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PVX-Q: an Infective Product of Potato Virus X and a Leaf *o*-Quinone

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SUMMARY

When potato virus X (PVX) is exposed to enzymically oxidizing chlorogenic acid, and re-isolated, some of its properties are changed. Its u.v. spectrum is modified and centrifuged pellets are coloured: its u.v. fluorescence is diminished, a longer-wave fluorescence is introduced: it produces less colour with 2,4,6-trinitrobenzene sulphonic acid, and moves faster during electrophoresis. There is, however, little or no loss of infectivity in the course of the reaction. The results suggest that PVX combines with chlorogenoquinone to produce a modified but infective virus (PVX-Q) and it is thought that this reaction may occur naturally.

INTRODUCTION

Some plant viruses are unstable in browning leaf extracts and can only be isolated in an aqueous medium containing a reducing agent or an inhibitor of *o*-diphenol oxidase: examples include cucumber mosaic virus (Harrison & Pierpoint, 1963), prune dwarf virus (Hampton & Fulton, 1961) and Tulare apple mosaic virus (Mink, 1965). It is usually assumed that inactivation is caused by *o*-quinones produced during the oxidation of leaf polyphenols which combine with the virus protein and either disrupt it or 'tan' it. Little is known of the specific reactions involved except that they may be complex (Pierpoint, 1971), depend on the virus and the quinone involved (Barnett & Fulton, 1971), and on the conditions of the reaction. Bancroft *et al.* (1971) have recently emphasized the role of virus –SH groups in the process, by isolating a mutant of cowpea chlorotic mottle virus, in which an introduced cysteine residue makes the virus sensitive to oxidizing phenols: the –SH groups of other proteins are known to react readily with chlorogenoquinone produced during the enzymic oxidation of chlorogenic acid (Pierpoint, 1969*b*).

The purpose of the present paper, however, is to demonstrate that the reaction of a virus with an enzymically generated *o*-quinone does not necessarily inactivate the virus. Potato virus X (PVX), although inactivated by some *o*-quinones, has been shown not to lose infectivity during the oxidation of chlorogenic acid (Saksena & Mink, 1970). This is confirmed; but evidence is presented suggesting that PVX reacts with chlorogenoquinone, and that its apparent resistance is due to the infectivity of the quinone-modified virus (PVX-Q). Tobacco mosaic virus is also resistant to chlorogenoquinone, but here no reaction between virus and quinone could be demonstrated.

METHODS

Preparation of PVX. PVX was a necrotic strain (PVX^N) originally isolated from potatoes by Dr D. Govier. It was cultured in glasshouse-grown tobacco plants (*Nicotiana tabacum*, var. Xanthi n.c.) by inoculating their bottom leaves with infected sap. After 2 to 3 weeks, the systemically infected upper leaves (about 100 g) were disrupted for 2 min in an Atomix, in 3·5 to 5 times their weight of phosphate buffer (50 mM; pH 7), containing sodium diethyldithiocarbamate (13·5 mM). The extract was filtered through muslin, centrifuged at 8000*g* for 30 min to remove cell debris, and then at 50000*g* for 2 h to pellet the virus. The pellet was resuspended overnight in more phosphate (usually 30 ml; 50 mM; pH 7) and the cycle of low- and high-speed centrifuging repeated 3 to 5 times. The final high-speed pellet, free from green material, was suspended in a few ml of phosphate buffer and stored on ice. In some preparations borate buffer (10 mM; pH 7·5) was used to suspend the pellet during intermediate stages; green material tended to pack less well during high-speed centrifuging in this buffer and could be removed by gently washing the surface of the pellet with a few ml of buffer. The concentration of PVX in suspension was estimated from its E_{265}, uncorrected for scattering, by assuming $E^{0{\cdot}1\%}_{1\,cm}$ for this strain is 3·5 (Bawden & Kleczkowski, 1959).

o-Diphenol oxidase (EC 1.10.3.1) was a partially purified preparation made from an acetone powder of leaves of Xanthi tobacco essentially as described previously (Pierpoint, 1966, 1969*a*). Buffer for the initial extraction contained benzene sulphinic acid (50 mM) instead of cysteine, and this extraction, as well as the first precipitation with ammonium sulphate, was done in an N_2-filled glove-bag to minimize the oxidation of phenols. The final solution, 0·04 ml of which would oxidize chlorogenic acid at 13 μl O_2 uptake/min, about half the maximal rate in the manometric assay, was divided into small samples and stored at −15 °C. This preparation has been used over a period of 2 years, and because its activity was decreasing, rather large amounts (0·2 to 0·5 ml) were used in the present experiments.

