Martin, J. R., Perun, T. J., and Girolami, R. L. (1966), *Biochemistry 5*, 2852.
Nelson, N. (1944), *J. Biol. Chem. 153*, 375.
Sicher, J. (1962), *Progr. Stereochem. 3*, 202.
Sugihara, J. M., and Bowman, C. M. (1958), *J. Am. Chem. Soc. 80*, 2443.
Tardrew, P. L., and Nyman, M. A. (1964), U. S. Patent 3,127,315.
Vanek, Z., Puza, M., Major, J., and Dolezilova, L. (1961), *Folia Microbiol. 6*, 408.
Wawszkiewicz, E. J., and Lynen, F. (1964), *Biochem. Z. 340*, 213.
Wiley, P. F., Gerzon, K., Flynn, E. H., Sigal, M. V., Jr., Weaver, O., Quarck, U. C., Chauvette, R. R., and Monahan, R. (1957), *J. Am. Chem. Soc. 79*, 6062.
Woodward, R. B. (1957), *Angew. Chem. 69*, 50.

Studies on the Interaction of *p*-Mercuribenzoate with Turnip Yellow Mosaic Virus. IV. Conformational Change, Exposure of Buried Prototropic Groups, and pH-Induced Degradation*

J. M. Kaper and F. G. Jenifer

ABSTRACT: The unstable derivative of turnip yellow mosaic virus (TYMV), resulting from its reaction with *p*-mercuribenzoate (PMB) at pH 4.6, disintegrates in neutral media of finite ionic strength. This reaction was investigated by means of potentiometric and ultracentrifugal methods. Upon mixing the mercurial-TYMV derivative with KCl, an immediate exchange of protons for K^+ ions took place, causing a relatively large acid pH shift. Approximately 3600 protons were released per particle under the experimental conditions. No exchange occurred with untreated TYMV.

This indicated that the incorporation of PMB into the virus structure, *via* the protein sulfhydryl groups, had induced a conformational change sufficient to expose previously buried prototropic groups of the protein, or the ribonucleic acid (RNA), or both. Subsequent upscale titration of PMB-substituted TYMV caused the majority of the virus particles to collapse in the relatively narrow range of pH 6–7. The potentiometric titration curves of PMB-substituted TYMV and untreated TYMV allowed for the construction of a difference titration curve in the pH range above 3.8; this represented the titration properties of the newly exposed prototropic groups. Since the removal of PMB with mercaptoethanol restabilized most of the virus preparation, and also restored its titration properties, these phenomena appeared to be interrelated, justifying attempts to correlate the titration curves and the degradation curves. The protein capsids of TYMV did not retain their structural integrity upon reaction with PMB at pH 4.6, indicating an involvement of the RNA in stabilizing TYMV's structure. Such a possible involvement is discussed with respect to the correlation between titration and degradation properties of PMB-substituted TYMV.

Chemical studies of biologically significant proteins have frequently established a link between enzymatic properties and the reactivity of protein sulfhydryl groups. A number of excellent review articles have been written on this subject (Boyer, 1959; Cecil and McPhee, 1959; Cecil, 1963). In recent years, more and more reports have appeared in which sulfhydryl groups were assigned a strategic role in maintaining conformational stability of proteins. This provided another, though less direct, link between sulfhydryl groups and biological activity. Such a spatially more distant involvement of SH in the enyzmatic activity is reminiscent of an allosteric site (Gerhart and Schachman, 1965).

Among the pioneer studies which concerned the relationship of sulfhydryl reactivity, protein conformation, and enzymatic activity were those of Madsen and Cori (1956), Madsen (1956), and Madsen and Gurd (1956). They found a reversible inactivation of muscle phosphorylase as a result of its reaction with the mercurial *p*-mercuribenzoate (PMB),[1] which

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[1] Abbreviations used: TYMV, turnip yellow mosaic virus; ATC, artificial top component (empty protein shells or capsids); PMB, *p*-mercuribenzoate; ME, mercaptoethanol.

was accompanied by a dissociation of the enzyme into distinct subunits. More recently, studies have been conducted with liver alcohol dehydrogenase (Li *et al.*, 1962), dihydrofolate reductase (Kaufman, 1964; Perkins and Bertino, 1965), and phosphotransferase (Kress *et al.*, 1966). While there are other reports of cases where protein conformation is influenced as a result of mercurial–sulfhydryl group interaction, the above publications were singled out because they appear to represent examples in which mercurial-induced changes of biological activity could be associated with changes of conformation more subtle than outright dissociation into subunits or denaturation with loss of native structure. In this sense the findings to be presented here are related to the above-mentioned studies.

