Disruption of Herpes Virus Nucleocapsids using Lithium Iodide, Guanidine and Mercaptoethanol

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SUMMARY

The nature of the covalent and non-covalent bonds which are responsible for the maintenance of the morphological integrity of the herpes simplex virus type I nucleocapsid was examined. Virus particles were treated at various temperatures with mercaptoethanol, guanidine, or lithium iodide under neutral and alkaline conditions. After treatment with mercaptoethanol or lithium iodide, the virus particles morphologically resembled collapsed amorphous structures composed of loosely bound fibrous strands, which no longer retained the capsomeric detail. Subsequent exposure of these treated preparations to alkaline conditions or high concentrations of guanidine was required to break the bonds that held the strands together. It was not possible under the conditions employed in this study to selectively break the intercapsomeric bonds and thus release free intact capsomeres.

The detailed mechanisms of herpes virus morphogenesis require an understanding of (a) the nature of the virus structural components, (b) the mechanism by which these components are synthesized, and (c) the means by which these components interact to form the virus particle. The nature of the herpes virus structural polypeptides and their synthesis has been examined in some detail. Various reports have suggested the presence of at least eight major polypeptide components in pseudorabies virus (Shimono, Ben-Porat & Kaplan, 1969; Ben-Porat, Shimono & Kaplan, 1970), and herpes simplex virus (McCombs & Courtney, 1970; Olshevsky & Becker, 1970; Robinson & Watson, 1971) capsids, and as many as 24 total polypeptides (Spear & Roizman, 1972) to be present within the herpes simplex enveloped particles. Electron microscopic examination of herpes virus-infected cells has shown the apparent assembly of the virus capsid around a core and subsequent envelopment at the inner nuclear membrane (Darlington & Moss, 1968; Nii, Morgan & Rose, 1968). However, very little is known of the mechanisms by which the virus structural polypeptides interact and are assembled to form the nucleocapsid. The present report represents studies undertaken to gain an understanding of the covalent and non-covalent bonds which determine how the various polypeptide subunits interact to form the virus nucleocapsid.

Herpes simplex virus (HSV) type I, strain XIII, was a large-plaque mutant obtained from Dr F. Rapp and grown in BHK-21 cells as described previously (Courtney, McCombs & Benyesh-Melnick, 1970). The virus nucleocapsids were purified as described previously (McCombs, 1969; Dreesman et al. 1972). The morphology of the virus nucleocapsids after the various treatments was examined by electron microscopy using the droplet-pseudo-replication method and negative staining with potassium phosphotungstate described previously (McCombs, Benyesh-Melnick & Brunschwig, 1966).

The role of disulphide bonds in maintaining the integrity of the virus nucleocapsid was examined by treatment with mercaptoethanol. Nucleocapsids in either RSB (pH 7.4, Warner, Knopf and Rich, 1963) or in carbonate–bicarbonate buffer (0.1 M; pH 8, 9 or 10)
Fig. 1. Effect of mercaptoethanol and guanidine hydrochloride treatment on the morphology of the herpes simplex virus nucleocapsid. A, B, morphology of virus particles maintained in the presence of mercaptoethanol under alkaline conditions at 4 °C for 30 min. Arrows indicate capsomers in the process of being released from the nucleocapsid. C, morphology of virus particles heated at 90 °C in the presence of mercaptoethanol under alkaline conditions for 5 min. D, morphology of virus particles heated at 90 °C in the presence of mercaptoethanol under alkaline conditions for 10 min. E, morphology of virus particles heated at 90 °C in the presence of 4 M-guanidine hydrochloride for 5 min. F, morphology of virus particles heated at 90 °C in the presence of 2 M-guanidine hydrochloride and mercaptoethanol for 5 min. Arrows indicate structure which possibly represents virus core.
were mixed with mercaptoethanol (final concentration 0.13 M) and either held at 4 °C or heated at 37, 56, 70, 80 or 90 °C for 1 to 5 min. The morphology of virus nucleocapsids, as determined by electron microscopy, held at 4 °C or heated up to 70 °C in either buffer for a total of 30 min showed essentially no alterations in the ultrastructure of the virus nucleocapsid. Occasional virus particles were observed that had apparently lost (Fig. 1A) or appeared to be in the process of losing (Fig. 1B) individual capsomeres.