Reaction of PVX with chlorogenoquinone. PVX (10 to 20 mg) was incubated for 30 min at 25 °C in 2 to 3·5 ml of solution containing chlorogenic acid (6 μ-mol), excess *o*-diphenol oxidase (0·1 to 0·2 ml) and phosphate buffer (20 to 45 mM; pH 7 to 7·3). Usually five more lots of neutralized chlorogenic acid (6 μ-mol in 0·1 ml) were added at half-hourly intervals, the third and the final one being accompanied by more (0·1 ml) *o*-diphenol oxidase. After the final incubation the solution was stored on ice, often overnight, before the virus was separated by chromatography. One control solution, containing chlorogenic acid and enzyme but no PVX, and another containing only virus and phosphate were made in the same way; they were mixed just before chromatography.

Virus was separated from the oxidized chlorogenic acid on a freshly prepared column (usually 2·2 × 35 cm) of Sepharose 2B that had been prepared and washed in phosphate buffer (10 mM; pH 7·0). The chromatogram was developed in the same buffer, running at about 15 to 20 ml/h and the effluent monitored on a Uvicord 1.

Infectivity was estimated by the production of local lesions on *Chenopodium amaranticolor*. Twelve plants were stripped of all but four leaves and dusted with carborundum. Virus solutions were rubbed on by a finger which was rinsed between each inoculation. Usually two PVX solutions, diluted in borate buffer, 10 mM, pH 7·0, were compared with solutions of quinone-treated virus of identical E_{265}. All solutions were tested on each plant and tested the same number of times at each leaf position. Lesions, when fully developed, were usually counted on two successive days and the counts averaged.

P content of PVX. Dialysed suspensions of virus were precipitated with enough cold

trichloroacetic acid (50 %, w/v) to bring the final concentration to 17 to 25 %, cooled on ice for 10 min, and centrifuged at 3000 rev/min for 10 min. The precipitates were suspended in cold trichloroacetic acid (25 %, w/v), cooled on ice again and re-centrifuged. Occasionally they were washed a second time. The pellets were heated overnight at 100 °C with 1 ml of 2 N sulphuric acid, digested with perchloric acid and the total P estimated as described by Holden & Pirie (1955).

Spectral measurements. Absorption spectra were recorded on an Optica CF4 spectrophotometer. Fluorescence spectra were measured on a Fluorispec SF 1 fluorescence spectrophotometer attached to an $X-Y$ recorder (Advance, Electronics Ltd, HR 100). For the spectra in Fig. 2, gain was set at 300 and the recorder potentiometer at 0·1 V/in. In Fig. 2A the slits on the excitation beam were set in the medium and narrow positions, and both those on the emission beam at medium; in Fig. 2B each beam had one slit set in the medium position and the other wide open.

Electrophoresis. Virus preparations were dialysed overnight against 67 mM-phosphate buffer ($Na_2HPO_4+KH_2PO_4$; pH 7·0) and examined in a Perkin-Elmer electrophoresis apparatus, model 38A, with Philpot-Svenson optics, using a 2 ml Tiselius cell. Movement of peaks was usually measured in ascending and descending limbs and averaged graphically. The conductivity of the buffer was not measured; mobilities were calculated assuming the potential gradient to be 9·5 V/cm, as was found for this buffer in this apparatus by Bawden & Kleczkowski (1959).

Ultracentrifuging was done by Dr J. Carpenter on a Spinco model E analytical centrifuge operated at 29500 rev/min and 21·9 °C.

Serology was done by Dr B. Kassanis using an anti-PVX serum prepared from rabbits by Dr D. Govier.

Buffers. Phosphate buffers were usually made from Na_2HPO_4 solutions adjusted to the appropriate pH with M-HCl or M-NaOH; those used for electrophoresis were a mixture (10:15·5) of 67 mM-KH_2PO_4 and 67 mM-Na_2HPO_4. Borate buffer was a 10 mM solution of $Na_2B_4O_7$ adjusted to pH 7·5 with M-HCl.

RESULTS

Reaction of chlorogenoquinone with PVX and spectra of the product

When PVX was present during the oxidation at pH 7 of small amounts of chlorogenic acid added successively, the reaction mixture was initially redder than controls, and finally bluer. Similar colour changes occur when chlorogenoquinone reacts with amino acids (Pierpoint, 1969*a*). Virus pellets centrifuged out of the mixtures at 50000*g* were bluish-brown or bluish-green, and this colour was not removed when the pellets were resuspended and then resedimented from large volumes of buffer. By contrast, previously oxidized chlorogenic acid which was added to PVX was readily removed by this treatment leaving colourless virus pellets. The coloured pellets, however, were often difficult to handle; after being firmly packed by sedimentation many of them would not resuspend, and electron micrographs showed the virus particles to be aggregated randomly instead of into the more familiar 'ropes'. More amenable preparations were obtained by separating quinone-treated PVX (PVX-Q) from the reaction mixtures by chromatography.