In several preceding publications (Kaper and Houwing, 1962a,b; Kaper and Jenifer, 1965), we have been able to show that PMB, upon reaction with the sulfhydryl groups of TYMV, interferes profoundly with the physical stability of this virus. This has led us to believe that perhaps this mercurial could be used to probe certain structural features of TYMV. Therefore, the investigations described in the present report can be viewed as one of the directions we have taken in our attempts to establish the foundations of a farther-reaching inquiry into the nature of the inter- and intracomponent interactions which govern the structure of TYMV (see also alkaline degradation studies of TYMV (Kaper, 1960a,b; Kaper, 1964; Kaper and Halperin, 1965)). Experimental evidence will be given for the PMB-induced conformational change in the above-mentioned TYMV. This conformational change is expressed as an "unmasking" of prototropic groups of the viral protein and/or nucleic acid. Furthermore, it will be shown that the availability of these groups for subsequent titration apparently is responsible for the pH–ionic strength-induced degradation of the mercurial-virus derivative.

Experimental Section

Materials. The source of purified TYMV and of PMB were the same as described earlier (Kaper and Jenifer, 1965). ATC was prepared from purified TYMV by means of alkaline degradation (Kaper, 1960a,b, 1964). All other chemicals obtained from commercial sources were of analytical quality.

Apparatus. Ultraviolet spectra and preparative and analytical ultrifugation were done with the same instruments described before (Kaper and Jenifer, 1965). For potentiometric titrations and pH-Stat reactions, an all-glass thermostated reaction vessel of 5-ml maximal capacity was used (TTA 31 from Radiometer,[2] Copenhagen) in conjunction with the TTT1 automatic titrator and the SBR2-SBU1 titrigraph recorder from the same manufacturer. Water was pumped into the jacket of the reaction vessel from a constant-temperature bath.

Methods. PMB-substituted virus was prepared and its concentration estimated by means of the methods described previously (Kaper and Jenifer, 1965). The concentration of untreated TYMV was estimated from its ultraviolet absorption spectrum, using a specific extinction coefficient $E^{1\%}_{260}$ 86 (Kaper and Litjens, 1966).

Potentiometric titrations and pH-Stat reactions were all subjected to the following protocol. KCl solution (1 ml of 0.6 M) was introduced into the reaction vessel thermostated at 30°. The pH of this solution generally varied between 5.5 and 6.0. After temperature equilibration, a 1-ml solution of PMB-substituted TYMV or of TYMV was introduced. Both types of sample, prior to their titration, had undergone exhaustive dialysis *vs.* glass-distilled water. If used to obtain a difference-titration curve, the samples were dialyzed together with the same dialysis fluid. Concentration of the samples was estimated after dialysis and subsequently adjusted with dialysis fluid to a final value of 0.5%. The sample volume to be added to the KCl solution was 1.0 ml, thus providing an initial concentration of 0.25% of material in 0.3 M KCl. The introduction of PMB-substituted TYMV into the KCl solution caused a sharp drop of pH to a value around 3.8. This was attributed to an exchange of protons of the mercurial-virus derivative with K^+ ions from the environment. The pH assumed was the starting point of the titration. The absence of a similar acid pH shift upon mixing untreated TYMV and KCl necessitated preliminary acidification of the TYMV controls. This was performed manually with 0.1 N HCl, taking great care to reduce overshooting to a minimum. The use of a relatively concentrated HCl solution (0.1 N) was required to keep the dilution of the reaction mixture minimal (about 1%). Upscale titration was performed with a solution of 0.02 N KOH in 0.3 M KCl delivered from the titrigraph-driven syringe of 0.5 ml. In titrations to about pH 7, PMB-substituted TYMV and the virus control usually exhibited a differential KOH consumption of 0.075 ml, leading to a dilution error of, at the most, 3% which, moreover, was partly compensated for by 1% from the acidification procedure. A direct subtraction of the experimentally obtained titration curves to produce the difference curve, therefore, seemed warranted. All titrations were performed in a nitrogen atmosphere. pH-Stat reactions had a total duration of 8 min except in the time series (this included the time it took the pH-Stat to deliver titrant to the desired pH). Potentiometric titrations were performed using the highest speed settings available on the equipment. This resulted, on the average, in a 6-min duration of a titration covering the range of pH 3.8–10.5.

