THE EFFECT OF CONFORMATIONAL CHANGES IN BROME MOSAIC VIRUS UPON ITS SENSITIVITY TO TRYPSIN, CHYMOTRYPSIN AND RIBONUCLEASE

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1. Introduction

It has been shown recently that the pH-induced conformational change in Brome Mosaic Virus (BMV) [1] is a two-step process comprising a true pH-induced transition with no temperature-dependence, and a thermal expansion step postulated to be a result of changes in RNA conformation, the latter contribution being eliminated in the presence of Mg^{++} ions [2]. In the absence of Mg^{++} ions however, raising the pH from 6 to 7.5 yields completely swollen BMV, which, in contrast to native virus, is sensitive to pancreatic RNase [3,4] and to trypsin treatment [5,6]. We have investigated the correlation between sensitivity to nucleolytic and proteolytic enzymes and the various steps of the conformational change.

2. Materials and methods

2.1. *Virus preparation*

This has already been described in detail [7]. Purified BMV suspensions were freed from adventitiously bound divalent cations by dialysis against 0.05 M EDTA adjusted to pH 5, followed by extensive dialysis against water.

2.2. *Incubation with enzymes*

BMV at 3–5 mg/ml was incubated in 0.05 M Na cacodylate buffer (enventually supplemented with 0.005 M $MgCl_2$) with : 1% (w/w) trypsin (12 500 BAEE units/mg), 2% (w/w) chymotrypsin (50 units/mg), or 20 μg/ml pancreatic ribonuclease (100 Kunitz units/mg). All dialysis and incubations were performed at room temperature (22 ± 1°C).

2.3. *Analytical ultracentrifugation and electron microscopy*

A Beckman 'Model E' analytical ultracentrifuge equipped with Schlieren optics and a Siemens Elmiskop 101 were used throughout. Experimental details are given in [7] and [8].

3. Results

3.1. *Swelling of the virus in the presence and absence of Mg^{++} ions as followed by the appearance of sensitivity towards trypsin and chymotrypsin*

We found that native BMV is unaffected by incubation with trypsin and chymotrypsin at pH 6, whether MG^{++} ions are present or not, but reacted with these enzymes above pH 6.5 in the absence of Mg^{++} ions (fig.1a and b). Controls showed however that dissociated BMV protein was cleaved by trypsin and chymotrypsin even at pH 6, proving thus that the lack of reaction of these enzymes with BMV at this pH value was not merely due to an effect of pH on enzyme activity.

The main difference between the action of trypsin and chymotrypsin at pH 7.4, is that the former caused progressive collapse of the virus structure into small particles sedimenting in the range 32–36 S (as already described by Agrawal and Tremaine [5]), while the latter converted BMV into a slower component sedimenting with 56 S (fig.1b, lower pattern). This component was stable to prolonged incubation with chymotrypsin, while the 53 S component obtained after moderate action of trypsin (see fig.1a, lower pattern), was gradually converted into a 32 S component after increased reaction time.

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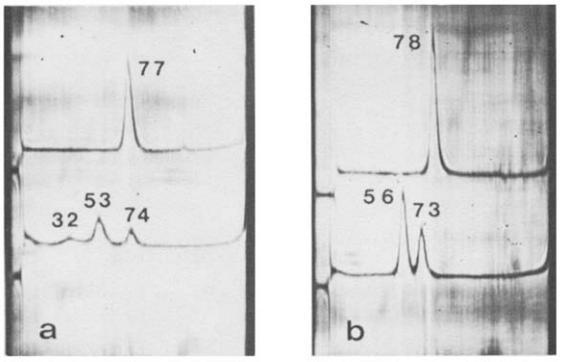

Fig.1. Effect of proteolytic enzymes on BMV. (a), *Lower pattern*: BMV (3 mg/ml) incubated for 4 hr at 22°C with 1% (w/w) trypsin in pH 7.40 0.05 M Na cacodylate–HCl buffer. *Upper pattern*: the same, but in presence of 5×10^{-3} M $MgCl_2$. (b), *Lower pattern:* BMV (5 mg/ml) incubated with 2% (w/w) chymotrypsin: conversion into Chy-BMV. *Upper pattern*: the same, but in presence of 5×10^{-3} M $MgCl_2$. Numbers on these and subsequent sedimentigrams are $S_{20,w}$ values, but without correction for virus concentration.