PVX can be satisfactorily separated from added oxidized chlorogenic acid on Sepharose 2B: the virus emerges in the break-through volume of the column with a u.v. spectrum identical to that of untreated PVX, and the phenol is eluted near the bed volume. PVX-Q also emerges in the break-through volume of the column but its spectrum is modified (Fig. 1).

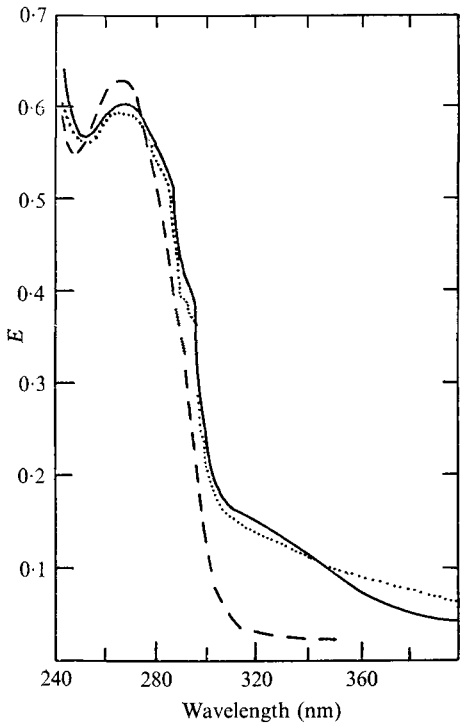

Fig. 1 Absorption spectrum of chlorogenoquinone-treated PVX (PVX-Q). PVX-Q was separated chromatographically from the reaction mixture in phosphate buffer at pH 7·0 (——), and its pH adjusted to about 9·0 with dilute NaOH (.....). PVX (– –) was separated chromatographically from previously oxidized chlorogenic acid.

Table 1. *Estimates of the fraction of E_{265} which is due to bound quinone in PVX-Q*

	PVX			PVX-Q			
Virus preparation	$E_{265}^{1\,cm}$	P (μg/ml)	P content (%)	$E_{265}^{1\,cm}$	P (μg/ml)	$E_{265}^{1\,cm}$ expected for virus component	$E_{265}^{1\,cm}$ due to quinone component (%)
9	1·55	2·56	0·59	1·65	2·35	1·42	14
	4·1	6·85	0·58	6·9	10·7	6·4	7
10	27·5	43·0	0·55	31·4	43·3	27·6	12
	4·9	5·85	0·42	1·16	1·41	1·18	0

Suspensions of PVX and PVX-Q, of similar $E_{265}^{1\,cm}$, were precipitated with TCA and the P of the precipitates estimated after digestion. The extinction expected from the virus component of PVX-Q was estimated from the ratio $\frac{\text{P of PVX-Q } (\mu\text{g/ml})}{\text{P of PVX } (\mu\text{g/ml})} \times E_{265}^{1\,cm}$ of PVX. Comparing these values (column 6) with the observed absorbance of PVX-Q (column 4) gave an estimate (last column) of the absorbance due to the quinone component of PVX-Q.

