THE STRUCTURE OF MOUSE-ELBERFELD VIRUS: A MODEL*

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Abstract.—It is proposed that the capsid structure of mouse-Elberfeld (ME) virus, a small icosahedral ribonucleic acid containing picornavirus, is determined by 60 identical protein subunits; each of the latter is composed of several non-identical polypeptide chains. Icosahedral symmetry in a 60-subunit shell allows three types of specific intersubunit bonding contacts which establish its axes of two-, three-, and fivefold symmetry, respectively. Of these three bonding types, two are sufficient to specify a complete shell. We interpret the possible identity of some discrete supramolecular structures involved in the biosynthesis of poliovirus and in the degradation of ME virus in the context of stepwise formation or disruption of two different types of specific intersubunit bonding contacts.

Introduction.—Theories relevant to the architectural design of icosahedral viruses containing one type of protein subunit are well developed.1–4 The central element of these theories is that the protein shell is constructed from identical subunits symmetrically packed into equivalent or nearly equivalent environments so that the same intersubunit bonding contacts are used repeatedly.1,2 However, the existence in many picornaviruses of several non-identical polypeptide chains has left open to speculation the organizational principles governing their structure. Indeed, it has been suggested6 that their architecture may differ fundamentally from that of viruses containing identical polypeptide chains. Our studies on ME virus indicate that the structure of this murine picornavirus10 is determined by 60 identical multichained protein subunits and suggest that the principles governing picornaviral architecture are the same as those operating on the small plant and bacterial viruses.

Materials and Methods.—Isotopically labeled and unlabeled ME virus was prepared and purified as described previously.7 Virus to be electrophoresed was subjected to disulfide interchange11 by incubation in 0.1% sodium dodecyl sulfate (SDS), 0.005 M ethylenediaminetetraacetic acid, 0.1 M N,N'-diacetyl cystamine, $10^{-4}$ M dithiothreitol for 45 min at 45°C. Methods for electrophoresis, staining, and determination of radioactivity on SDS polyacrylamide gels have been described previously.12 Estimation of the molecular weights of the viral breakdown fragments was done by the Yphantis method of sedimentation equilibrium.13

Results.—The polypeptide elements of ME virus and their molecular weights: The radioactivity profile of $^3$H-leucine-labeled capsid polypeptides from disrupted virus reflects the same pattern as that observed on stained gels (Fig. 1). The apparent molecular weights of these components (Table 1, column 2) were obtained by comparison of their electrophoretic mobility on the SDS-containing gels14 with those of well-characterized proteins. Included for comparison are the molecular weight values obtained by Maizel and Summers15 for the polypeptides of poliovirus.

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**TABLE 1. Polypeptide elements of the ME virion.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Molecular Weight</th>
<th>Fraction of total (^4\text{C})†</th>
<th>Molar ratio (\times 10^5)</th>
<th>Chains per virion§</th>
<th>Cysteine residues per chain**</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)</td>
<td>33,000</td>
<td>35,000</td>
<td>0.340</td>
<td>1.03</td>
<td>59</td>
</tr>
<tr>
<td>(\beta)</td>
<td>30,500</td>
<td>28,000</td>
<td>0.309</td>
<td>1.01</td>
<td>58</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>25,000</td>
<td>24,000</td>
<td>0.277</td>
<td>1.10</td>
<td>63</td>
</tr>
<tr>
<td>(\delta)</td>
<td>10,000</td>
<td>6,000</td>
<td>0.059</td>
<td>0.59</td>
<td>34</td>
</tr>
<tr>
<td>(\epsilon)</td>
<td>41,000</td>
<td>41,000</td>
<td>0.015</td>
<td>0.037</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Estimated by electrophoresis on SDS-containing polyacrylamide gels. Included in column 3 are the apparent molecular weights of the electrophoretically analogous chains of poliovirus.\(^1\)
† Calculated from the radioactivity content of each peak derived from virus labeled with \(^4\text{C}\)-amino acid mixture.
‡ Moles of component per gram of total protein; calculated by dividing the mass ratio (column 4) by the molecular weight (column 2).
§ The number of chains, \(n\), per virion was calculated from the relation
\[
W = \frac{14C - \text{content}}{\text{protein mass}} \times \frac{\text{mass fraction of each polypeptide component}}{\text{molar proportion of each component}}
\]
where \(W\) represents the total weight of the capsid protein in daltons and \(R\) is the mass fraction of each polypeptide component (moles per gram of total protein) calculated on the assumption that the \(^4\text{C}\)-content is directly proportional to mass. \(W\) was estimated on the assumption that each virus particle contained 60 each of the \(\alpha\), \(\beta\), and \(\gamma\) chains and that these constitute 93% of the total protein. Then \(W\) is approximately 60 \((35,000 + 30,500 + 25,000)/0.93\) or 5.7 \(\times 10^6\) daltons of protein per virion.
** Determined from the radioactivity content of each peak derived from virus labeled with \(^35\text{S}\)-cysteine.