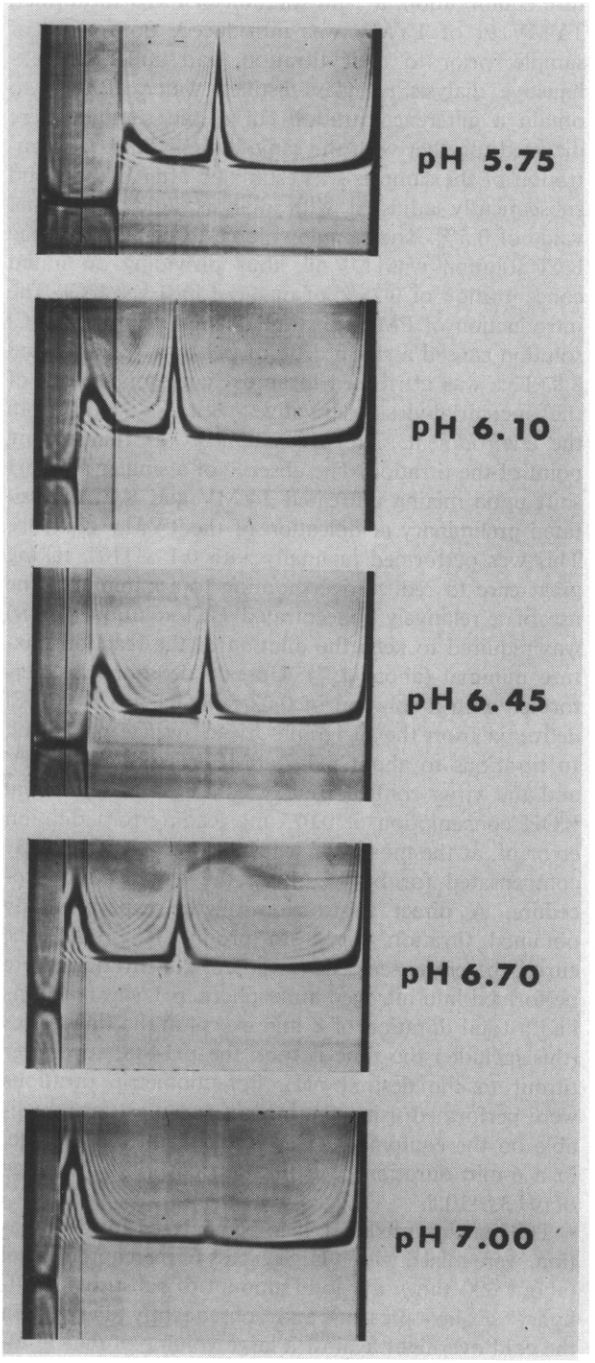
The reaction mixtures, if to be analyzed for degradation, were mixed with a large excess of mercaptoethanol (about 300 times the total amount of substituted sulfhydryl groups present) and subsequently dialyzed in the cold overnight against a large volume of mercapto-

[2] Mention of specific equipment, trade products, or a commercial company does not constitute its endorsement by the U. S. government over similar products or companies not named.

ethanol solution. This insured the removal of PMB from the protein (Kaper and Jenifer, 1965). Finally, the reaction mixtures were dialyzed against distilled water and diluted for ultracentrifugal analysis.

Ultracentrifugation was carried out using ultraviolet optics. Samples were in distilled water and possessed an OD_{260} of 1.0. This was regarded safer than analyzing the samples in the presence of salts because of some residual instability in mercaptoethanol-treated, PMB-substituted TYMV (see Results).

The extent of degradation that had occurred in the different treatments was estimated from densitometer tracings of the ultraviolet sedimentation patterns. Assuming equality of extinction coefficients of the nucleoprotein before and after the degradation, and allowing for a radial dilution correction, direct estimates could be made of the relative concentrations of undegraded virus.



