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On the Release of Viral Nucleic Acid from the Icosahedral Capsid

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SUMMARY

A hypothesis that the nucleic acid of an icosahedral virus is released through a capsomere at the vertex of the icosahedral capsid is presented in this paper. The hypothesis is based on the morphological observation on cytoplasmic polyhedrosis virus showing that the core substance can be released through a capsomere at the vertex of the icosahedral capsid and on the presence of a morphological apparatus, which is regarded as involved in the release of the viral core substance, at vertices of the icosahedral capsid or core membrane of other icosahedral viruses.

The nucleic acid configuration within icosahedral capsids, the alteration of the capsid protein associated with the release of the nucleic acid, and the early events during infections with these viruses are discussed in relation to this hypothesis.

INTRODUCTION

T-even phages attach to host cells by the tips of their tails and their tail fibers and inject viral nucleic acids into the host cells through their tail cores, while the viral protein shells remain extracellularly on the surface of the host cell (Hershey and Chase, 1952; Kellenberger and Arber, 1955; Barrington and Kozloff, 1956; Brenner *et al.*, 1962). The contractile system of the tail sheath is thought to be involved in the injection mechanism (Kozloff and Lute, 1957; Brenner *et al.*, 1959).

However many animal viruses enter into host cells as virions and intracellularly release the viral nucleic acid (Higashi, 1959; Dales and Siminovitch, 1961; Dales, 1962; Mussgay and Weibel, 1962; Mandel, 1962; Holland and Hoyer, 1962; Silverstein and Marcus, 1964 and Joklik, 1964). There is little information concerning the mechanism of the release of the viral nucleic acids of these viruses during the early stage of viral infection. However recently data has been accumulated showing that "spherical" or icosahedral viruses can release their nucleic acids from the capsids *in vitro*, without breaking down the capsids. Alkaline degradation of RNA of turnip yellow mosaic virus leaves structurally intact empty shells (Kaper, 1960). Urea treatment (Cooper, 1962) and heat treatment (Hummeler and Hamparian, 1958; Hinuma *et al.*, 1963) of poliovirus results in empty shells of intact virions. Heating of $\phi \times 174$ expels a fibrous core, leaving a more or less intact outer coat (Maclean and Hall, 1962). Recently Philipson and Lind (1964) have shown that the interaction of ECHO 7 virus and the receptor *in vitro* resulted in the uncoating of the infectious RNA, without gross alteration of the morpholo-

gical appearance of the ECHO 7 virus particle. These findings indicate that the nucleic acid of many icosahedral viruses may be released from the capsids without the capsids being broken down during the early stage of the viral infections.

This paper describes how the nucleic acids of icosahedral viruses (nucleocapsids) are able to be released from the capsids, without break down of the capsids, though the case is only concerned with icosahedral viruses without envelope, to avoid complication.

RESULTS AND DISCUSSION

1. *Morphological considerations on liberation of the core substance of the cytoplasmic polyhedrosis virus (CPV) of the silkworm, Bombyx mori (Linnaeus) from the capsid*

Fig. 1 shows a morphological model of CPV (Hosaka and Aizawa, 1964). It is seen from the model that there are connecting tubes between the vertices of the capsid and the core membrane, and that these, together with the capsomere and surface projection of each vertex of the icosahedral capsid form a continuous morphological passage connecting from within the core to outside the virion of CPV. The inner diameter of the passage was estimated as 20-40A, although it is difficult to measure exactly because the inner diameters of the small tubes vary considerably due to phosphotungstic acid embedding.

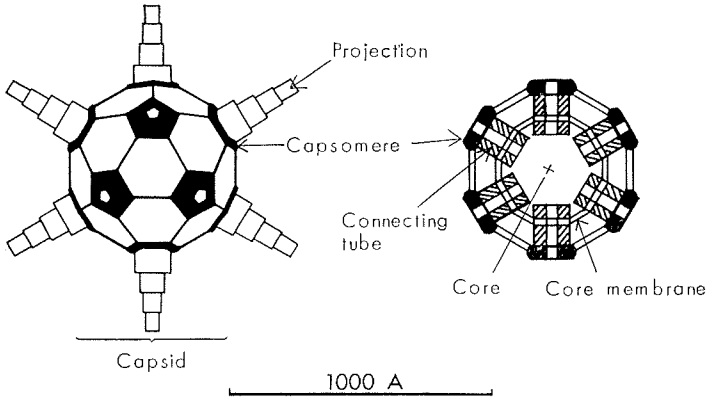

Fig. 1. Morphological Models of CPV. Left; surface structure, Right; internal structure. The 3 forward projections on the surface are omitted. The model shows that the connecting tube, the capsomere and the projection form a continuous passage from within the core to the outside of the virion.