Incubation of the virus particles in RSB (pH 7.4) at 80 °C or 90 °C for 1 to 5 min in the presence of mercaptoethanol resulted in the partial collapse of the virus nucleocapsid (similar to Fig. 1C). The fibrous strands that probably represented the polypeptide subunits as well as perhaps DNA, appeared to have partially unfolded. Further incubation under these conditions did not result in any further breakdown of the nucleocapsid. Virus particles in RSB (pH 7.4) heated under the same conditions in the absence of mercaptoethanol showed no morphological alterations. These results suggested that some breakage of hydrogen bonds (by heat) was necessary to unmask the disulphide bonds or, alternatively, that both disulphide bonds and hydrogen bonds were necessary for the maintenance of the morphologic integrity of the herpes virus nucleocapsid.

Treatment of virus nucleocapsids under alkaline conditions (pH 9 or 10), at 80 or 90 °C for 1 to 5 min in the presence of mercaptoethanol (final concentration 0.13 M) also resulted in the partial collapse of the virus particle (Fig. 1C). After incubation for 10 min at 80 or 90 °C the fibrous strands had further unfolded. Morphologically the nucleocapsids resembled either particles that had expanded in size to approximately 140 nm in diam. or disorganized aggregates of the fibrous strands (Fig. 1D). Virus particles treated under the same alkaline conditions in the absence of mercaptoethanol were also partially disrupted into aggregates of the fibrous strands which resembled the particles that were expanded in size (similar to Fig. 1D). Since in the absence of mercaptoethanol the virus nucleocapsids were affected only under alkaline conditions (pH 9 or 10) and not under neutral conditions (pH 7.4), ionic bonds would appear to be of importance in stabilizing the herpes virus nucleocapsid.

Substitution of DTT (final concentration 0.01 M) for mercaptoethanol under either neutral (pH 7.4) or alkaline (pH 10) conditions gave the same results as mercaptoethanol when virus was heated for 5 min at 90 °C.

The role of hydrogen bonds in maintaining the nucleocapsid structure was further examined by treating virus in carbonate-bicarbonate buffer (pH 10) with guanidine hydrochloride (Schwarz/Mann, Orangeberg, N.Y.) to give a final concentration of 2, 4, 6, or 8 M. The virus was incubated at either 4 °C or heated to 90 °C. The addition of guanidine crystals to the virus preparation reduced the pH from 10 to approximately 8, the pH at which the incubation was carried out. Incubation of the virus particles at 4 °C for 30 min had little or no effect on the morphology. After 5 min at 90 °C in 2 M-guanidine there was little alteration in the virus particle, but in 4 or 6 M-guanidine the virus particles were disrupted into amorphous masses (Fig. 1E). These masses appeared to be more compact than after mercaptoethanol and heat treatment. In the presence of 8 M-guanidine the virus particles were reduced to amorphous masses in 5 min without heating (i.e. at 4 °C), while heating for 5 min resulted in the complete dissolution of the virus particles into morphologically unrecognizable components.

In order to more closely examine the influence of pH on guanidine treatment, virus particles were dialysed overnight at 4 °C against 5 M-guanidine in carbonate-bicarbonate buffer at pH 9, 10 and 11.2 (adjusted with 1 N-NaOH). It was found that the virus particles were disrupted into fibrous strands similar to those observed after treatment with mercaptoethanol (Fig. 1D) rather than the amorphous masses observed after heating in the presence of
Fig. 2. Effect of lithium iodide and storage on the morphology of the herpes simplex virus nucleocapsid. A, morphology of virus particles heated under neutral conditions (pH 7.4) at 90 °C in the presence of 10 % lithium iodide for 5 min. B, C, D, morphology of virus particles heated at 90 °C in the presence of 10 % lithium iodide for 5 min and subsequently dialysed overnight against carbonate-bicarbonate buffer (pH 10). E, F, effect of storage at 4 °C in RSB on the morphology of the herpes simplex virus nucleocapsid.
4 or 6 M-guanidine (Fig. 1E). There appeared to be little difference in the morphological appearance of the material whether it was treated at pH 9, 10 or 11.2. Virus particles dialysed overnight at 4 °C against the alkaline buffer under the same conditions in the absence of guanidine showed no morphological alterations.