Results

Instability of PMB-Substituted TYMV. The preparation of structurally intact PMB-substituted TYMV at pH 4.6 was described in a previous publication (Kaper and Jenifer, 1965). It was shown that incubation of this material for 1 hr at 30° in a 0.1 M phosphate buffer of pH 7 caused its virtually complete degradation. This could be attributed to either the change of pH from about 5.5 (approximate initial pH of a distilled water solution of PMB-substituted TYMV) to 7, or to the interaction of the mercurial-virus derivative with salt ions. To test the alternatives, a water-dialyzed solution of PMB-substituted TYMV was mixed with KCl to make the solution 0.3 M. The reaction mixture turned heavily opalescent and developed a precipitate. Simultaneously, a drop in pH was noticed from 5.5 to 3.8. The precipitate could easily be redissolved in distilled water. The ultraviolet spectrum of this solution was characteristic of a nucleoprotein with a depressed 260:240 mμ ratio due to PMB substitution. Only a small amount of nucleoprotein was found in the supernatant. The ultracentrifuge pattern of the redissolved precipitate exhibited the virus nucleoprotein peak plus a minor amount of degraded material. Thus, the PMB-substituted virus had been precipitated as a result of the presence of KCl and the concomittant acid shift in pH of the reaction mixture. The alternative possibility that neutral pH alone caused degradation of PMB-substituted TYMV was tested by neutralizing a water-dialyzed solution of this material with KOH. Degradation was observed in the ultracentrifuge under these conditions, although it was not quite as complete as with 0.1 M phosphate buffer of pH 7. However, if TYMV was neutralized to pH 7 in the presence of 0.3 M KCl, complete degradation was observed. It was concluded that the combined effect of a finite ionic strength and neutral pH was necessary to degrade PMB-substituted TYMV. Figure 1 illustrates these relationships.

FIGURE 1 (left): Degradation of PMB-substituted TYMV as demonstrated by the sedimentation characteristics after titration to different pH values in the pH-Stat. All titration experiments were of 8-min duration and were performed at 30° in the presence of 0.3 M KCl. Sedimentation from left to right. Concentration of samples approximately 0.225%. Temperature, 20°. Pictures taken at 40° Schlieren angle approximately 4 min after attaining speed of 33,450 rpm (picture representing pH 7.0 experiment was taken 8 min after reaching speed).

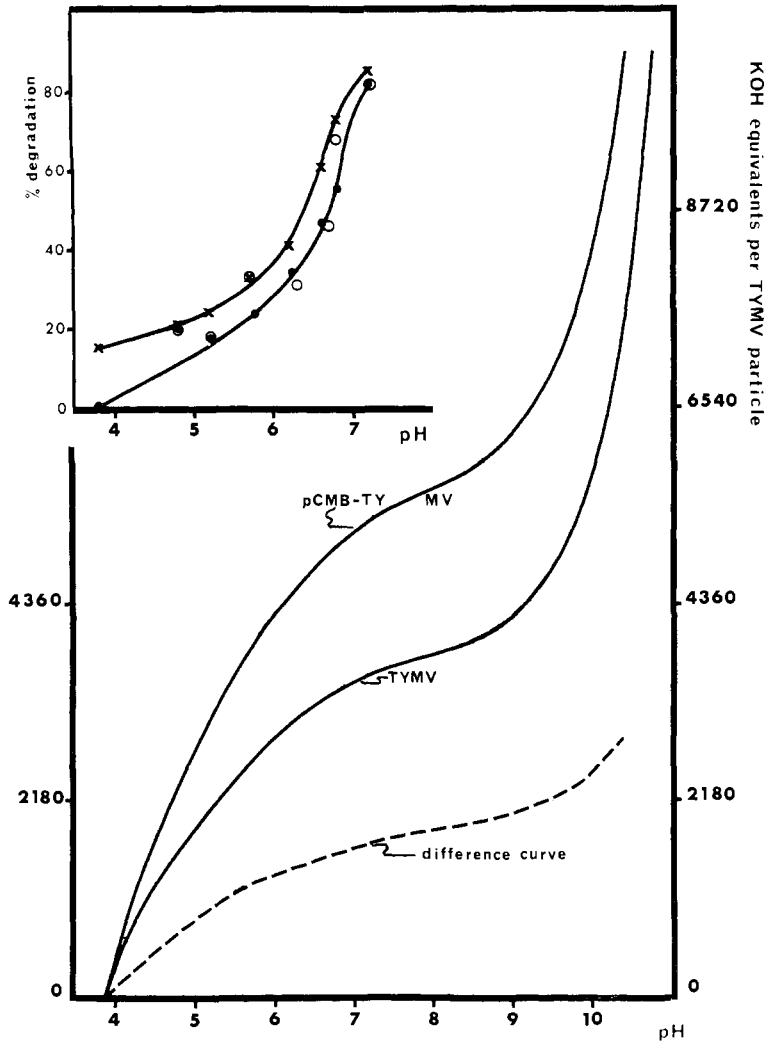


FIGURE 2: Potentiometric titration curves of PMB-substituted TYMV and of untreated TYMV plus their difference curve. (insert) Determination of the extent of degradation of PMB-substituted TYMV as a function of pH in pH-Stat experiments. Experimental points ●●, ○○, and xx represent three sets of experiments with different PMB-TYMV preparations. Titrations and pH-Stat experiments were in 0.3 M KCl at 30° with a 0.25% initial sample concentration.