In the previous report (Hosaka and Aizawa, 1964), it was described that CPV has two icosahedral shells and the region within the inner shell correspond to the core. In the present paper, the outer shell will refer to the capsid and the inner shell, the core membrane.

Fig. 2 shows a purified preparation of CPV by the negative staining technique.

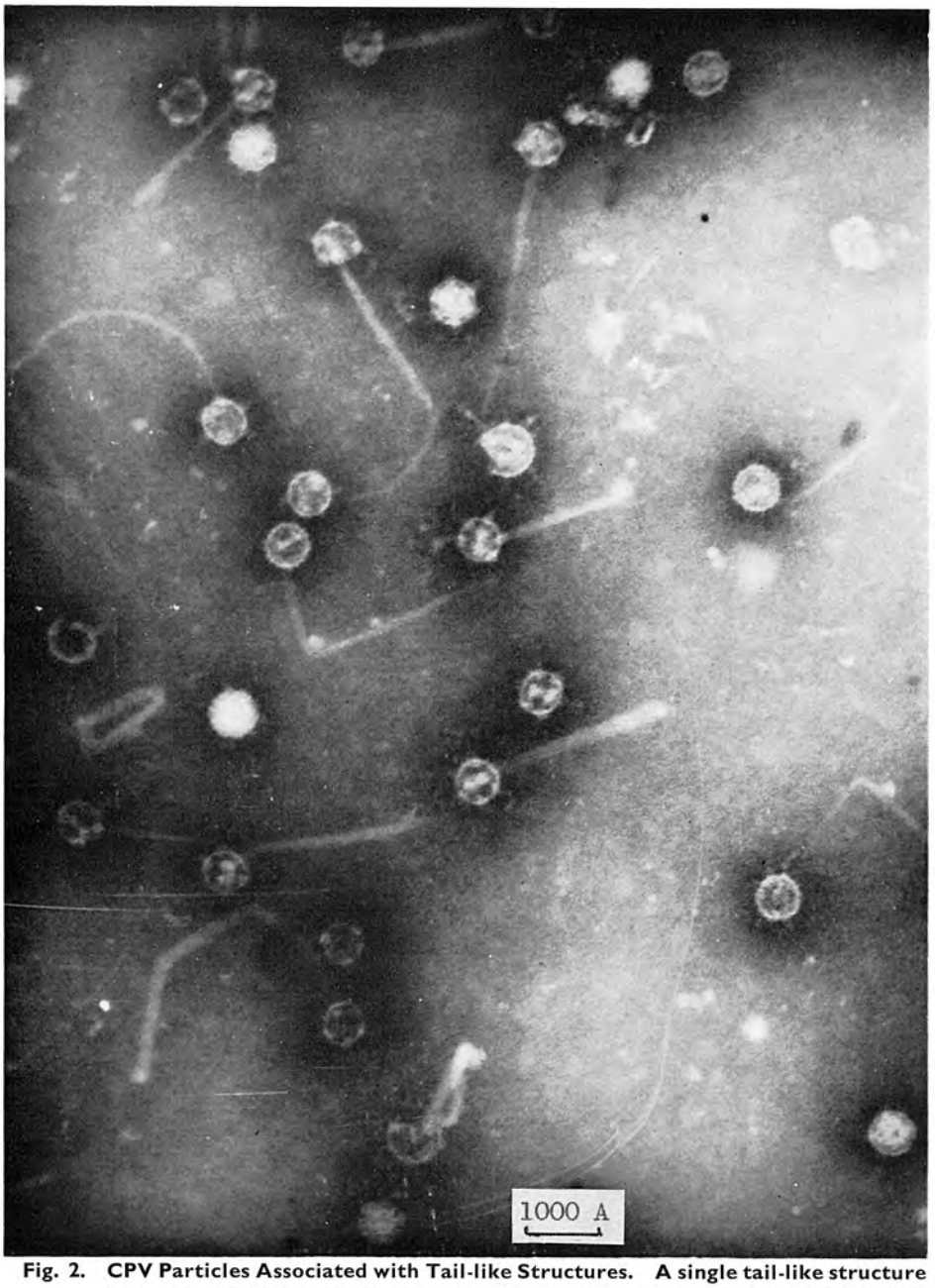

Fig. 2. CPV Particles Associated with Tail-like Structures. A single tail-like structure is associated with a single empty shell but never with an intact virion. Tail like structures are seen running from capsomeres.

