Studies on the Interaction of Mercuric Ions with Tomato Bushy Stunt Virus*

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ABSTRACT: Tomato bushy stunt virus reacts with $HgCl_2$ without degradation of the protein shell. Upon addition of $HgCl_2$, the ultraviolet spectrum of tomato bushy stunt virus exhibits a shift toward longer wavelengths related to the molar ratio r_a, the number of moles of Hg(II) added per mole of phosphorus. The spectra display an isosbestic point for the range $0 < r_a < 1$ and another one for $r_a > 1$. The determinations of the number of moles of Hg(II) bound per mole of phosphorus (r_b) at pH 4.8 and 5.8 provide evidence that three kinds of complexes are formed: the first two tomato bushy stunt virus–Hg complexes are obtained in the range $0 < r_a < 1$ and are responsible for the binding of 0.5 mole of Hg(II) each, but the second complex does not exist at pH 4.8. For $r_a > 1$ there is a third complex. It has been concluded, from the stoichiometry of the mercury binding and the spectrophotometric changes of tomato bushy stunt virus in comparison to the reaction of $HgCl_2$ with tomato bushy stunt virus–ribonucleic acid, that Hg(II) is mainly bound to the tomato bushy stunt virus–ribonucleic acid inside the virus capsid. The sedimentation coefficient of tomato bushy stunt virus greatly increases upon addition of $HgCl_2$. We have found that the s value is a linear function of r_b for the first two complexes. It has been possible through theoretical considerations to assume that this variation is due to the increase of the molecular weight of virus by mercury binding, without configurational change. This point of view is in agreement with density and viscosity measurements.

With the formation of the third tomato bushy stunt virus–Hg complex, in the presence of an excess of Hg(II), the particles undergo a slight increase in size. The reaction of tomato bushy stunt virus with $HgCl_2$ can be reversed by adding complexing agents for Hg(II). Mercaptoethanol is more effective than KCN in reversing completely the reaction. The stability of tomato bushy stunt virus in regard to mercury binding to the ribonucleic acid has been discussed in relation to the arrangement of the ribonucleic acid inside the virus capsid. Some considerations are made about the probable substitution of SH groups by Hg(II) and the involvement of aspecific protein groups in the mercury binding to tomato bushy stunt virus.

In a previous publication, we have reported that neither PMB[1] nor mersalyl dissociates TBSV (Dorne and Hirth, 1968a) under conditions where several viruses such as TYMV (Kaper and Jenifer, 1968), PVX (Reichmann and Hatt, 1961), and poliovirus (Philipson, 1965) are disintegrated into subunits with liberation of RNA. However, mercurials cause a conformational change of TBSV to such an extent as to reduce the sedimentation coefficient to less than half the original value. While the degradation of TYMV has been attributed to the substitution of sulfhydryl groups by organic mercurials (Godschalk and Veldstra, 1965), it has been suggested that the structural change of TBSV is mainly due to interactions of mersalyl with RNA inside the virus capsid (Dorne and Hirth, 1968b).

In this paper it is shown by means of spectrophotometry and ultracentrifugation studies that mercuric ions react with TBSV. This reaction does not lead to a disintegration of the virus particles. The possible involvement of RNA and protein groups in the mercury binding affinity of virus is discussed. Some considerations are made about the stability and the structure of virus particles.

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PMB, *p*-mercuribenzoate; TBSV, tomato bushy stunt virus; TYMV, turnip yellow mosaic virus; PVX, potato X virus.

Materials and Methods

Materials. TBSV was isolated from infected *Datura stramonium* plants and purified according to a combination of Stanley's (1940) and Bawden and Pirie's (1938) methods. The sap of *Datura* is clarified by precipitation of plant proteins (at pH 4.8) in the cold. The virus is concentrated by precipitation with ammonium sulfate at 30% of saturation at room temperature and resuspended in 0.02 M Na acetate (pH 4.8); at this stage most of the impurities are removed by a low speed centrifugation. The purification is finished by differential centrifugation in the same buffer. The virus is finally resuspended in water and stored at 2°.

Analytical grade mercury chloride was used. The solutions are unstable and therefore freshly prepared for each experiment. ^{203}Hg was purchased from the Commissariat à l'Energie atomique (Paris) in the acetate form.