Titration Properties of PMB-Substituted TYMV and Analysis of Degradation as a Function of pH. The relationship of degradation to pH within the range of pH 5.5–7.0 was studied in somewhat more detail. To this end pH-Stat reactions were performed in which PMB-substituted TYMV was titrated to a number of preset pH values after which the reaction mixtures were run in the ultracentrifuge. In Figure 1, a set of sedimentation patterns is presented showing that degradation takes place predominantly between pH 6 and 7. In Figure 2, representative titration curves for PMB-substituted TYMV and for virus control are given. The difference-titration curve of the two, which was obtained by direct subtraction of the latter from the former, is also shown. This procedure is justified because the two titrations were performed essentially identically. Out of some 3600 protons/particle liberated at pH 5.5, approximately 1150 were titrated in the range of pH 3.85–5.5. Such a calculation can be applied at any pH; and, as the difference curve shows, there is throughout the pH range investigated, a more or less continuous titration of protons in excess over the ones available on the untreated virus. In order to establish more precisely the pH region in which the increase in degradation of PMB-TYMV was maximal, pH-Stat experiments of 8-min duration were performed at different pH values. This length of treatment slightly exceeded that of the continuous titrations. However, alkali consumption values at the preset pH values were registered and matched those of the titration curves closely. This justified a direct correlation of the degradation data with the titration curves. The degradation data were obtained from ultracentrifugal analysis with ultraviolet optics (see Methods). The

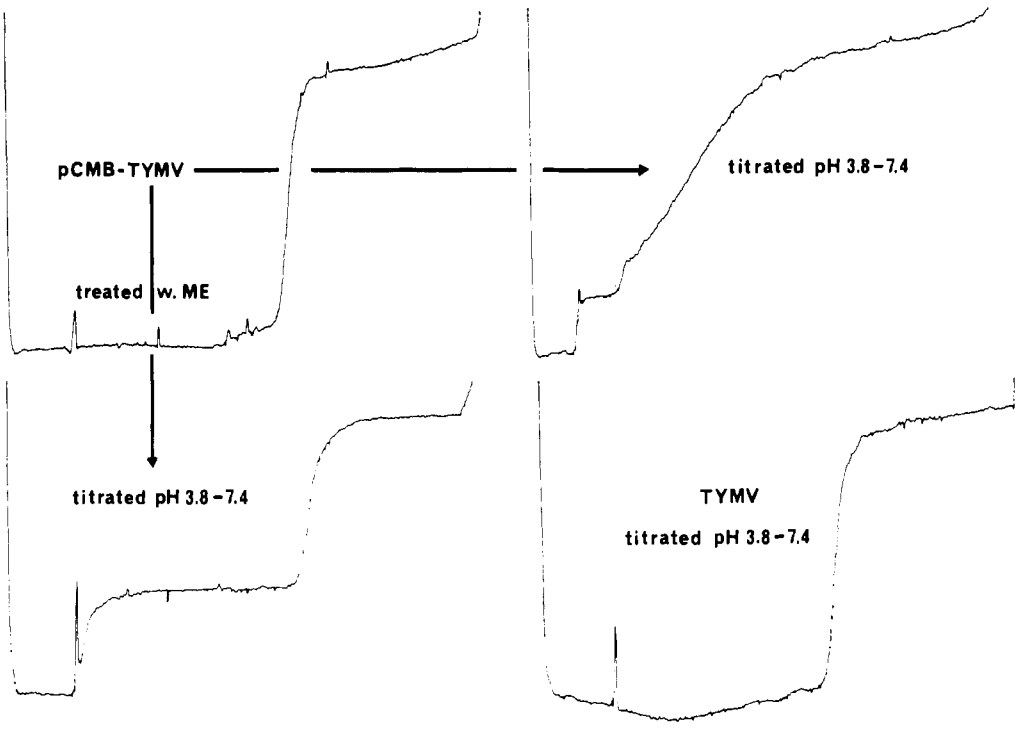


FIGURE 3: Densitometer tracings of ultraviolet sedimentation patterns of PMB-substituted TYMV and of TYMV after different treatments. Sedimentation from left to right. Pictures were taken at about 16 min after the centrifuge attained a speed of 29,500 rpm. (upper left tracing) PMB-substituted TYMV, untreated. (upper right tracing) PMB-substituted TYMV after titration to pH 7.4 in 0.3 M KCl. (lower left tracing) Same as in upper right tracing, but after initial removal of PMB with mercaptoethanol. (lower right tracing) TYMV acidified and titrated from pH 3.8 to 7.4 in 0.3 M KCl.