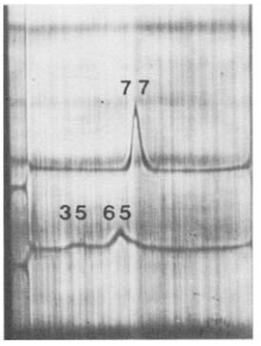

Fig.3. Effect of ribonuclease on native BMV as influenced by the presence of Mg^{++} ions. *Lower pattern*: BMV (3 mg/ml) incubated for 5 hr at 22°C with 20 μg/ml pancreatic ribonuclease in pH 7.4 0.05 M Na-cacodylate buffer. *Upper pattern:* the same, but in the presence of 5×10^{-3} M $MgCl_2$.

In the electron microscope (fig.2), chymotrypsin-converted BMV (Chy-BMV) apparently retained the overall aspect of native BMV, with its characteristic icosahedral symmetry and surface morphology, but detailed structural studies are required for analysis of the fine structural details.

However, as shown on the upper patterns of fig.1a and b, the presence of 0.005 M $MgCl_2$ abolished completely the action of both trypsin and chymotrypsin, in the same way as it abolished that of RNase on BMV at pH 7 and above (3,4, and fig.3). The Mg-stabilized, partially expanded form of BMV is thus like the native virus in its compact configuration, resistant to trypsin, chymotrypsin, and also to RNase, while the sites for proteolytic cleavage become unmasked and simultaneously the RNA contents become accessible to RNase in the completely swollen virus

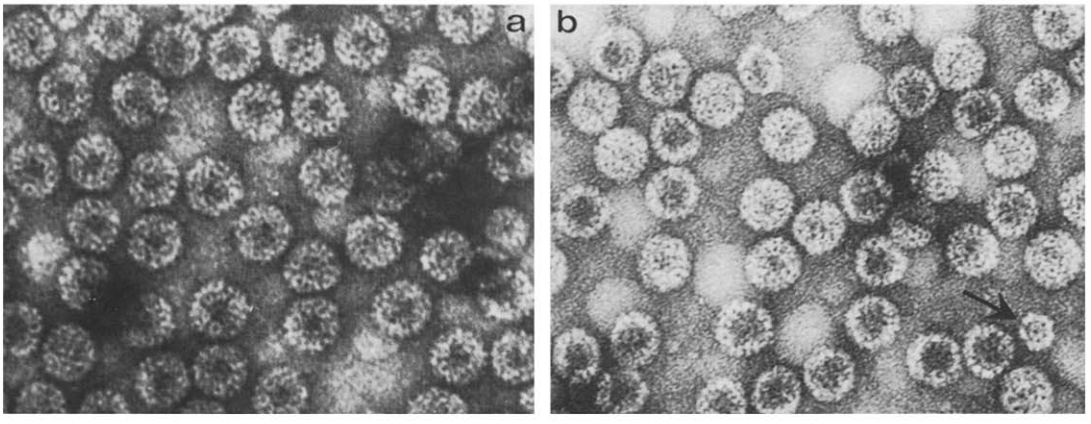

Fig.2. Electron micrographs of: (a) Native BMV in pH 7.4 0.05 M Na-cacodylate; (b) Purified Chy-BMV under the same conditions. The arrow points to a small particle (built on the T = 1 model) which results from the breakdown and reassembly of BMV particles, the RNA of which was already cleaved in situ: such structures form in increasing amount with increasing pH, temperature, and reaction time with chymotrypsin. Uranyl formate staining. Final magnification × 270 000.

obtained at neutral or slightly alkaline pH in the absence of Mg^{++} ions.