Spectrophotometry. Measurements of the ultraviolet spectra were performed at room temperature with a Beckman DK2A recording spectrophotometer, in quartz cells of path length 1.00 cm. For routine readings, a Zeiss PMQ II spectrophotometer was used. The specific absorption of TBSV was taken to be 5.0/mg per ml at 258 mμ (Bradish and Crawford, 1960). From the phosphorus content of TBSV of 1.58% (De Fremery and Knight, 1955), the extinction coefficient at 258 mμ calculated per mole of P was found to be equal to 9810. The amount of mercury added or bound to the virus is given as the number of moles of mercury per mole of phosphorus or nu-

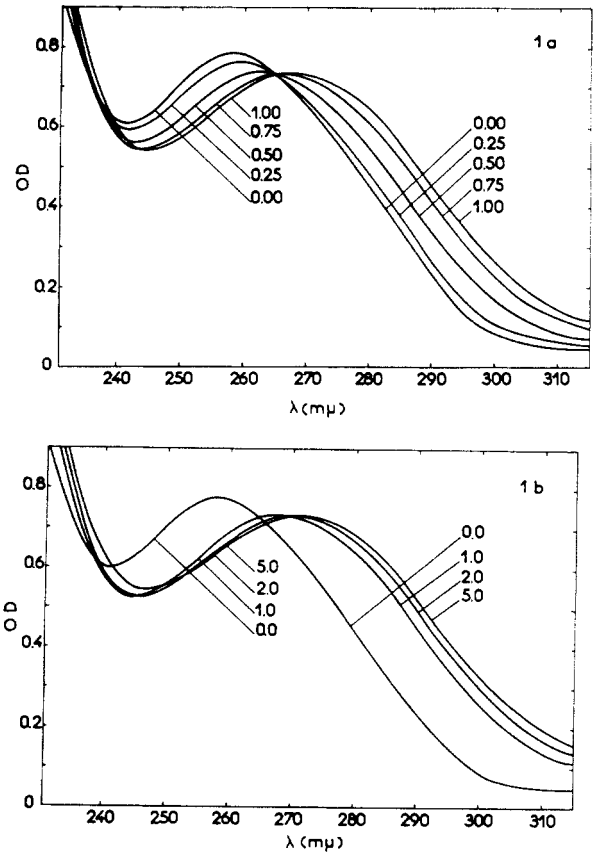

FIGURE 1: Ultraviolet spectra of the reaction products of TBSV and $HgCl_2$ in 0.1 M sodium acetate (pH 5.8) as a function of r_a defined in the text: (1a) for various values of r_a between $r_a = 0$ and $r_a = 1$; (1b) for $r_a > 1$. The successive additions of $HgCl_2$ to spectrophotometer cells containing 2 ml of TBSV solution were made up with 5-μl micropipets.

cleotide. These molar ratios are called r_a and r_b, respectively. The additions of $HgCl_2$ and reversing reagents were made in equal amounts to both reference and sample cells in such a way that the dilution effect never exceeded 1% and therefore could be ignored. The reaction of TBSV and $HgCl_2$ ($r_a = 1$) was reversed with KCN or mercaptoethanol in the molar ratio to total Hg(II) added equal to 4.0 and 20, respectively; spectral measurements and analytical ultracentrifugation were performed after 30 min of reaction. Results do not change if reversing reagents in addition to the previously stated are added.

Mercury Determinations. In binding experiments, 10 ml of the TBSV solution of concentration 750 μg/ml were placed in polycarbonate centrifuge tubes, and varying amounts of $HgCl_2$ dissolved in the same buffer were added so that r_a had different values ranging from 0 to 5. This set of tubes was adjusted to a final volume of 20 ml with the buffer and ultracentrifuged at 30,000 rpm for 3 hr. As will be seen later, the reaction of Hg(II) with TBSV does not liberate slow sedimenting components; thus all of the virus material was sedimented. If care was taken to avoid resuspension of the pellets, the supernatants contained only the unbound $HgCl_2$, as it has been controlled spectrophotometrically. The amounts of unbound $HgCl_2$ were measured on known aliquots of the upper fractions of the supernatants by dithyzone mercury determination according to Laug and Nelson (1942). We have verified that no others metals interfered; thus inorganic mercury salts analyses were simplified accordingly. From these data, the amounts of Hg(II) bound to TBSV can be calculated and expressed in values of r_b.

Direct determinations of the amounts of mercury bound or remaining bound to TBSV after reversal reactions were obtained by liquid scintillation counting of the labeled virus–^{203}Hg complexes with a Beckman LS 200 B apparatus. The labeled virus was isolated from the reaction mixture by three cycles of ultracentrifugation at 30,000 rpm for 3 hr and resuspension in the same buffer. Since the third supernatant contained no more labeled mercury, data for moles of Hg(II) remaining bound are derived from the counts of radioactive metal found in the last virus suspension.

Analytical Ultracentrifugation. Sedimentation velocity experiments were carried out in a Spinco Model E ultracentrifuge. Virus concentrations were 0.5 mg/ml unless specified otherwise. In all cases the sedimentation coefficients were converted into standard temperature and solvent conditions. The s values obtained at the virus concentration of 0.5 mg/ml have been assimilated to the s values at infinite dilution since it has been determined experimentally that the s values of TBSV and TBSV–Hg complexes are almost independent of the concentration. We have found the following concentration dependences in 0.1 M Na acetate (pH 5.8)

$$\text{TBSV } (r_a = 0)\ s_{20,w} = s^0_{20,w}\,(1-6 \times 10^{-3}C)$$

$$\text{TBSV } (r_a = 1)\ s_{20,w} = s^0_{20,w}\,(1-8 \times 10^{-3}C)$$

where C is the virus concentration in milligrams per milliliter. For our purpose it was assumed that the molecular weight of TBSV is 8.9×10^6 daltons and its diffusion coefficient 1.26×10^{-7} cm²/sec (Cheng and Schachman, quoted by Schachman and Williams, 1959). The partial specific volumes of Hg(II) and mercury ($\bar{V}$ Hg) are not very different and have been considered to be equal to 0.0738 ml/g.