insert of Figure 2 shows the pH region of maximal degradation increment, which was obtained from three separate analyses. Two pH *vs.* degradation curves could be fitted through the experimental points. They resembled each other closely except for some scatter of points in the second experiment. In the third experiment, some degradation was already present in the sample prior to titration. Taking into account a certain degree of aspecific degradation prior to titration or at low pH, the pH region of maximal degradation increment for PMB-substituted TYMV seems to lie in the range of 6.5–7.0. To ascertain whether the degradation reaction followed the titration immediately, a series of reactions was performed in which PMB-substituted TYMV was titrated to pH 6.8 and kept at that pH for various lengths of time. Maximum degradation (60%) was noted in the first aliquot, which was taken immediately after titration. This demonstrated the practically instantaneous nature of the degradation.

Partial Restabilization of PMB-Substituted TYMV. Partial restabilization of the mercurial-substituted TYMV was accomplished through removal of PMB with mercaptoethanol. In Figure 3 are presented the sedimentation diagrams of PMB-substituted TYMV titrated to pH 7.4 prior to, and after removal of, the mercurial and of the appropriate controls; *i.e.*, PMB-substituted TYMV without titration as well as unsubstituted TYMV titrated from pH 3.8 to 7.4. Since the removal of PMB restored the stability of some 68% of the particles, the titration curves of this preparation and of its control were also examined. Mercaptoethanol-treated, PMB-substituted TYMV did not exhibit such acidity as the material which was not treated with mercaptoethanol, although a significant drop in pH was still noticed upon mixing the preparation with KCl. No precipitate was formed, however. Upscale titration was performed after initial acidification of sample and control to pH 3.85. The results of these titrations, as well as their difference curve, are given in Figure 4. It can readily be seen that removal of PMB also reversed the titration behavior of PMB-substituted TYMV toward that of the unsubstituted virus. It is likely that the apparent incompleteness of the restabilization and of the reversal of titration properties (Figures 3 and 4) are related to each other.

The Reaction of PMB with ATC at pH 4.6. ATC was submitted to standard reaction conditions normally applied to prepare PMB-substituted TYMV. In a matter of minutes, a turbidity started to develop which gained rapidly in intensity (see Kaper and Houwing, 1962a). After 1 hr of incubation at 30°, the precipitate was removed by low-speed centrifugation. The supernatant was devoid of proteinacous material, as tested by the addition of ammonium sulfate to 40% saturation. The

precipitate was washed extensively with distilled water in which it was insoluble. It was also insoluble in buffer solutions of neutral pH. Subsequent dialysis against buffers of increasing pH resulted in dissolution of the material in a 0.04 M K_2HPO_4–NaOH buffer of pH 11.0. Examination in the ultracentrifuge revealed that complete degradation had taken place, while it is known that ATC alone under such pH conditions would have retained its quaternary structure (Kaper, 1960a,b, 1964). Unfortunately, this is still no unequivocal evidence that the degradation already took place during the reaction at pH 4.6. However, since the reaction product was insoluble in water, while ATC, TYMV, as well as PMB-TYMV are easily soluble, it is very likely indeed that complete degradation (and denaturation) had taken place during the reaction with PMB.

Discussion

Three experimental facts of fundamental importance underlie any conclusions to be drawn from the current study. (1) A major portion of PMB-substituted TYMV remains structurally intact at lower pH values. (2) In contrast, the RNA-devoid capsid or ATC degrades upon reaction with PMB at pH 4.6. (3) PMB-substituted TYMV is unstable in ionic media of neutral pH. These observations suggest that the introduction of mercuribenzoate substituents has reduced the thermodynamical stability of the TYMV structure. A simple titration over a relatively narrow range of pH is now sufficient to make a different state of the nucleoprotein more favorable and cause degradation. They suggest, furthermore, that the RNA is essential to maintain the precarious balance in the structure after PMB has been introduced.

The manner in which mercurials can destabilize the TYMV structure is not understood as yet. Boyer (1959) proposed a blocking of transient conformational changes by means of mercurials, preventing a return to thermodynamically more favored conformations. The experimental data given by Godschalk and Veldstra (1965, 1966) for the interaction of aliphatic organic mercurials with TYMV can be blended into Boyer's hypothesis, if it is assumed that the presence of such apolar substituents interferes with the previously existing bonding patterns. We have recently extended this idea to explain the biological inactivation observed in PMB-substituted TYMV (Kaper and Jenifer, 1965). No further clarification can be offered at this point with respect to the destabilizing action of PMB. However, the experimental separation of the reaction of PMB with TYMV into two main stages: (a) destabilization induced by the presence of PMB on the virus particle, resulting in conformational changes; and (b) subsequent degradation of the mercurial-virus derivative as a result of the titration of previously "buried" prototropic groups should facilitate further studies on various aspects of TYMV's structure and on the mechanism of action of protein denaturation by PMB.