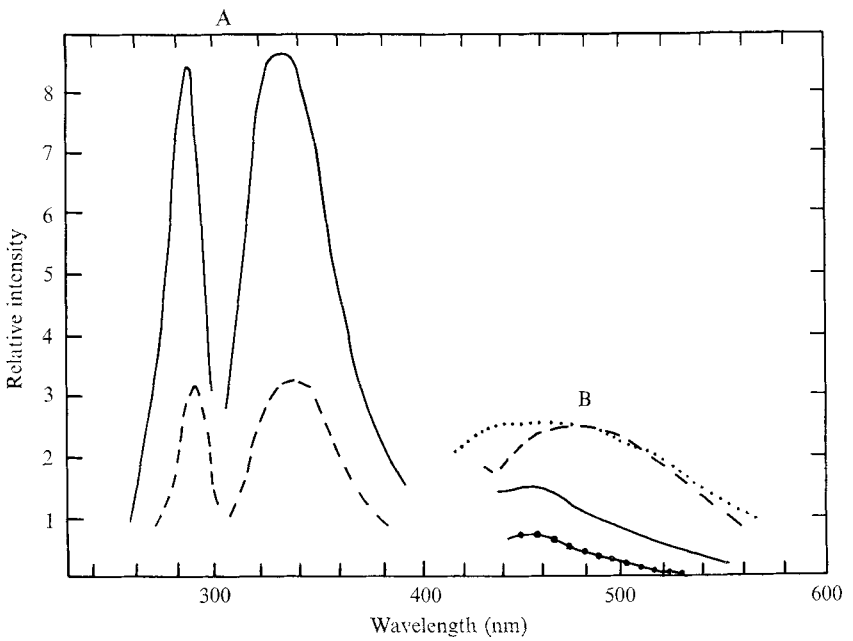

Fig. 2. Fluorescence spectra of PVX (——) and PVX-Q (– –) solutions whose E_{265} were 1·08 and 1·42 respectively. (A) Excitation spectra measured by emission at 335 nm, and fluorescence spectra excited by 295 nm. (B) Fluorescence of the same solutions as well as that of phosphate buffer, 10 mM, pH 7·0, excited by light of wavelength 380 nm. For comparison the fluorescence of oxidized chlorogenic acid excited at 340 nm (.....) is shown. Instrument slits are wider for B than for A (see Methods).

It has a shoulder at 300 to 320 nm which may absorb up to a quarter (typically about 20 %) as much as the main peak, and which is diminished, and shifted to longer wavelengths by NaOH. The minimum near 250 nm is also less marked. These spectral changes resemble those of albumin which has reacted with chlorogenoquinone (Pierpoint, 1969*b*).

Chlorogenic acid absorbs about a quarter as much at 265 nm as it does at 320 nm, and oxidized chlorogenic acid absorbs equally at both wavelengths. Some of the absorption of the PVX-Q at 265 nm may therefore be due to bound phenolic material. As E_{265} is used to estimate the concentration of PVX and PVX-Q when their properties are compared, an attempt was made to estimate this absorption by comparing the spectra of preparations containing equal amounts of P. The results (Table 1) were variable: this is probably because the absorption measurements were not corrected for light-scatter, and the TCA extracts some virus P (Pirie, 1958). Nevertheless they suggest that, by assuming PVX-Q to have the same E_{265} as PVX, the PVX-Q concentration is probably overestimated by about 10 %.

The fluorescence spectrum of PVX (Fig. 2A) is like that of other tryptophan-containing proteins (Teale, 1960) and, like that of TMV (Shore & Pardee, 1956), it is probably uninfluenced by the presence of nucleic acid. As both chlorogenic and, to a lesser degree, oxidized chlorogenic acid fluoresce, the spectrum of six preparations of PVX-Q were examined. The results in Fig. 2A are typical: the tryptophan-excitation and emission curves are not altered in shape or wavelength, but their intensity is decreased to 30 to 40 % of that of a PVX solution with the same u.v. absorption at 265 nm. The relative decrease is independent of exciting wavelength and the sensitivity settings on the fluorimeter; it is probably a little exaggerated in Fig. 2 because the higher E_{265} of the PVX-Q solution produces a larger

Table 2. *Reaction of PVX-Q with TNBS*

Virus preparation	Amount ($E^{1\,cm}_{2\,65}$ × sample vol.)	Colour with TNBS E_{430}	Colour / Amount virus
PVX	1·53	0·72	0·47
	0·76	0·32	0·42
PVX mixed with oxidized chlorogenic acid and re-isolated	1·03	0·57	0·55
	0·41	0·23	0·55
PVX-Q	1·64	0·51	0·31
	0·82	0·26	0·31

PVX, PVX which had been mixed with oxidized chlorogenic acid and separated by chromatography, and PVX-Q, were incubated in 3·5 ml phosphate buffer (0·12 M; pH 7·0) containing TNBS (2·0 mg) at 40 °C. E_{430} was measured until a stable value was attained (3 h).

'inner filter' effect. PVX which had been mixed with oxidized chlorogenic acid and then reseparated showned no such decrease.

In addition to this protein fluorescence, a broad peak of fluorescence excited by longer wavelengths (380 nm) was detected in all the PVX-Q preparations (Fig. 2B). It was much less than the tryptophan fluorescence and only detected when the optical slits on the fluorimeter were open wide and bandwidths were consequently large. In these conditions, there was often (e.g. Fig. 2B) detectable background fluorescence from buffer alone and from PVX which had been recovered after mixing with oxidized chlorogenic acid. This fluorescence of PVX-Q is not appreciably increased by adding sodium borohydride, nor, like that of chlorogenic acid, by adding alkali. It does, however, resemble a fluorescence that can be observed in fractions of the brown oxidized chlorogenic acid eluted from Sepharose (Fig. 2B).