Viscosity. The viscosity of virus solutions was measured with an Ubbeholde suspended level capillary viscosimeter. The temperature was controlled to ± 0.1°. Dust was removed from the samples by centrifugation.

Results

Spectrophotometry. Addition of mercuric chloride to TBSV produces a great change of the virus spectrum. In Figure 1, the course of the reaction in 0.1 M Na acetate (pH 5.8) may be seen as a function of r_a (the number of moles of $HgCl_2$ added per mole of phosphorus). Upon addition of $HgCl_2$ dissolved in the same buffer, for values of r_a ranging from 0 to 1 (Figure 1a), there is a red shift of the wavelength of maximum absorption, initially accompanied by a decrease of the maximum absorbancy; an isosbestic point is located at 264.5 mμ. On increasing the $HgCl_2$ concentration above $r_a = 1$ (Figure 1b), the absorption curves display a new isosbestic point at about 269.5 mμ, but do not pass through the first one at 264.5 mμ. As r_a increases above $r_a = 1$, the absorption at the new maxima (between 268 mμ and 273 mμ) does not change noticeably.

The shift in the absorption spectra of TBSV brought about

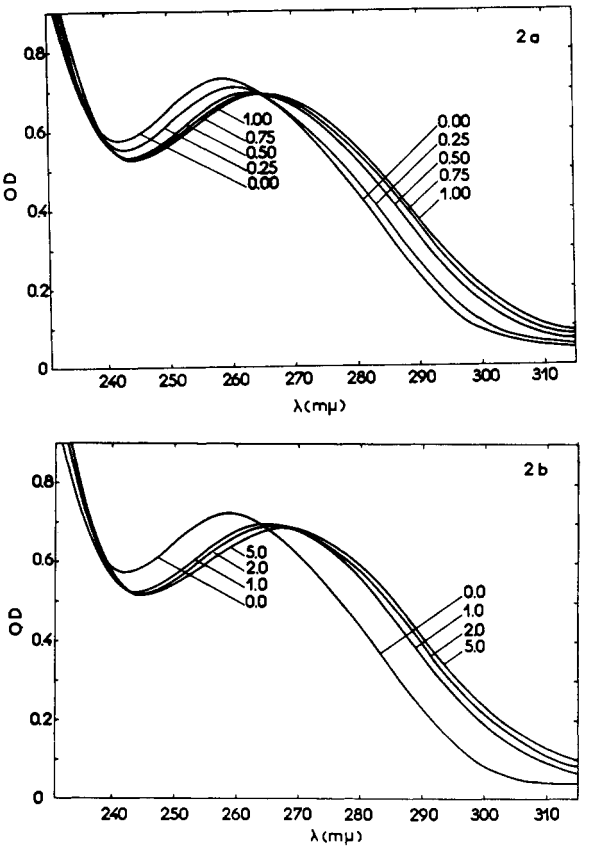

FIGURE 2: Ultraviolet spectra of the reaction products of TBSV and $HgCl_2$ in 0.1 M sodium acetate pH 4.8 as a function of r_a: (2a) for $0 < r_a < 1$; (2b) for $r_a > 1$.

by the addition of $HgCl_2$ resembles similar spectral shifts obtained when mercuric ions interact with RNA (Yamane and Davidson, 1962; Kawade, 1963) and DNA (Katz, 1952; Yamane and Davidson, 1961). The comparison of these results provides evidence that interaction of mercuric ions takes place on the RNA level (Thomas, 1954). The existence of two reasonably sharply demarcated isosbestic points in the absorption curves displayed by TBSV suggests that the reaction gives rise to at least two types of complexes (Katz and Santilli, 1962): the first for $0 < r_a < 1$, and the second for $r_a > 1$.

The addition of $HgCl_2$ to TBSV in 0.1 M Na acetate at pH 5.8 (Figure 1a,b) and pH 4.8 (Figure 2a,b) produces very similar spectral changes: at both pH values, the absorption curves pass through the same isosbestic points for the same range of values of r_a, suggesting the formation of the same kinds of complexes. However the spectral shift toward longer wavelengths as a function of r_a is less at pH 4.8 than at pH 5.8. It can be evaluated for $0 < r_a < 1$, independently from initial virus concentration, by the optical ratio of $OD_{290\,m\mu}$ to $OD_{264.5\,m\mu}$ (isosbestic point). Figure 3 shows that for $0 < r_a < 0.5$, the spectral shifts are closely related, but for $0.5 < r_a < 1$, the optical ratios increase more quickly at pH 5.8 than at pH 4.8; for $r_a > 1$, the red shifts are relatively weak and similar for both pH values. The difference in spectral shifts between the two pH values could be indicative of more than one complex in the range $0 < r_a < 1$, despite the single isosbestic point.