Direct experimental evidence for a conformational change as a result of PMB-substitution is given by the difference titration curve of the mercurial-virus derivative and that of TYMV itself (Figure 2). PMB-substituted TYMV exchanges a large number of its protons with K^+ ions as soon as it is introduced into KCl solution. These protons, which are responsible for the acid pH shift, must have been buried inside the virus structure before the introduction of the PMB substituents, as the untreated TYMV control does not exhibit an acid pH shift. An additional source of protons would be the newly introduced PMB-carboxyl groups (maximum 720), although they could never account for the total number of protons released per PMB-substituted virus particle, which is considerable (about 3600 at pH 5.5). Potential candidates for prototropic groups with pK's below 5.5 would be the aspartic acid and glutamic acid residue carboxyl groups, the amino substituents on the nucleic acid bases adenine, guanine, and cytosine, and the phosphoric acid residues of the nucleic acid. It was pointed out previously (Kaper and Jenifer, 1965) that since only partial charge compensation could be provided by basic amino groups of the protein and the reported polyamine (Johnson

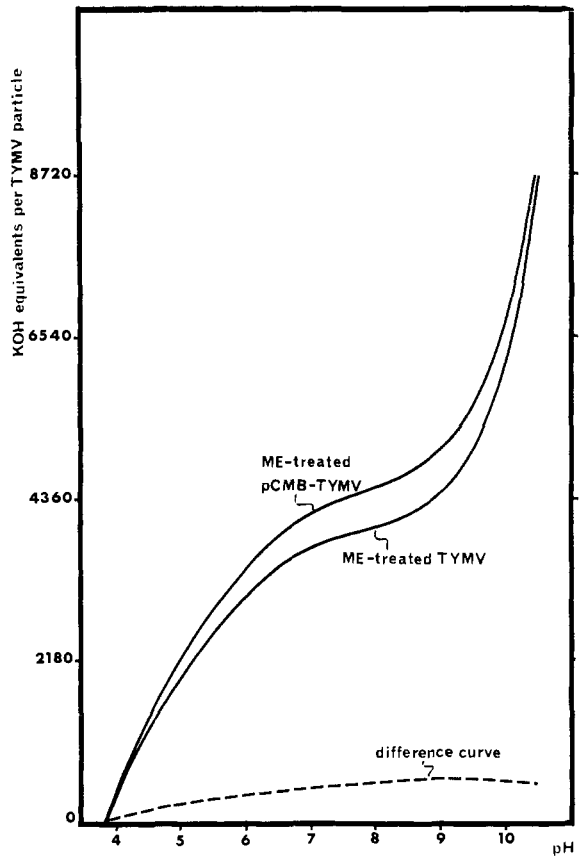


FIGURE 4: Potentiometric titration curves of PMB-substituted TYMV from which mercurial was removed with mercaptoethanol and of the corresponding TYMV control, plus their difference curve. Titrations were in 0.3 M KCl at 30° with an initial sample concentration of 0.25%.

and Markham, 1962), part of the nucleic acid could very well be located in the presumably hydrophobic interior of the capsid, a situation which would favor RNA–phosphoric acid residues in their undissociated state. Titrations covering the pH range below 3.8 have, however, not been made so far; therefore, no further speculations will be made regarding this point. The difference curve resulting from the titration of PMB-substituted TYMV and the TYMV control in the pH range above 3.8 shows a considerable amount of alkali uptake between pH 3.8 (starting point) and 5.5 for which some of the above-named groups are undoubtedly responsible. However, more interesting from a point of view of structural instability is the range of pH 6–7. Relatively little alkali is taken up, although a small, reproducible discontinuity can be noticed, which suggests the titration of a small number of groups between pH 6.5 and 7.0. How significant the titration of these groups is, in relation to the degradation observed in the same pH range, remains unknown at the present time. The rapidity of the degradation reaction, and the correlation of degradability and anomalous titration behavior (also after removal of PMB), suggest that the two phenomena are interrelated.