These changes in the colour and spectra of PVX were not brought about by the *o*-diphenol oxidase preparation in the absence of chlorogenic acid, neither were they induced by long incubations (2·5 h at 25 °C) of PVX with enzyme + phenol mixtures in which the oxidation had gone to completion. They suggest therefore that PVX combines chemically with chlorogenoquinone. If this is so, PVX-Q should, by analogy with quinone-treated serum albumin (Pierpoint, 1969*b*), have fewer free amino groups than PVX, and a different surface charge. These properties of PVX-Q were therefore examined.

Reaction of PVX and PVX-Q with 2,4,6-trinitrobenzene sulphonic acid (TNBS)

The accessible $-NH_2$ groups of PVX and PVX-Q can be estimated by reaction with TNBS; the amounts of nucleic acid present are unlikely to produce any colour (unpublished experiments), and this strain of PVX contains no –SH groups that can be readily detected with Ellman's reagent. The results in Table 2 show that PVX-Q gave 55 % of the colour given by the same amount of PVX which had been mixed with oxidized chlorogenic acid and then re-isolated. However, the conclusion that PVX-Q has about half the available amino groups of PVX ought to be treated cautiously. Untreated PVX consistently gave a little less colour than PVX that had been separated from the reaction mixture (Table 2): moreover, in view of unpublished experience with TMV, it seems unlikely that all the free amino groups of PVX and PVX-Q react with TNBS in these mild conditions.

Table 3. *Electrophoretic mobilities of PVX and PVX-Q*

Virus preparation	Virus concentration (approximate E_{265})	Mobility (μ/sec/V/cm) PVX	PVX-Q	Mobility PVX-Q/PVX
6	3·0	−0·15	—	—
7	3·3	−0·18	−0·31	1·7
9	3·0	−0·12	−0·30	2·5
10	8·2	−0·097	−0·20	2·1

In each experiment PVX-Q was compared with PVX which had been exposed to already-oxidized chlorogenic acid. Solutions containing approximately equal amounts of virus were dialysed and analysed electrophoretically in the same batch of phosphate buffer (67 mM; pH 7·0). Mobilities were calculated assuming the potential gradient to be 9·5 V/cm.

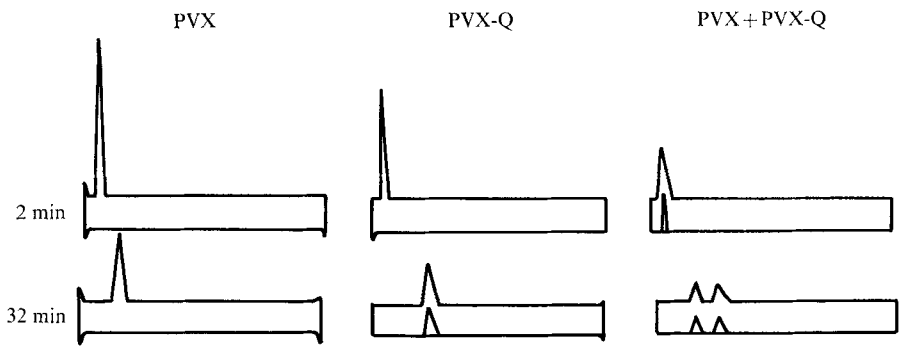

Fig. 3. Electrophoresis of PVX, PVX-Q and a mixture (1·5:2) of PVX+PVX-Q. The three solutions had an E_{265} of 2·7, and were dialysed and analysed in the same sample of phosphate buffer (pH 7·0; 67 mM). Peaks produced by the ascending (left to right) boundary were photographed after 2 and 32 min.

Electrophoresis of PVX and PVX-Q

PVX which had been mixed with oxidized chlorogenic acid and re-isolated gave a single boundary peak when examined electrophoretically in phosphate buffer (67 mM; pH 7·0). It migrated towards the anode and remained sharp for at least 90 min, usually much longer. Its mobility varied from one preparation to another (Table 3), but was 2 to 3 times greater than that of the strain of PVX examined by Bawden & Kleczkowski (1959) in these conditions. Only a little of the variability is thought to be due to differences in experimental technique, and most of it may reflect differences between the PVX preparations: the least mobile preparation (Table 3; preparation 10) had been re-isolated from oxidized chlorogenic acid by centrifuging, rather than chromatography.