3.2. *Localization of the trypsin and chymotrypsin-sensitive sites in BMV*

Trypsin treatment of completely swollen BMV has already been shown to cause a specific split at Arg^{25} accompanied by the release of 25 aminoacyl residues [6], of which eight are arginyl, and one a lysyl residue. Since the loss of this peptide bearing high positive charge causes collapse of the virus structure, the N-terminal region becomes a good candidate for a strong interaction site between RNA and protein subunits in the virus.

We have not yet localized with precision the site for chymotrypsin cleavage in BMV, but we have found by MW estimation upon polyacrylamide gel electrophoresis (PAGE) that the largest, and in actual fact the major residual polypeptide had a mol. wt in the range 14 000, compared with 20 300 for native BMV protein [9]. Fig.4 shows that minor bands are found in addition to this major product: the rate of cleavage was found to increase with pH, but no additional bands appeared with increasing pH values. The expected complementary peptide of some 5 to 6000 mol. wt could not be evidenced, either because it was not retained on the 10% gels, or because it was further cleaved into smaller peptides after release from the virus.

Preliminary peptide analysis, both by cation exchange resin chromatography and two-dimensional fingerprinting revealed however that both C-terminal and N-terminal peptides are conserved in Chy-BMV, which strongly favours a two-split action of chymotrypsin with retention of both ends of the polypeptide chain in Chy-BMV, owing to tertairy and quaternary structure of the coat protein chains in the virus particle. From the MW of the major residual polypeptide obtained by PAGE under denaturing conditions, one of the cleavage sites can be expected to be located at approximately 60 residues from one or the other end of the polypeptide chain.

3.3. *Physico-chemical properties of chymotrypsin-converted BMV*

While Chy-BMV retains the overall aspect and morphology of native BMV, its surface properties are evidently altered, since in contrast to native BMV, it

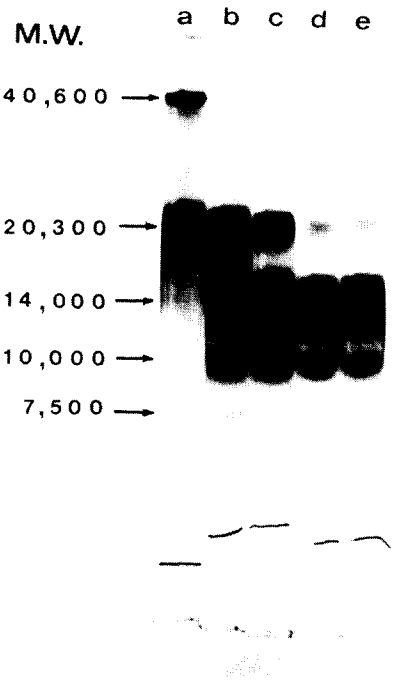

Fig.4. Analysis on 10% polyacrylamide gels of the coat protein of BMV after 8 hr of incubation with 2% (w/w) chymotrypsin at 22°C and at increasing pH values. Fifty micrograms of virus (native or chymotrypsin-treated) were dissociated by 5 min. heating at 100°C in electrophoresis buffer containing 1% SDS and 1% mercaptoethanol. (a) Native BMV, dissociated without mercaptoethanol, so that small amounts of dimers of the protein subunit are found (upper band); (b): BMV incubated with chymotrypsin at pH 7.25; (c): pH 7.60; (d) pH 7.85; (e): pH 8.05. Gels were overloaded to detect minor bands. The position reached by the tracking dye (bromophenol blue) is indicated by a wire inserted in the gel.

precipitates below pH 5 as the probable consequence of the removal of aminoacyl residues bearing a charged side-group.

