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EFFECTS OF PH AND IONIC STRENGTH ON PRECIPITATION OF PHYTOPATHOGENIC VIRUSES BY POLYETHYLENE GLYCOL

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Summary. — The effects of ionic strength of the solution (changed by varying NaCl concentrations or buffer molarity) on the precipitation with polyethylene glycol (PEG) 6000 were studied on phytopathogenic viruses of different morphology: the isometric red clover mottle virus (RCMV), rod-shaped tobacco mosaic virus, flexuous potato virus X (PVX) and bacilliform alfalfa mosaic virus. With increasing NaCl concentration or buffer molarity up to a certain level (0.1 mol/l), the efficiency of PEG precipitation increased. This relationship did not apply to PVX. The effects of pH on PEG precipitation were studied on RCMV. The efficiency of precipitation increased with decreasing difference between pH of the solution and pI of the virus.

Key words: *plant viruses*; *virus purification*; *polyethylene glycol precipitation*; *ionic strength*; *effect of pH*

Introduction

In protein and virus purification, their precipitation has been frequently achieved by polyethylene glycol (PEG), a non toxic polymer well soluble in water. The molecular mechanism of this precipitation has not yet been elucidated but it has been assumed that it is connected with steric exclusion of proteins from those regions of the solvent which become occupied by inert molecules of the synthetic polymer. The effective concentration of protein thus increased and leads to precipitation of the latter (Atha and Ingham, 1981). Several authors proposed equations expressing the quantitative relations in the course of precipitation (Juckes, 1971; Polson, 1977; Atha and Ingham, 1981). The dependence of protein or virus solubility on PEG concentration can be expressed by the simple equation (Ingham, 1984)

$$\log S = \log S_0 - \beta C$$

where S = solubility at the given PEG concentration (W/v), S_0 = solubility in the absence of PEG, β = constant depending on precipitation conditions

like, e.g., ionic strength, pH, temperature of the solution, size of the protein or virus particle, size of PEG molecule, and C = PEG concentration.

Since the first use of PEG for plant virus purification by Herbert (1963), the PEG precipitation has become a widely used routine method for the concentration and purification of a variety of phytopathogenic viruses. Several authors attempted to elucidate the conditions of precipitation. For example Leberman (1966) studied the effects of NaCl concentration and pH on the precipitation of tobacco mosaic, turnip yellow mosaic and turnip crinkle viruses and Juckes (1971) the effects of PEG 6000 concentration on the precipitation of brome mosaic virus. Clark and Lister (1971) described precipitation of some plant viruses with PEG 6000 by the method involving centrifugation of PEG-precipitated virus trough a reverse concentration gradient of PEG 6000. A number of plant virus model systems was dependent on their surface/volume ratio and their surface charge characteristics and on the pH and ionic conditions. We carried out a quantitative study on the precipitation of selected viruses of a different size and morphology by PEG 6000 at different buffer molarities, various NaCl concentrations and various pH values. We used the isometric red clover mottle virus (RCMV; Comovirus group), the flexuous potato virus X (PVX; Potexvirus group) and the bacilliform alfalfa mosaic virus (ALMV).

Materials and Methods

Viruses. RCMV isolate TpM 25 was propagated and purified as described by Marcinka (1971); its isoelectric points are 5.95 and 6.40 (Marcinka, 1983). TMV strain vulgare has an isoelectric point of 3.5 (Schramm, 1953). PVX was propagated and purified as described by Koenig *et al.* (1970). The isoelectric point of PVX is 4.4 (Berck, 1970). ALMV isolate 425 (Hagedorn and Hanson, 1963) was propagated and purified as described by Clark (1986); its isoelectric point is below pH 6.0 (Jaspars and Bos, 1980).

Conditions of polyethylene glycol precipitation. In all suspensions of purified viruses used in the present experiments, the virus concentration was adjusted to 1 mg/ml. Precipitation with PEG 6000 (Sigma) was carried out at room temperature with exception of ALMV, which was precipitated at 4 °C to avoid its thermal disintegration. After 30 min, the precipitates were removed by centrifugation (3000 × g 30 min, 4 °C) and the amount of virus remaining in the supernatant was estimated spectrophotometrically at 260 nm. The pH of sodium phosphate buffers used selected so as differ sufficiently from the pI of the given virus.