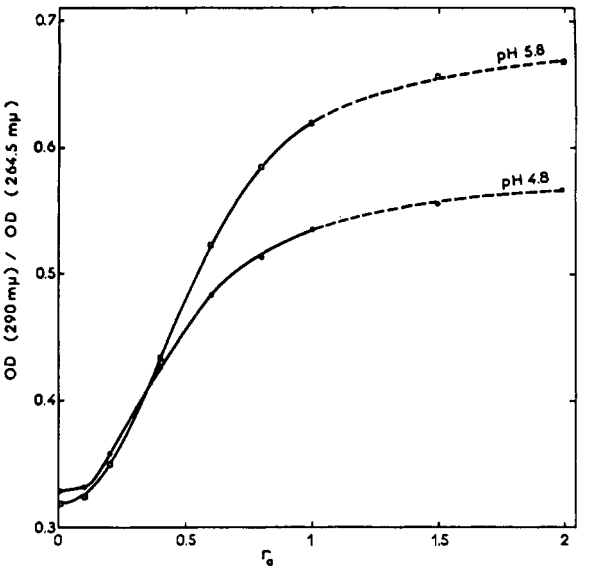

FIGURE 3: Variation of the optical ratio $OD_{290\,m\mu}/OD_{264.5\,m\mu}$ of TBSV as a function of the molar ratio r_a at pH 4.8 and 5.8 in 0.1 M sodium acetate. The optical ratio up to $r_a = 1$ is evaluated relative to the absorbance at 264.5 mμ of uncomplexed TBSV.

Binding Experiments. The amounts of Hg(II) bound to TBSV have been measured in order to correlate the data obtained at different pH values from spectral measurements. These experiments have been performed by dithyzone mercury determination as mentioned in Materials and Methods. The results are shown in Figure 4.

For $0 < r_a < 0.5$ the binding curves are closely related; practically the total amount of added mercury is bound by the virus, whatever the buffer may be.

In the range of $0.5 < r_a < 1$, the extent of reaction is appreciably influenced by pH, as previously mentioned: we obtain $r_b = 0.54$ at pH 4.5 instead of $r_b = 1$ at pH 7.0 for the value $r_a = 1$. It would appear that in the range $0 < r_a < 1$, there are

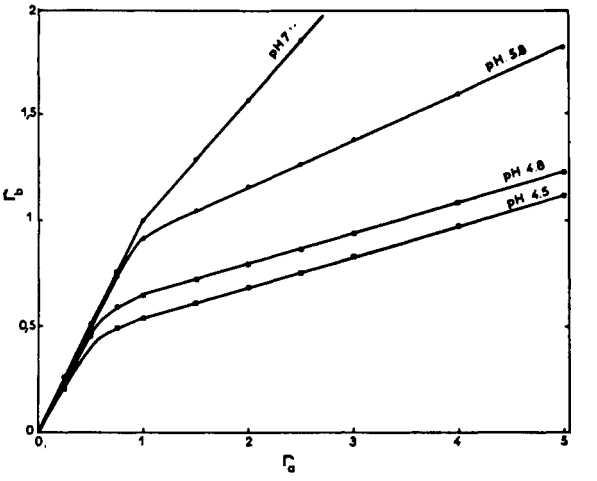

FIGURE 4: Binding of $HgCl_2$ by TBSV in the following buffers: 0.1 M Na acetate pH 4.8 and 5.8; 0.01 M phosphate pH 7. The number of moles of Hg(II) bound per mole of phosphorus (r_b) is given as a function of the molar ratio added (r_a).

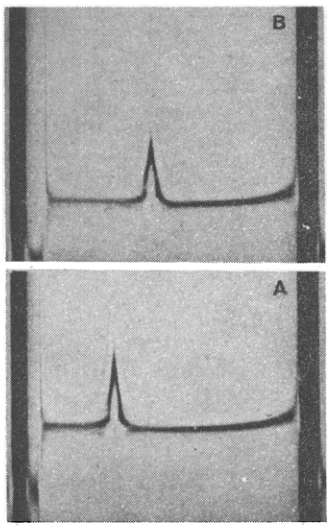

FIGURE 5: Sedimentation pattern of TBSV in 0.1 M sodium acetate pH 5.8 before (A) and after (B) treatment with $HgCl_2$ in such a way to have $r_a = 1$. The virus concentration was 0.5 mg/ml and each picture was taken at a Schlieren angle of 25° approximatively 20 min after reaching 23,150 rpm (A) $s_{20,w} = 135$ S; (B) $s_{20,w} = 174$ S.