PVX-Q preparations also gave a single boundary peak, but it migrated towards the anode about twice as fast as did the control preparation of PVX (Table 3). They showed no trace of residual PVX. Electrophoresis of mixtures of PVX+PVX-Q readily resolved the two components (Fig. 3), each migrating with its characteristic mobility; the presence of 17 % PVX could readily be detected in mixtures made from preparation 9 (Table 3), and about half this amount in the more concentrated preparation 10.

Table 4. *Infectivity in PVX-Q preparations*

Preparation	Concentration (μg/ml)	Lesions (average/leaf) PVX	Lesions (average/leaf) PVX-Q	PVX-Q infectivity (% of PVX)
5	5·0	—	49	—
	1·0	26	19	65
	0·2	6·7	8·0	125
7	1·0	57	72	155
	0·2	27	36	180
9	0·5	37 (102)	29 (42)	75 (35)
	0·1	8·3 (28)	7·6 (20)	90 (70)
10	0·5	66 (38)	36 (21)	40 (40)
	0·1	26 (14)	14 (9)	45 (50)

PVX-Q preparations, and PVX that had been mixed with oxidized chlorogenic acid and re-isolated, were diluted in borate buffer (10 mM; pH 7·0) and assayed for local lesion production on *Chenopodium amaranticolor*. The concentration of both preparations was estimated from E_{265} assuming $E^{0.1\%}_{1\,cm}$ to be 3·5. Rough infectivity–dilution curves for PVX were drawn, and the infectivity of each dilution of PVX-Q (last column) judged from its position on this curve. Results of repeated assays are given in parentheses.

Infectivity of PVX-Q

The infectivity of PVX was not destroyed during the course of its exposure to oxidizing chlorogenic acid. Thus, when about 12 mg PVX was incubated with 36 μmoles of chlorogenic acid and the brown reaction mixture diluted with borate buffer (10 mM; pH 7·0), dilutions containing 1·0 and 0·2 μg virus/ml produced an average of 114 and 36 lesions/leaf on *Chenopodium amaranticolor*. A control solution of PVX to which previously oxidized chlorogenic acid was added just before dilution gave virtually identical numbers of lesions (112 and 35). Infectivity assays on four preparations of PVX-Q after they had been isolated from the reaction mixtures are summarized, and compared with appropriate controls in Table 4. In spite of the variability expected in this type of assay, it is clear that the PVX-Q preparations are highly infective, and probably as much so as the parent PVX preparations. The one preparation (prep. 10; Table 4) that was consistently less infective than the control in two assays had been re-isolated by centrifuging rather than chromatography, and once pelleted, resuspended less readily than did PVX. Sedimentation apparently increased the amount of PVX inactivated by tetrachloro-*o*-benzoquinone (Mink & Saksena, 1971).

The infectivity of PVX-Q (prep. 5) was also confirmed qualitatively on Xanthi tobacco plants. There was little or no difference in the number of lesions, their time of development or their ringspot appearance between leaves inoculated with equivalent solutions of PVX and PVX-Q.

Sedimentation and serology of PVX-Q

Examination in an analytical ultracentrifuge failed to show any appreciable difference between PVX and PVX-Q. Both showed a main peak which sedimented in phosphate buffer (10 mM; pH 7·0) with an $s_{20,w}$ value of 126 to 130 S, and a minor peak whose slightly larger $s_{20,w}$ value (approximately 15 % more) suggested that it was due to rods aggregated in pairs. Similarly, there was little or no difference in serological properties of the two virus forms: at equal concentrations, as judged by E_{265}, PVX-Q precipitated a PVX-antiserum from rabbits as effectively as did PVX.

DISCUSSION

When PVX is re-isolated after exposure to chlorogenic acid which is being oxidized by a crude preparation of *o*-diphenol oxidase, some of its properties are changed. Its colour and u.v. spectrum are modified; its typtophan fluorescence is decreased, and a small longer-wave fluorescence introduced; its readily available $-NH_2$ is decreased and its negative surface charge is increased. These changes are not likely to be the consequence of physical absorption of oxidized phenolic material, nor are they the effect of the enzyme preparation acting directly on the virus. This last point needs emphasis, because a plant enzyme removes a large polypeptide of about 4000 from the protein subunits in PVX (Koenig *et al.* 1970) and could conceivably produce some of these changes. Additional proof that this did not happen in the present experiments is the demonstration by Dr J. Carpenter (personal communication) that the protein subunits of PVX-Q preparations are in the high mol. wt. form. The most likely explanation of the changes therefore is that PVX reacts with an intermediate formed during the oxidation of chlorogenic acid.