In Fig. 2, several tail-like structures are seen running from empty shells of CPV and some of them clearly seen running from capsomeres of the empty shells. One tail-like structure is associated with one empty shell but not with an intact virion, although the tail-like structure separated from the virus particle also occurs. This finding leads to the interpretation that the tail-like structure is the core substance of CPV and the release of the core substance from the intact vition results in the production of an empty shell, some of which are still connected with the core substance. According to this interpretation, the finding that the tail-like structure runs from the capsomere at the vertex of an empty shell indicates that the core substance of CPV is released from the capsomere at a vertex of the icosahedral capsid.

Thus the hypothesis of the core substance passing through the morphological passage of CPV, postulated from the model in Fig. 1, is verified by the findings shown in Fig. 2. The maximum length of the tail-like structure is approximately 0.5 μ. This structure may be a bundle of nucleic acid fibers. Tail-like structures are frequently observed when purified CPV is suspended in hypertonic solutions (Hosaka and Aizawa, unpublished data). In this *in vitro* case, the core substance of CPV is considered to be released as a bundle of nucleic acid fibers.

In the present study, "the core substance" is used as a morphological term. Morphologically an icosahedral virion without an evelope, that is an icosahedral nucleocapsid, consists of a core and a capsid. The capsid is a protein shell and the core is the area within the protein shell. The core substance is a nucleic acid only or otherwise may be a nucleic acid associated with a trace of protein. An empty shell is a capsid without the core substance.

2. *The morphological apparatus on the vertices of capsids or core membranes of icosahedral viruses and its biological significance*

Morphological studies were made on whether the liberation of the viral core substance from the icosahedral capsid is possible in other icosahedral viruses.

Markham *et al.*, (1963) reported that the core membrane of carnation mottle virus was an icosahedron, with a capsomere-like morphological structure of a hollow polygon at each vertex. This morphological structure at the vertex of the icosahedral core membrane must be connected with the capsomere at the vertex of the icosahedral capsid. The inner diameter of the morphological structure is 30 A. Thus the carnation mottle virus has morphological passages, from within the core to outside the capsid at the vertices of the icosahedral capsid. These passages can similarly be interpreted as a releasing canal for the core substance of carnation mottle virus.

The icosahedral capsid of rice dwarf virus (RDV) consists of 5 capsomeres per edge (Total, 162 capsomeres) (Hosaka, in preparation). The core membrane of RDV is also icosahedral and has a morphological unit at each vertex. Therefore the morphological unit at the vertex of the core membrane must be in contact with

the capsomere at the vertex of the capisd. Thus it is possible that the capsomere at the vertex of RDV capsid forms a morphological passage from within the core to outside the capsid.

Tromans and Horne (1961) reported that an icosahedral capsid of $\phi \times 174$ phage had projections at its vertices and the projections might be involved in the mechanism of the injection of the DNA of $\phi \times 174$ phage.

Thus the above morphological data indicate that in general, the capsomere at the vertex of an icosahedral capsid may function as the passage for the core substance from within the core to outside the capsid.

The capsids of icosahedral viruses other than above described, also consist of capsomeres of hollow prisms, although such a specialized organ as the projection is not yet known. The capsomere at the icosahedral vertex seems to be a hollow pentagonal prism while others are hollow hexagonal prisms (Widly *et al.*, 1960; Horne and Wildy, 1961). The inner diameter of the capsomeres was estimated to be 15-40A. (Howatson and Grawford, 1963; Vasquez and Tournier, 1962; Wildy *et al.*, 1960a; Wright *et al.*, 1964). Therefore the hypothesis of the liberation of the core substance through the capsomere on other icosahedral capsids is not impossible.

In some icosahedral viruses, as for example in poliovirus, the capsomere arrangement on the capsid has not yet been identified by electron microscopy (Horne and Nagington, 1959). In such cases, the arrangement of a number of structure units at the five fold symmetrical axes is tentatively assumed as the morphological apparatus involved in the release of the core substance.