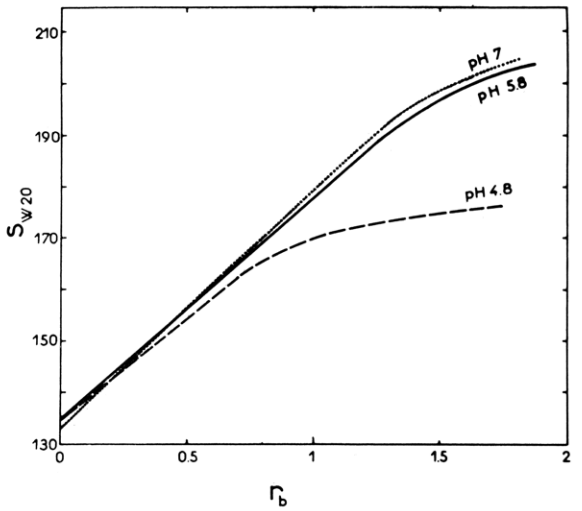

FIGURE 7: Variation of the sedimentation coefficient of TBSV as a function of r_b in 0.01 M Na phosphate pH 7 (.....), 0.1 M Na acetate pH 5.8 (———) and pH 4.8 (– – – –).

two complexes of TBSV–Hg for the same isosbestic point at 264.5 mμ. The first complex ($0 < r_a < 0.5$) exists at any pH investigated, while the second one ($0.5 < r_a < 1$) does not exist at acid pH.

The break-off points of the binding curves make evident the existence of a third complex for $r_a > 1$ at pH above 5.8 and for $r_a > 0.5$ at pH below 4.8.

Direct determination of the amount of mercury bound to TBSV by counting of labeled virus-^{203}Hg complex after isolation from the reaction mixture leads to results identical with those obtained previously, except in the range of r_a where the third complex exists: at pH 5.8 the direct determination gives $r_b = 1.02$ instead of $r_b = 1.17$ for $r_a = 2$ by the dithyzone method; at pH 4.5 we obtain $r_b = 0.75$ for $r_a = 5$ instead of $r_b = 1.12$. In the case of the third complex, the diminution of the amount of mercury bound indicates that there is a major fraction of Hg(II) which can be removed by simple washing out and which is probably weakly bound to the virus.

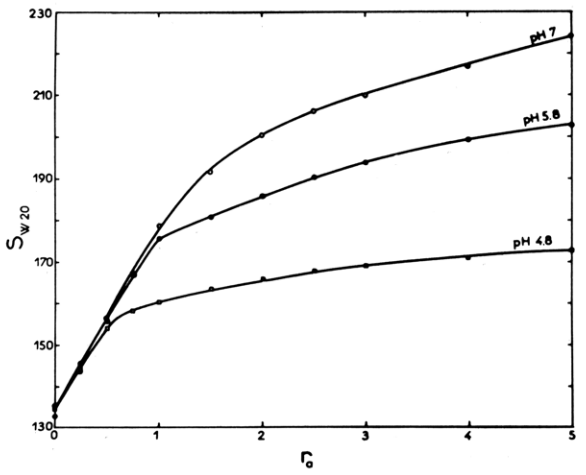

FIGURE 6: Variation of the sedimentation coefficient of TBVS as a function of r_a in the following buffers: 0.1 M Na acetate (pH 4.8 and 5.8); 0.01 M phosphate (pH 7).

Sedimentation Experiments. The addition of $HgCl_2$ to a TBSV solution under the above described conditions does not degrade the virus into its components as indicated by the single homogeneous peak of TBSV–Hg observed on the sedimentation patterns (Figure 5). However, mercury increases the sedimentation rate of native TBSV.

The increase of the sedimentation coefficient of TBSV–Hg examined by the Schieren method in various buffers has been plotted as a function of r_a (Figure 6). The variation of the s value closely parallels the results of binding measurements (Figure 4) since the changes in the slopes of the curves appear in the same range of values of r_a for the different buffers used. This fact suggests that there is a simple relationship between the increase of the s values and the amount of mercury bound by the virus measured by the dithyzone method.

Figure 7 shows that the sedimentation coefficient of TBSV–Hg increases at the inception in a linear fashion with increasing amounts of Hg(II) bound until certain values of r_b are reached: at pH 5.8 and 7 the limited value of linear variation is reached for about $r_b = 1.3$ and at pH 4.8 for $r_b = 0.8$. The linear relationships between s and r_b are represented by the following equations.

$$\text{pH } 7.0,\ s_{20,w} = 45r_b + 133$$

$$\text{pH } 5.8,\ s_{20,w} = 43r_b + 135$$

$$\text{pH } 4.8,\ s_{20,w} = 40r_b + 135$$

On comparing the validity ranges of the linear variations with the ranges of r_a corresponding to the three complexes previously described, it may be observed that the first TBSV–Hg complex ($0 < r_a < 0.5$) and the second ($0.5 < r_a < 1$) are responsible for the linear variation of the s value. This suggests that similar hydrodynamic changes occur for the first two

complexes. The position of the break-off points in the s curves indicates that different hydrodynamic changes occur for the third complex and that at pH 4.8 the formation of the first complex is immediately followed by the third one; the second complex does not exist, as has already been seen.