Two such likely intermediates are an *o*-quinone and a semi-quinone. Both have been identified in the oxidation of catechol by *o*-diphenol oxidase, but it has been argued that the quinone is the primary product and the semi-quinone is produced secondarily by dismutation (Mason, Spencer & Yamazaki, 1961). The proportion of semi-quinone produced and its effect on the subsequent polymerization probably increase in more concentrated solutions (Forsyth & Quesnel, 1957). An *o*-quinone, chlorogenoquinone, is produced during the oxidation of chlorogenic acid and can be trapped with benzene sulphinic acid (Pierpoint, 1966). In the absence of any evidence for the formation of semi-quinone during the oxidation, the effects of oxidizing chlorogenic acid on amino acids and proteins were interpreted as reactions of chlorogenoquinone (Pierpoint, 1966, 1969*a*, *b*). It is proposed to regard the modification of PVX by oxidizing chlorogenic acid in the same way.

Previous experience with serum albumin (Pierpoint, 1969*b*) suggests that the parts of a protein likely to react with enzymically generated chlorogenoquinone are thiols, α-amino and lysine ϵ-amino groups. No evidence was found for a reaction with the S of methionine, in spite of the recent demonstration that methionine S reacts with *o*-benzoquinone in acid solution (Vithayathil & Murthy, 1972). Preliminary analysis of the present strain of PVX (Dr J. Carpenter, unpublished result) suggest that it has about 11 lysine residues/subunit, but that its amino terminus is not readily detected and could well be acetylated, as are those of other strains of PVX (Miki & Knight, 1968). Preliminary experiments with Ellman's reagent have also failed to detect any readily available –SH, even though this reagent does so in other PVX strains (Reichmann & Hatt, 1961). This needs confirmation because the thiol group of serum albumin (Pierpoint, 1969*b*) and cowpea chlorotic mottle virus (Bancroft *et al.* 1971) react with enzymically generated quinones. It would therefore be premature to conclude that the reaction of PVX with chlorogenoquinone involves only its lysine groups. But whatever the details of the reaction, it clearly does not affect the *in vivo* removal of the virus protein coat; PVX-Q preparations retain a high degree of infectivity (Table 4) which cannot be accounted for by residual PVX (Fig. 3).

Although the conditions under which PVX combines with chlorogenoquinone were chosen so as to encourage the reaction, they are such as to suggest that the reaction occurs under less deliberate circumstances. It may, for instance, have occurred in the concentrated leaf saps from which early preparations of PVX were made (Bawden & Pirie, 1936) and although most of the PVX-Q would probably have been preferentially removed because of its reluctance to resuspend after centrifugal pelleting, it may have contributed to the colour

and handling difficulties of the final preparations. PVX-Q may indeed be one of the minor components observed by Bawden & Kleczkowski (1957) in their electrophoretic analysis of ammonium sulphate precipitates of extracts of PVX-infected leaves. It is also possible that PVX-Q is formed 'naturally' in necrosing and senescing leaves of infected plants: if so it would be interesting to know if any of its properties are so different from those of PVX as to affect the survival of the virus in the field and its transfer to new hosts.

The infectivity of tobacco mosaic virus (TMV) is unaffected by oxidizing phenols (Harrison & Pierpoint, 1963; Saksena & Mink, 1970). However, in many unreported experiments similar to those described for PVX, there was no detectable change in the spectra, the reaction with TNBS or the electrophoretic mobility of TMV after exposure to chlorogenoquinone. This was also true for the U_2 strain of TMV whose unacetylated amino terminus is proline, a group which in protamines (Mason & Peterson, 1955) and peptides (Pierpoint, 1969*a*) reacts readily with *o*-quinones. Moreover, native, but disaggregated, TMV protein, whose lysine $-NH_2$ groups are less unreactive than those in intact virus (Perham & Richards, 1968; Scheele & Lauffer, 1969), also did not react with chlorogenoquinone as judged by its ability to reaggregate and by the spectra of the polymer. The resistance of TMV to chlorogenoquinone is probably due, therefore, to the unavailability of its amino and thiol groups and contrasts with the tolerance, rather than resistance, of PVX.

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REFERENCES

BANCROFT, J. B., MCLEAN, G. D., REES, M. W. & SHORT, M. N. (1971). The effect of an arginyl to a cysteinyl replacement on the uncoating behaviour of a spherical plant virus. *Virology* **45**, 707–715.

BARNETT, O. W. & FULTON, R. W. (1971). Differential response of prunus necrotic ringspot and tulare apple mosaic virus to stabilizing agents. *Virology* **46**, 613–619.