In regard to the above hypothesis, it is interesting to recall that T-even phages have their injection apparatus of DNA, tail, at the vertices of hexagonal pyramids of their heads.

3. *Configuration of viral nucleic acid within an icosahedral capsid and its relation to the release of nucleic acid.*

Based on the above interpretation, an icosahedral capsid must have 12 morphologically equivalent passages for the core substance, because an icosahedral capsid has 12 vertices and the 12 capsomeres at the vertices are arranged symmetrically. If an icosahedral capsid contains a unit of nucleic acid, the core substance would be released from one of the 12 capsomeres, as a single intact unit. It seems unlikely that disrupted pieces of a core substance are released from different capsomeres. Recently, it has been shown that the parent viral nucleic acid remains intact even in later stages of mengovirus (Tobey, 1964) and RNA phage infection (Doi and Spiegelman, 1964).

The problem arises of how the capsomere used for the release of the core substance is determined among the 12 possible capsomeres, if the core substance is released through only one capsomere as a single unit. The capsomere may be determined by the configuration of the core substance within the icosahedral

capsid or by factors other than the primary configuration of the icosahedral nucleocapsid, as for example, successful contact with the uncoating receptor. At present, it is impossible to give a conclusive answer to this problem. However, if the latter is the case, the core substance must become rearranged within the icosahedral capsid, so that the end of the core substance can be adapted for release through the capsomere with successful contact with an uncoating receptor. The evidence that the viral nucleic acid has a regular structure within the icosahedral capsid (Klug and Finch, 1960) seems to exclude the possibility of free rearrangement of the core substance within the icosahedral capsid.

The lengths of the viral nucleic acids in the icosahedral capsids must be in the order of μ judging from their nuleic acid contents, but the diameters of most icosahedral capsids are less than 100 mμ. Therefore the nucleic acid must be folded an possibly form a bundle of fibers within the capsid. The tail-like structures of CPV shown in Fig. 2 would represent one such form of bundle of nucleic acid. Klug and Finch (1960) studied the configuration of the RNA of turnip yellow mosaic virus (TYMV) within the capsid by X-ray diffraction and showed that the RNA within the particle must possess some kind of regular structure and may have 12 subunits. "This does not mean that the RNA cannot be a single chain, for the "subunits" would refer to the mode of winding of the RNA chain". The most probable explanation for the presence of 12 "subunits" of the RNA is that some kind of folding of the RNA bundle occurs corresponding to the 12 vertices of the icosahedral capsid, so that the RNA conforms to the caspid. If this is so, the ends of the RNA bundle would not be distributed at random but possibly would be at points relative to the folding points of RNA, that is, at the vertices of the icosahedral capsid. Thus it is possible that the end of the bundle of the viral nucleic acid fiber is at a vertex of the icosahedral capsid and therefore the nucleic acid may be easily released through the capsomere at the vertex. According to this idea, it is possible that the configuration of the nucleic acid determines which one of the 12 capsomeres the core substance is released from. The fact that some of the fiber of T2 phage DNA within the head are arranged parallel to the tail, (North and Rich, 1961) seems to support the idea that viral nucleic acid is oriented favorably for its release through the releasing apparatus.

4. *Release of nucleic acid from the icosahedral capsid and rearrangement of the capsid protein subunits*

The N antigenicity of the poliovirus capsid containing nucleic acid is converted to the H antigenicity of the empty shell by heating, with simultaneous liberation of the nucleic acid (Hummeler and Hamparian, 1958; Hinuma *et al.*, 1963). Hummeler *et al.*, (1962) clearly demonstrated by electron microscopy that two antigenic particles were agglutinated by respective specific antisera and did not cross-react.

This antigenic conversion of the poliovirus capsid, of course, does not mean

a change of its amino acid composition. There must be an alteration of the whole arrangement of the capsid protein subunits, because a N antigenical particle is completely converted to a H antigenical particle and a mixed antigenical particle does not occur. It is anticipated that the structure of the capsomere through which the nucleic acid is released, must be altered somehow on release of the nucleic acid. However, the symmetry of the capsid structure as a whole probably would not allow an alteration of only a small part of the capsid protein subunits.