The questions which then remain are whether there exist certain interactions of electrostatic nature that maintain the precarious balance between structural intactness and complete collapse of the mercurial-virus derivative, and, if so, to what kinds of groups they can be attributed. Are these groups on the nucleic acid, on the protein, or on both? Is it a matter of loss of electrostatic attractions, or is it an augmentation of electrostatic repulsions, which finally causes the particle to collapse? The fact that structural collapse can be prevented at pH 4.6, only if the RNA is present, seems to indicate a role for the nucleic acid. It has been proposed earlier (Kaper and Jenifer, 1965) that dissociation of PMB and other carboxyl groups could conceivably cause collapse of the structure through their mutual repulsion at neutral pH. Due to the finding of an anomalous titration behavior of PMB-substituted TYMV, the spectrum of possibilities has been enlarged. Focusing on the protein in the pH 6–7 range, conceivably only histidinyl residues are converted from a protonated to an uncharged state. It is here that perhaps existing salt links with nucleic acid phosphate could be broken. As far as the titration of the nucleic acid is concerned, the following could be suggested. TYMV-RNA is known to have a disproportionately high content of cytosine (Symons *et al.*, 1963). It has also been known for some time that the homopolymer polycytidylic acid possesses a greatly ordered structure (double helix) between pH 3.0 and 5.7 (Hartman and Rich, 1965), while Mitra and Kaesberg (1965) have given some evidence for the probable existence of limited regions with polycytidylic acidlike stabilization in TYMV-RNA. In view of the fact that with TYMV structural regularities or periodicities of the RNA have now been shown to exist *in situ* (Kaper and Halperin, 1965; Klug *et al.*, 1966), it seems likely that these RNA regions would also possess considerable secondary structure resulting from intrachain interactions as well as from interactions with the protein. It is conceivable that polycytidylic acidlike regions are a contributing stabilizing factor in this complex system of inter- and intracomponent interactions. The titration of such regions above a pH of approximately 5.7 would unfold hydrogen bond stabilized double-helical structures according to Hartman and Rich (1965). If this should happen to TYMV-RNA *in situ*, the nucleic acid would not be restrained from expanding any more and contribute to a collapse of the whole structure. Several of the questions posed above are now under investigation in our laboratory.

References

Boyer, P. (1959), *Enzymes 1*, 511.

Cecil, R. (1963), *Proteins 1*, 379.

Cecil, R., and McPhee, J. R. (1959), *Advan. Protein Chem. 14*, 256.

Gerhart, J. C., and Schachman, H. K. (1965), *Biochemistry 4*, 1054.

Godschalk, W., and Veldstra, H. (1965), *Arch. Biochem. Biophys. 111*, 161.

Godschalk, W., and Veldstra, H. (1966), *Arch. Biochem. Biophys. 113*, 347.

Hartman, K. A., Jr., and Rich, A. (1965), *J. Am. Chem. Soc. 87*, 2033.

Johnson, M. W., and Markham, R. (1962), *Virology 25*, 508.

Kaper, J. M. (1960a), *Nature 186*, 219.

Kaper, J. M. (1960b), *J. Mol. Biol. 2*, 425.

Kaper, J. M. (1964), *Biochemistry 3*, 486.

Kaper, J. M., and Halperin, J. E. (1965), *Biochemistry 4*, 2434.

Kaper, J. M., and Houwing, C. (1962a), *Arch. Biochem. Biophys. 96*, 125.

Kaper, J. M., and Houwing, C. (1962b), *Arch. Biochem. Biophys. 97*, 449.

Kaper, J. M., and Jenifer, F. G. (1965), *Arch. Biochem. Biophys. 112*, 331.

Kaper, J. M., and Litjens, E. C. (1966), *Biochemistry 5*, 1612.

Kaufman, B. T. (1964), *J. Biol. Chem. 239*, PC669.

Klug, A., Longley, W., and Leberman, R. (1966), *J. Mol. Biol. 151*, 558.

Kress, L. F., Bono, V. A., Jr., and Noda, L. (1966), *J. Biol. Chem. 241*, 2293.

Li, T. K., Ulmer, D. D., and Vallee, B. L. (1962), *Biochemistry 1*, 114.

Madsen, N. B. (1956), *J. Biol. Chem. 223*, 1067.

Madsen, N. B., and Cori, C. F. (1956), *J. Biol. Chem. 223*, 1055.

Madsen, N. B., and Gurd, F. R. N. (1956), *J. Biol. Chem. 223*, 1075.

Mitra, S., and Kaesberg, P. (1965), *J. Mol. Biol. 14*, 558.

Perkins, J. P., and Bertino, J. R. (1965), *Biochemistry 4*, 847.

Symons, R. H., Rees, M. W., Short, M. N., and Markham, R. (1963), *J. Mol. Biol. 6*, 1.