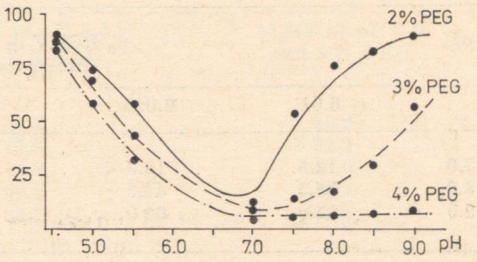

Fig. 1.
Precipitation of RCMV at various pH and PEG concentrations in 0.01 mol/l sodium phosphate buffer
Ordinate: % RCMV in the supernatant fluid.

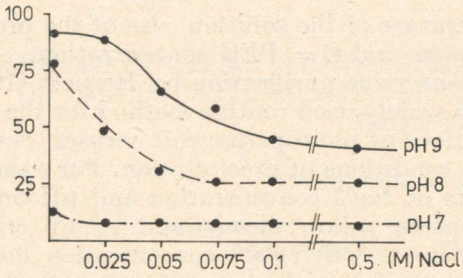

Fig. 2.
Precipitation of RCMV by 2 % PEG 6000 in 0.01 mol/l sodium phosphate buffer at various pH and in the presence of different concentrations of NaCl
Ordinate: % RCMV in the supernatant fluid.

Results

Precipitation of RCMV

The effects of pH of the buffer at its low molarity (0.01 mol/l) and at low PEG 6000 concentrations (2, 3 and 4 %) showed (Fig. 1) that the smaller the difference between pH of the buffer and pI of the virus, the lower the PEG concentration necessary for efficient precipitation. Precipitation in alkaline pH region proved to be more efficient than in the acid region. This was most marked with precipitation by 4 % PEG in alkaline environnment, when the effect of pH was no more manifested.

When precipitation by 2 % PEG 6000 proceeded in buffer of low molarity (0.01 mol/l), it was markedly enhanced with increasing NaCl concentration, but only up to a certain level (0.1 mol/l) (Fig. 2). This enhancement effect of NaCl was at pH 8 and 9 more marked than at pH 7. There was nearly no difference between 0.1 and 0.5 mol/l NaCl. The effect of increasing buffer molarity on RCMV precipitation was similar to that of NaCl. Enhancement was manifested mainly at pH 8 and 9. The more remote the pH of buffer from the pI of virus, the higher the molarity of buffer necessary for efficient precipitation of virus (0.05 mol/l at pH 8, 0.075 mol/l at pH 9) (Table 1).

Precipitation of TMV

The efficiency of TMV precipitation was greatly affected by ionic strength of the solution, both by buffer molarity and NaCl concentration (Table 2).

Table 1. Effect of buffer molarity on the precipitation of RCMV with 2 % PEG 6000 in sodium phosphate buffer at various pH

pH	% RCMV in supernatant fluid Buffer molarity (mol/l)				
	0.01	0.025	0.05	0.075	0.1
7.0	12.5	12.3	24.5	24.0	24.5
8.0	85.5	42.3	27.7	29.5	32.5
9.0	95.0	92.0	68.5	51.3	54.0

Table 2. Precipitation of TMV with PEG 6000 in sodium phosphate buffer at pH 5.2

Buffer molarity (mol/l)	% TMV in supernatant fluid			
	% PEG 6000			
	2	3	4	2 + 0.1 mol/l NaCl
0.01	100	100	100	13.5
0.025	100	100	100	8.2
0.05	100	100	100	6.6
0.075	72.5	64.2	51.2	4.3
0.1	63.5	31.7	20.6	4.8

With PEG 6000 in the presence of NaCl (Table 4), efficient precipitation (over 90 %) occurred at minimal NaCl concentration (0.075 mol/l). Higher NaCl concentrations (up to 0.5 mol/l) had no substantial effect on TMV precipitation. Increasing buffer molarities had similar, but less pronounced effects. Precipitation with 3 % PEG 6000 in the presence of 0.075 mol/l NaCl or 0.075 mol/l sodium phosphate buffer resulted in 6.8 or 64.2 % of TMV remaining in the supernatant, respectively.