Here the question arises of whether the rearrangement of the capsid protein subunits of poliovirus is required for liberation of the nucleic acid or is a result of their liberation. Holland and Hoyer (1962) found that the interaction of poliovirus and the plasma membrane receptor results in alteration of the capsid antigenicity and loss of its resistance to proteolysis, its specific attachment sites and its infectivity but not in the release of the nucleic acid. Since these alterations of the poliovirus capsid must be due to a rearrangement of the protein subunits, this indicates that a rearrangement of the capsid protein subunits, can occur without release of the nucleic acid. Holland and Hoyer suggested that these reactions might be the first stage ones for the release of the nucleic acid. Although it is not yet known whether or not the rearrangment of the capsid protein subunits triggered by the membrane receptor is the same as that accompanied by nucleic acid release on heating, the two rearrangements are very similar. The above findings seem to favor the idea that the rearrangement of the capsid protein is a prerequisite rather than a result of the release of the nucleic acid.

The empty shell of poliovirus produced by heating was slightly smaller than the intact capsid (Hosaka and Hinuma, unpublished). The contraction of the polio capsid, associated with the alteration of the capsid protein, may indicate a mechanism analogous to the injection mechanism of T-even phages. Empty shells of smaller sizes than the intact capsids were observed with other icosahedral capsid, polyoma virus (Wildy *et al.*, 1960) and cytoplasmic polyhedrosis virus (Hosaka and Aizawa, 1964). Further Holland and Hoyer (1962) showed that the alteration of the capsid can be prevented by neutralizing antibody, possibly because the antibody reactive sites bridge capsid subunits (Almeida *et al.*, 1963) in such a way as to hinder subunit rearrangement. This is one explanation for the neutralization mechanism of one icosahedral nucleocapsid by one antibody (Dulbecco *et al.*, 1956). Mandel (1958, 1962) showed that the antibody did not prevent the attachment of poliovirus to host cells, when the concentration of antibody is not so high.

5. *Release of the core substance during early stages of icosahedral virus infection.*

Many icosahedral viruses attach to, and enter host cells and release their nucleic acid from the capsid into the host cells, during early stages of infection. Recently, these early stages have been extensively studied with the enterovirus-host cell systems.

The first attachment of poliovirus is temperature independent and reversible by high concentration of salt, acid and urea and is inhibited by high concentration of antibody (Holland, 1962; Mandel, 1962b). The subsequent entrance is temperature dependent and the virus during this reaction is progressively resistant to antibody and to treatments which dissociate the first virus-cell complex (Holland 1962; Mandel, 1962b; Philipson and Bengtsson, 1962). The final reaction releasing nucleic acid is probably also temperature dependent and beyond reach of antibody. Recently, Philipson and Lind (1964) have succeeded for first time in the isolation of the uncoating receptor for ECHO 7 virus from human erythrocyte membranes and demonstrated that the high molecular and infectious RNA of ECHO 7 virus is released from the capsid following the interaction of the virus with this receptor *in vitro* and that this reaction is temperature dependent.

The capsid of poliovirus altered, by the plasma membrane receptor becomes sensitive to proteolytic enzymes (Holland and Hoyer, 1962). The acquisition of this sensitivity does not necessarily mean that capsid degradation is a prerequisite for the release of the nucleic acid, because the criterion for the acquisition of such lability is the sensitivity of the RNA infectivity to RNase after the capsid has been treated with proteolytic enzymes and is not a test of the degradation of the capsid itself. The further interaction of the altered capsid with uncoating receptors within host cells naturally would expose its RNA. There are many examples of empty shells that still retain their morphological integrity after releasing the core substance. Thus, the most probable sequence of events involved in release of the core substance during infection is the first rearrangement of the capsid protein subunits associated with slight contraction of the capsid, next, the release of the core substance and then the degradation of the altered capsid.

Eluted poliovirus (Joklik and Darnell, 1961) has similar properties to the capsid which has been altered by the plasma membrane receptors. The eluted poliovirus is considered to be altered virus which did not contact with uncoating receptors and was desorbed from cell membranes, being affected by unknown factors.

For an intact icosahedral nucleocapsid to be infectious, the nucleocapsid would have to follow the sequence of reactions described above, during the early stages of viral infection. This means that, if one of these reactions is imcomplete or defective, the nucleocapsid would not be infectious and this seems to be one factor leading to low plating efficiency reported for icosahedral viruses.

In this paper, the release of viral nucleic acid from icosahedral capsids were considered, irrespective of animal, plant and insect viruses. There is no basal difference in the morphology and the chemical and physical nature among these viruses. Therefore it is possible to assume some common aspects in the mechanism of the release of the viral nucleic acid from the icosahedral capsids.

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