Interpretation of Sedimentation Data. In order to interpret the linear increase of the sedimentation coefficient of TBSV in terms of configurational changes, the experimentally determined relations between s and r_b have been compared with the theoretical variation of s induced by an increase in the molecular weight of virus without configurational change.

When mercuric ions are bound by the virus, the molecular weight of TBSV–Hg may be regarded as a linear function of the amount of mercury bound:

$$M = mr_b + M_0 \quad (1)$$

where M and M_0 are the molecular weights of TBSV and TBSV–Hg, respectively; m is the weight of mercury bound by 1 mole of virus for $r_b = 1$. This value is equal to 9.1×10^5 g.

If we assume that mercury binding does not modify the shape and size of the virus, the diffusion coefficient of complexed TBSV (D) would be equal to the diffusion coefficient of uncomplexed TBSV (D_0). This assumption allows us to write eq 1 in terms of apparent molecular weights:

$$M(1 - \bar{V}\rho) = m(1 - \bar{V}\mathrm{Hg}\rho)r_b + M_0(1 - \bar{V}_0\rho) \quad (2)$$

where ρ is the solvent density and $\bar{V}_0$, $\bar{V}$, $\bar{V}\mathrm{Hg}$, the partial specific volumes of TBSV, TBSV–Hg and Hg(II), respectively.

From the relation between M and r_b (eq 2) and the assumption concerning the equality of the D's, the sedimentation coefficient of TBSV–Hg (S) represented by the classical Swedberg equation (3) can be calculated as a function of r_b; eq 4, which works out numerically to eq 5, shows that the sedimentation coefficient of TBSV–Hg is a linear function of r_b.

$$s = \frac{D}{RT} M(1 - \bar{V}\rho) \quad (3)$$

$$s = \frac{D_0}{RT} m(1 - \bar{V}\mathrm{Hg}\rho)r_b + s_0 \quad (4)$$

$$s = 43.6r_b + s_0 \quad (5)$$

where s_0 is the sedimentation coefficient of native TBSV.

Since eq 5 works out to be essentially identical with the equations experimentally derived in the previous section, the initial assumption concerning the equality of the D's is correct, and hence there is no mercury-induced configurational change within the validity ranges of the experimental equations. As can be noticed from the experimental equations, the slope for the different pH experiments varies a bit; it has been calculated that this difference corresponds to a change of the D's no greater than 3% which could account for a variation of 3% in the radius of the particle. However such a small variation of the slope is hardly significant relative to the experimental errors in determining r_b as a function of r_a.

For the values of r_b up to the validity ranges, the experimental s values are noticeably inferior to the s values given by the theoretical eq 5 assuming no configurational change. Therefore the virus seems to show an increase in size with the formation of the third complex. For example, it has been calculated that the radius of the particle would increase by about 16% at pH 4.8 for $r_b = 1.7$.

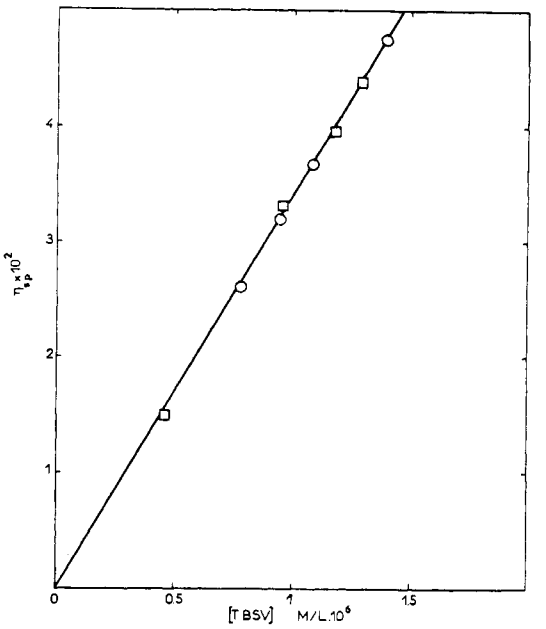

FIGURE 8: Concentration dependence of the specific viscosity of TBSV (□) and TBSV–Hg ($r_a = 0.8$) (○). Viscosity measurements were made at 20° in 0.1 M sodium acetate (pH 5.8). The virus concentration is calculated from the absorbance of TBSV and TBSV–Hg solutions at 264.5 mμ and evaluated in M from the molecular weight of TBSV equal to 8.9×10^6 daltons.