Precipitation of PVX

As distinct from RCMV, TMV and ALMV, precipitation of PVX with 2 % PEG 6000 was not enhanced either by NaCl or ionic strength of buffer (Tables 3 and 4).

Precipitation of ALMV

A higher PEG concentration (5 %) was required for ALMV precipitation as compared with RCMV, PVX and TMV. Increasing NaCl concentration or buffer molarity displayed an enhancement effect, though not as marked as with RCMV or TMV (Tables 3 and 4).

Table 3. Precipitation of PVX and ALMV with PEG 6000 in sodium phosphate buffer at various buffer molarities

Buffer molarity (mol/l)	% virus in supernatant fluid	
	PVX at pH 5.4 and 2 % PEG	ALMV at pH 7.0 and 5 % PEG
0.01	40.7	89.0
0.025	27.8	82.7
0.05	41.3	93.9
0.075	49.2	68.7
0.1	84.7	61.9

Table 4. Effect of NaCl concentration on the precipitation of TMV, PVX and ALMV with PEG 600 in 0.01 mol/l sodium phosphate buffer

NaCl concentration (mol/l)	% virus in supernatant fluid		
	TMV at pH 5.2 and 3 % PEG	PVX at pH 5.4 and 2 % PEG	ALMV at pH 7.0 and 5 % PEG
0	100	41.0	90.0
0.025	79.7	16.8	85.3
0.05	35.6	35.6	53.5
0.075	6.8	36.5	60.0
0.1	5.0	27.0	46.1
0.5	3.4	88.0	29.9

Discussion

In each experiment we used such PEG concentration so as to achieve only partial precipitation of the given virus. In this way the effects of pH and ionic strength of the solutions used in virus precipitation were clearly manifested.

The effects of pH were investigated only in RCMV precipitation. The efficiency of the latter was indirectly proportional to the difference between pH of the buffer and pI of virus (in absolute values). This means that smaller the difference between pH and pI, the more efficient the precipitation. In aplying these results to virus purification it should be borne in mind that in close vicinity of the isoelectric points the virus yields could be low due to eventual isoelectric precipitation of virus before its separation from plant materials. On the other hand the efficiency of virus precipitation at the same PEG concentration decreased with increasing distance between pH and pI of the virus. In most cases the efficiency of precipitation could be enhanced by increasing either the concentration of NaCl or buffer molarity.

In our experiments we used NaCl concentrations not higher than 0.5 mol/l. High molarities (5 mol/l) could lead to artifacts in PEG precipitation due to salting out. With the exception of PVX, NaCl enhanced the efficiency of precipitation. This effect was the most marked with TMV, which is in accordance with Leberman (1966). At low buffer molarity (0.01 mol/l sodium phosphate, pH 5.2) in the absence of NaCl, TMV was not precipitated even with 10 % PEG (results not shown).

Like NaCl, also buffer molarity enhanced the precipitation of RCMV, ALMV and TMV, but not of PVX. The precipitation curve of PVX had no uniform tendency. The varying with increasing NaCl concentration or buffer molarity (Tables 3 and 4) were confirmed in several repeated experiments. But as distinct from the other viruses, on increasing the limiting PEG 6000 concentration (3 % and higher), PVX precipitation was complete irrespective of ionic strength of the solution. By contrast, in the other viruses

studied the degree of precipitation depended on ionic strength of the solution. By contrast, in the other viruses studied the degree of precipitation depended on ionic strength of the solution also at higher PEG concentrations (e.g., TMV).

The question as to what should be preferred: adding NaCl or increasing buffer molarity, can be answered as follows: with TMV and ALMV the answer is to add NaCl. With RCMV the answer is equivocal, because the effects of either alternative were similar, especially at pH 8 and 9.

Our experiments showed that for plant virus purification with PEG an uniform protocol cannot be applied. One of the conditions affecting the purification appears to be the size of the virion and the surface of its structural proteins, i.e. the degree of their hydrophobicity.

We attempted to demonstrate the possibility of affecting virus precipitation with PEG by changing pH and ionic strength of the solutions. These factors should be selected so as to achieve virus precipitation at the lowest possible PEG concentration. At higher PEG concentrations also some plant proteins could precipitate along with the virus. By optimalizing the conditions of virus precipitation with PEG, this method can be effectively employed not only for virus concentration but also for virus purification.

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