BAWDEN, F. C. & KLECZKOWSKI, A. (1957). An electrophoretic study of sap from uninfected and virus-infected tobacco plants. *Virology* **4**, 26–40.

BAWDEN, F. C. & KLECZKOWSKI, A. (1959). Some properties of decomposition products of potato virus X. *Virology* **7**, 375–384.

BAWDEN, F. C. & PIRIE, N. W. (1936). Experiments on the chemical behaviour of potato virus X. *British Journal of Experimental Pathology* **17**, 64–74.

FORSYTH, W. G. C. & QUESNEL, V. C. (1957). Intermediates in the enzymic oxidation of catechol. *Biochimica et biophysica acta* **25**, 155–160.

HAMPTON, R. E. & FULTON, R. W. (1961). The relation of polyphenoloxidase to instability *in vitro* of prune dwarf and sour cherry necrotic ringsport viruses. *Virology* **13**, 44–52.

HARRISON, B. D. & PIERPOINT, W. S. (1963). The relation of polyphenoloxidase in leaf extracts to the instability of cucumber mosaic and other plant viruses. *Journal of General Microbiology* **32**, 417–427.

HOLDEN, M. & PIRIE, N. W. (1955). A comparison of leaf and pancreatic ribonuclease. *Biochemical Journal* **60**, 53–62.

KOENIG, R., STEGEMANN, H., FRANCKSEN, H. & PAUL, H. L. (1970). Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. *Biochimica et biophysica acta* **207**, 184–189.

MASON, H. S. & PETERSON, E. W. (1955). The reaction of quinones with protamine and nucleoprotamine: *N*-terminal proline. *Journal of Biological Chemistry* **212**, 485–493.

MASON, H. S., SPENCER, E. & YAMAZAKI, I. (1961). Identification by electron spin resonance spectroscopy of the primary product of tyrosinase-catalyzed catechol oxidation. *Biochemical and Biophysical Research Communications* **4**, 236–238.

MIKI, I. & KNIGHT, C. A. (1968). The protein subunit of potato virus X. *Virology* **36**, 168–173.

MINK, G. I. (1965). Inactivation of tulare apple mosaic virus by *o*-quinones. *Virology* **26**, 700–707.

MINK, G. I. & SAKSENA, K. N. (1971). Studies on the mechanism of oxidative inactivation of plant viruses by *o*-quinones. *Virology* **45**, 755–763.

PERHAM, R. N. & RICHARDS, F. M. (1968). Reactivity and structural role of protein amino groups in tobacco mosaic virus. *Journal of Molecular Biology* **33**, 795–807.

PIERPOINT, W. S. (1966). The enzymic oxidation of chlorogenic acid and some reactions of the quinone produced. *Biochemical Journal* **98**, 567–580.

PIERPOINT, W. S. (1969*a*). *o*-Quinones formed in plant extracts. Their reactions with amino acids and peptides. *Biochemical Journal* **112**, 609–618.

PIERPOINT, W. S. (1969*b*). *o*-Quinones formed in plant extracts. Their reaction with bovine serum albumin. *Biochemical Journal* **112**, 619–629.

PIERPOINT, W. S. (1971). Formation and behaviour of *o*-quinones in some processes of agricultural importance. *Rothamsted Experimental Station Report for* 1970, part **2**, 199–218.

PIRIE, N. W. (1958). The properties of fragments of tobacco mosaic virus. *Proceedings of the 4th International Congress of Biochemistry Symposium* No. **7**, 45–57.

REICHMANN, M. E. & HATT, D. L. (1961). The effect of the substitution of sulfhydryl groups on the macromolecular structure of potato virus X. *Biochimica et biophysica acta* **49**, 153–159.

SAKSENA, K. N. & MINK, G. I. (1970). The effects of oxidized phenolic compounds on the infectivity of four 'stable' viruses. *Virology* **40**, 540–546.

SCHEELE, R. B. & LAUFFER, M. A. (1969). Restricted reactivity of the epsilon-amino groups of tobacco mosaic virus protein toward trinitrobenzenesulfonic acid. *Biochemistry* **8**, 3597–3603.

SHORE, V. G. & PARDEE, A. B. (1956). Energy transfer in conjugated proteins and nucleic acids. *Archives of Biochemistry and Biophysics* **62**, 355–368.

TEALE, F. W. J. (1960). The ultraviolet fluorescence of proteins in neutral solution. *Biochemical Journal* **76**, 381–388.

VITHAYATHIL, P. J. & MURTHY, G. S. (1972). New reaction of *o*-benzoquinone at the thioether group of methionine. *Nature, London* **236**, 101–103.

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