Viscosity and Density. In order to have more accurate information about the structural effects of mercury binding, the specific viscosity of TBSV in 0.1 M Na acetate (pH 5.8) was compared to the specific viscosity of TBSV–Hg complex ($r_a = 0.8$) in the same buffer. Figure 8 shows that no differences have been found for complexed and uncomplexed material at the same concentration in moles/liter. According to Einstein's equation relative to the specific viscosity of spherical particles, this result would indicate that mercury binding does not cause a configurational change of the virus.

Addition of $HgCl_2$ to TBSV increases noticeably the buoyant density of the virus in Cs_2SO_4 gradient; the density calculated from the measured refractive index increases progressively from 1.31 for $r_a = 0$ to 1.40 for $r_a = 1$ in 0.1 M Na acetate (pH 5.8). In the absence of a mercury-induced conformational change, the increase of density may reflect an increase in particle weight.

Reversibility. To investigate the reversibility of the reaction of TBSV and $HgCl_2$, TBSV–Hg ($r_a = 1$) in 0.1 M Na acetate (pH 5.8) has been treated with KCN and mercaptoethanol as described in Materials and Methods.

Figure 9 shows that the spectral shift of TBSV induced by $HgCl_2$ is entirely reversible with both reagents. The slight general depression of the reversal curves was anticipated since the virus presents a similar spectral change when treated with reversing reagents alone.

Sedimentation properties of the reversal materials have

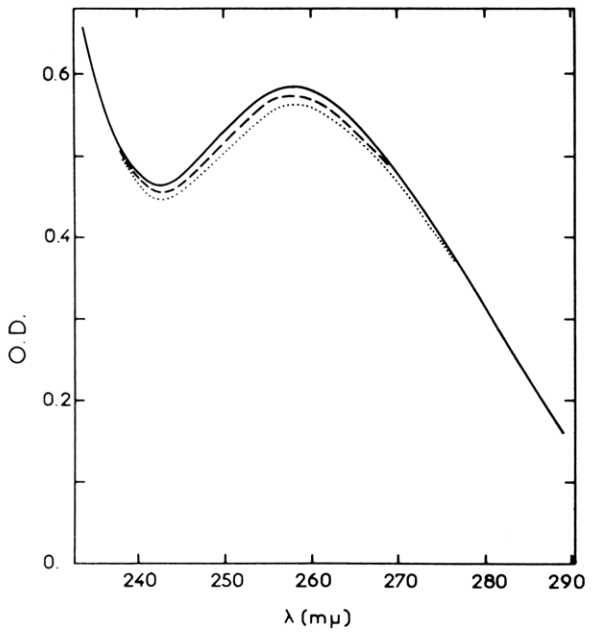

FIGURE 9: Spectra of TBSV–Hg (r_a = 1) in 0.1 M Na acetate (pH 5.8) after treatment with mercaptoethanol (----) and KCN (. . . .). The spectrum of native TBSV (———) is shown for comparison. Portions of some of the reversal curves have been omitted for clarity.

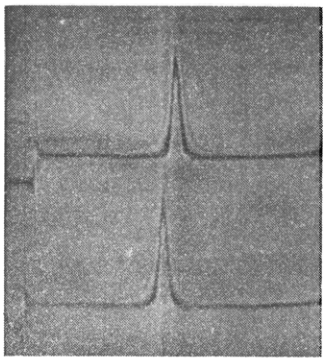

FIGURE 10: Sedimentation pattern of TBSV–Hg (r_a = 1) after reversion by mercaptoethanol (wedge cell). Standard cell: sedimentation pattern of TBSV control. The virus concentration was 2 mg/ml of 0.1 M sodium acetate buffer (pH 5.8). The photograph was taken 20 min after reaching 27,690 rpm, at a Schlieren angle of 55°.

been examined when the reactions were performed under the previous condition at the virus concentration of 2 mg/ml. As can be noticed in Figure 10, the sedimentation coefficient of TBSV–Hg after treatment with mercaptoethanol returns to *s* of 132.4 S which is identical with the *s* value of initial TBSV of 132.2 S; the *s* value of TBSV–Hg after treatment with KCN has been found to be 135.1 S.

In order to obtain a more quantitative comparison of the effectiveness of both chemicals, titration of the amount of mercury remaining bound to the virus has been performed with labeled ^{203}Hg(II). After treatment with KCN the virus still contains 0.074 mole of mercury/mole of P; this value is only 0.006 mole with mercaptoethanol. From the titration and sedimentation results, it may be concluded that mercaptoethanol is more effective than KCN in reversing completely the reaction of TBSV and Hg(II).

Discussion

The results presented here demonstrate that mercuric ions bind to TBSV. However some consideration must be made to determine the nature of the binding sites in the virus, since Hg(II) may form various complexes with protein and nucleic acid.