More interestingly, we found that the fundamental structure of BMV is maintained in Chy-BMV, as illustrated by the ability of Chy-BMV to undergo a pH-induced structural change similar to that of BMV: indeed, Chy-BMV is resistant to trypsin and RNase treatment at pH 6 (where it is in its native compact configuration) and at pH 7.5 in the presence of Mg^{++} ions (partially swollen, Mg^{++}-stabilized conformation). At pH 7.5 however, Chy-BMV is degraded by both

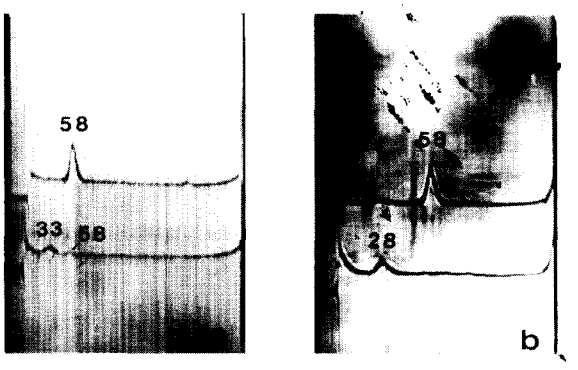

Fig.5. Behaviour of Chy-BMV towards trypsin and RNase in the presence and absence of Mg^{++} ions. (a) *Lower pattern*: Chy-BMV (3 mg/ml) was incubated for 5 hr at 22°C with 1% (w/w) trypsin at pH 7.5 in 0.05 M Na-cacodylate buffer; *Upper pattern:* the same, but in the presence of 5×10^{-3} M $MgCl_2$. (b) *Lower pattern:* Chy-BMV (3 mg/ml) incubated with 20 μg/ml pancreatic RNase (same conditions as before); *Upper pattern:* the same, but in presence of 5×10^{-3} M $MgCl_2$.

trypsin and RNase in the absence of Mg^{++} ions, and like for native BMV, there is a striking parallelism between the reduction of the sedimentation rate (which is the expression of the swelling) and the appearance of the susceptibility to proteolytic and nucleolytic enzymes. This provides additional evidence suggesting that neither the N-terminal region, which is compulsory for virus stability, nor the regions of the subunit which contain the presumed carboxyl-carboxylate pairs responsible for the conformational change in BMV [2,10], are lost upon chymotrypsin treatment.

4. Discussion

The finding that BMV becomes susceptible to trypsin, chymotrypsin and at the same time to RNase upon undergoing the thermal expansion step of the pH-induced structural change gives us a deeper insight into the changes occurring at the subunit level during this transition.

Exposure of RNA to RNase and unmasking of trypsin and chymotrypsin-sensitive sites are two intimately related and dependent phenomena. They are to correlate with the data obtained in recent neutron scattering experiments (B. Jacrot, personal communication) which show that in completely swollen BMV, the RNA loops expand much further from the center of the particle than in native BMV, and thus become accessible to RNase. On the contrary, the RNA retains the same structure in partially expanded, Mg^{++}-stabilized BMV, as in the native virus where it is completely 'buried'. We do know yet whether the expansion of the RNA loops is the cause or the consequence of the terminal expansion step, but it parallels in any case a repulsion of the protein subunits such as to unmask the trypsin and chymotrypsin-sensitive sites, and to leave enough space for the RNA loops to rise towards the surface of the virus particle.

A further interesting feature is that BMV retains many of its native properties after conversion by chymotrypsin treatment, in spite of alterations in the character of the particle surface (Pfeiffer, to be published). In particular, Chy-BMV undergoes the same conformational change as BMV, with the same dependence upon Mg^{++} ions. This swelling is accompanied by induction of sensitivity to RNase and trypsin, and Chy-BMV, like the native virus, collapses upon incubation with these enzymes in the absence of Mg^{++} ions. This proves that integrity of the RNA chain and the presence of the N-terminal region (which presumably interacts strongly with the RNA) are compulsory for the stability of both native and chymotrypsin-converted BMV.

The action of trypsin on Chy-BMV makes it almost certain that the N-terminus is retained in the modified virus, and since the C-terminal peptide has been detected upon peptide analysis on columns and two-dimensional fingerprints, we are strongly inclined to think that chymotrypsin cleaves the protein chain at two sites near one of the other end of the primary sequence. Work is in progress to confirm this and should permit us to assign some of the physico-chemical properties of BMV to the conserved and missing parts of the protein chain.

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