The effect of guanidine would appear to be primarily on intracapsomeric bonds, in as much as free capsomeres were not found nor was the capsomeric structure still discernible within the amorphous masses. However, treatment of the nucleocapsid with 5 M-guanidine under alkaline conditions without heating (i.e. at 4 °C) resulted in the disruption of the virus into fibrous strands. Again these results suggest that ionic bonds which are weakened under alkaline conditions are active in stabilizing the intercapsid interaction.

The effect of breaking both hydrogen and disulphide bonds was examined by treating virus in carbonate-bicarbonate buffer (pH 10) with 2 M-guanidine and mercaptoethanol (final concentration 0.13 M) and heated at 90 °C. After 5 min of heating, the capsomeres of the particles appeared to have collapsed around a central core structure (Fig. 1F). In some cases, structures measuring approximately 40 to 50 nm in diam. which morphologically resembled the virus core, were observed lying free (Fig. 1F). If such structural components do exist, whether or not they truly represent virus cores remains unknown, however, their size and morphology were similar to structures observed within partially disrupted virus particles.

Lithium iodide has been reported to cause conformational changes in polypeptides by the specific binding of the salt to peptide linkages (Kurtz & Harrington, 1966). The effect on the integrity of the herpes virus nucleocapsid was examined by mixing virus particles in RSB (pH 7.4) with crystals of lithium iodide (K and K Laboratories, Plainview, N.Y.) to give a final concentration of 10% or 15% in the presence of 2.5 x 10^-3 M-Na2S2O3 (Neurath, Stasny & Rubin, 1970) and heating at 90 °C for 5 min. The capsomeres appeared to lose their integrity and collapsed around the central core (Fig. 2A). There was little difference in the morphological appearance of the virus particles between 10 and 15% concentrations. Heating for up to 30 min did not result in further morphological changes. There appeared to be no morphological alterations of the nucleocapsid in parallel preparations held at 4 °C in the presence of lithium iodide. Exposure of the virus particles after treatment with 10% lithium iodide and heating at 90 °C for 5 min to alkaline conditions was achieved by dialysis against carbonate-bicarbonate buffer (pH 10) at 4 °C for 18 h. This treatment resulted in the further disruption of the nucleocapsid. The sequence of degradation apparently followed first the partial unfolding of the polypeptide chains which comprise the morphological subunits (Fig. 2B). The polypeptides continued to unfold (Fig. 2C) and finally were found to aggregate in large masses (Fig. 2D).

After purification, virus nucleocapsids were routinely stored in RSB (pH 7.4) at 4 °C. It was found that after several weeks the particles started to disintegrate spontaneously into both the fibrous material (Fig. 2F) typical of that found after either mercaptoethanol or lithium iodide treatment, and into sheets of intact capsomeres (Fig. 2E). In addition, an internal core component measuring 40 to 60 nm in diam. could be observed in many of the disrupted particles (Fig. 2E–2G) which resembled the structure found after guanidine and lithium iodide treatment. In rare cases, components which resembled the cores were found lying free (Fig. 2G).

The above results indicate that at least three distinct bonding forces appear to be important in the maintenance of the morphological integrity of the herpes virus nucleocapsid; namely, disulphide, hydrogen and ionic bonds. This is supported by the finding that intracapsomeric bonds were disrupted by heating in the presence of guanidine, mercaptoethanol
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or lithium iodide, while the intercapsomeric bonds were disrupted only under alkaline conditions or in the presence of high concentrations of guanidine. In as much as the precise nature of the bonds that are disrupted by each of these various treatments is not completely understood, it is difficult to define with absolute certainty the nature of the covalent and non-covalent bonds which are responsible for maintaining the integrity of the herpes virus nucleocapsid. However, the results do suggest that the integrity of the capsomeres was maintained primarily by disulphide and hydrogen bonds while the intercapsomeric structure was maintained primarily by ionic bonds.

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REFERENCES


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