:34-39.