Much work with protein provides evidence that SH groups have a stronger affinity for Hg(II) than other protein groups. In a preceding investigation (Dorne 1968c) it has been established that TBSV contains 600 SH groups per particle. On assuming that all the SH groups react with mercury, it may be calculated that they would not contribute to mercury binding to a larger extent than a ratio of 0.13 mole of mercury/mole of phosphorus, and half this value if $(Prot–S)_2Hg$ is formed. However it has not been proven that all the SH groups are reactive, especially since the extent of substitution of SH groups by PMB has failed to prove consistently their great reactivity and that the SH groups can react with *N*-ethylmaleimide only after denaturation of the virus (Dorne 1968c). Despite the absence of quantitative results, these considerations lead us to think that SH groups would only account for a small fraction of the amount of mercury bound to the virus.

On the other hand, there are a great number of various protein groups, such as carboxylate and amino groups, which may be involved in the mercury binding. Examination of the association constants for the complexing of Hg(II) leads to the conclusion that no proteins groups can compete very effectively with SH groups and nucleic acid for mercuric ions (Simpson, 1964; Webb, 1966). However, when all the binding sites having a great affinity for Hg(II) are fully saturated, binding of Hg(II) to aspecific groups may be expected: Haarmann (1943) and Perkins (1961) have observed that with increasing pH, more Hg(II) is bound to various proteins than is expected from their SH content; only a fraction is really tightly attached to the protein. As can be noticed in binding experiments, there is a fraction of Hg(II) which is relatively weakly bound to TBSV for the third TBSV–Hg complex; the slopes of the binding curves remain steep and no saturation of the sites is ever accomplished, especially at pH 7. Thus it may be postulated that interaction of Hg(II) with aspecific protein groups occurs for the third complex when excess mercury is present.

Since a large amount of Hg(II) which would not account for the SH groups content is tightly bound to TBSV, it may be assumed that complexing of Hg(II) with the bases of nucleic acid occurs. This assumption is largely supported by the fact that the spectral shifts of TBSV qualitatively resemble similar shifts obtained when Hg(II) interacts with TMV–RNA (Katz and Santilli, 1962; Yamane and Davidson, 1962); furthermore, preliminary experiments have shown that TBSV–RNA exhibits qualitatively similar spectral changes (Dorne, to be published). When the mercury concentration is not too high, the spectral shifts may be correlated to the mercury binding since the break-off point of the binding curve at pH 7 appears for r_a = 1 where the spectra begin not to pass through the first isosbestic point; it may also be noticed that a decrease in the pH has a similar influence on spectral shifts (Figure 3) and binding (Figure 4). Notwithstanding the probable small binding contribution of SH groups, the two types of com-

plexes which have been observed in the range $0 < r_a < 1$ would correspond to specific complexes with TBSV–RNA. This conclusion is in agreement with the results of Singer (1964), which show that TMV–RNA binds 1 mole of Hg(II)/mole of nucleotide on two different binding sites responsible for 0.5 mole each. For the third TBSV–Hg complex no correlation between binding and spectral shift may be found since Hg(II) starts to interact with aspecific protein groups and probably also with phosphate groups of the nucleic acid.

We have shown that the increase of the sedimentation coefficient and density of the virus upon addition of $HgCl_2$ corresponds only to an increase of its molecular weight; neither disintegration nor conformational change have been detected when Hg(II) reacts with SH groups and nucleic acid. Furthermore the reversibility by mercaptoethanol of the profound changes in the physical properties would be consistent with a nondegradation process. The stability of TBSV in the reaction with $HgCl_2$ or mercurials strongly contrasts with the dissociation of TYMV (Kaper and Jenifer, 1965). Since the dissociation of this virus is mainly due to the reaction of mercurials with SH groups, the structural stability of TBSV would indicate that SH groups are not essential for maintaining the virus structure. The stability of the virus when mercury is bound to RNA may be related to its strong general resistance to denaturing agents and would be due to the particular structure of TBSV with a protein core and an outer protein shell, as suggested by Harrison (1969); the mercury binding to TBSV–RNA inside the virus capsid would not bring about a conformational change since Harrison has shown that the region between the two protein shells has considerably greater solvent space than either layer.

On comparing the effects on TBSV structure of Hg(II) and organic mercurials which have been shown to form complexes with the RNA inside the virus capsid (Dorne and Hirth 1968b), it may be observed that mersalyl or PMB induces an important swelling of the virus particle. This difference may be explained on the basis either of steric effects of the greater bulk of organic compounds (Stols and Veldstra, 1965) or of hydrophobic interactions between the protein of the particle and the organic part of mercurials (Godschalk and Veldstra, 1965). This difference may also result from a difference in the nature of the conformational change of the RNA itself inside the capsid induced by the two types of mercury compounds.

It may be expected that the reaction of Hg(II) with RNA inside of the virus particle without conformational change of the latter would be a considerable help for an X-ray diffraction determination of the position of the RNA in TBSV.

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