Molar proportions of the polypeptides: Given its molecular weight, we can determine the molar ratio of each component by measuring its mass proportion in the viral capsid. We have attempted to make such a measurement from the electrophoretic radioactivity profile of disrupted whole virus, uniformly labeled with a \(^4\text{C}\)-amino acid mixture, assuming that radioactivity is a direct measure of protein mass. The \(\alpha\), \(\beta\), and \(\gamma\) peaks contained 34, 31, and 28 per cent, respectively, of the total recovered radioactivity (Table 1, column 4). It is apparent that the three large peaks are present in approximately equimolar proportions (Table 1, column 5). There appear, however, to be only about half as many delta chains and only about 1/30 as many epsilon chains.

Cysteine content of the polypeptides: \(^35\text{S}\)-cysteine-labeled virus was purified from infected cultures grown in the presence of the labeled amino acid. The relative molar proportions of the cysteine in the electrophoretically separated

**FIG. 1.—Polyacrylamide electrophorograms of the capsid polypeptides of ME virus.**

Stained gel insert: 25 \(\mu\)g of highly purified intact virus was disrupted as described in Materials and Methods. Electrophoresis (with the anode to the right) and staining are as cited. The five peaks are designated \(\epsilon\), \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\) in order of decreasing size. The minor components, \(\epsilon\) and \(\delta\), are not clearly visible in the photograph.

Radioactivity profile: Highly purified virus labeled with \(^1\text{H}\)leucine was disrupted, \(S\)-substituted, electrophoresed, and mechanically fractionated as cited in Materials and Methods.
\(\alpha, \beta,\) and \(\gamma\) peaks was determined by radioactive content to be 5.0:2.0:3.0 (Table 1, column 7). In order to check the validity of this experiment, the cysteine content of ME-virus protein was determined independently by disulfide interchange\(^{11}\) with \(^{35}\text{S-N, N'}\) diacetylcystamine. This determination, which will be described elsewhere in detail, indicated the presence of about ten cysteine residues per 86,000 daltons of whole virus protein. The \(\alpha, \beta,\) and \(\gamma\) chains of this protein, when electrophoretically resolved, contained \(^{35}\text{S}\) ratios of 5.0:2.0:2.4, which are in reasonable agreement with those obtained by the internal labeling procedure. It was also found by both methods that the \(\delta\) component contained no cysteine. Preliminary experiments indicate that the \(\epsilon\) component contains two to three cysteine residues per chain.

**Fragments from controlled dissociation of ME virus:** Ultracentrifugal studies on density gradients show that \(^{3}\text{H-leucine-labeled virus is converted by mild acid treatment to a product which sediments as a single peak at 14S. This subunit was further dissociated by a short treatment with 2 M urea to a product sedimenting as a single peak at about 5S on density gradients. We have isolated each of these fragments free of RNA by gel filtration after ribonuclease treatment. Both subunits contained all three of the nonidentical polypeptides in equimolar proportions (Table 2) and under suitable conditions behaved in sedimentation equilibrium studies\(^{13}\) as homogeneous materials with molecular weights of 420,000 and 86,000, respectively. Neither fragment contained appreciable amounts of \(\delta\) or \(\epsilon\) chains. Details will be reported elsewhere in full. We conclude that the 5S subunit must correspond to a molecule (protein "monomer") containing one of the three nonidentical polypeptides and that the 14S subunit represents an oligomer composed of five of the smaller 5S subunits.

**Discussion.—The model:** There is a close crystallographic similarity between poliovirus\(^4\) and turnip yellow mosaic virus (TYMV).\(^{16}\) TYMV appears to contain 180\(^7\) identical polypeptide chains consisting of 32 capsomeres\(^{17-19}\) arranged as 12 sets of five and 20 sets of six polypeptides.\(^{17}\) As shown by Klug and Finch (see Fig. 1b in their paper),\(^{16}\) it is impossible to put more than 60 identical subunits on the surface of a sphere so that all are identically situated. In fact, they have pointed out that 180 identical chains must necessarily be distributed into three sets of structurally distinct bonding domains (60 of each kind). It was this circumstance—that identical bonding contacts cannot be established by the same subunit in different environments—which forced Caspar and Klug to formulate the concept of quasi-equivalence,\(^4\) i.e., the existence of slight deformations of the bonding contact patterns between subunits.

<table>
<thead>
<tr>
<th>Component</th>
<th>Molecular weight*</th>
<th>Per cent of Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5S</td>
<td>86,000</td>
<td>((\alpha + \beta)) 76</td>
</tr>
<tr>
<td>14S</td>
<td>420,000</td>
<td>(\gamma) 24</td>
</tr>
<tr>
<td>Virus</td>
<td></td>
<td>74</td>
</tr>
</tbody>
</table>

* Determined by sedimentation-equilibrium measurements.
† Expressed as per cent of total radioactivity in the three components derived from \(^{3}\text{H-leucine-labeled virus. The alpha and beta components were incompletely resolved. The delta component contained about 10% of the total activity in whole virus, less than 2% in the delta and 14S fragments.**
A natural alternative to 180 identical chains in quasi-equivalent positions is three different sets of 60 identical polypeptide chains in equivalent positions corresponding to the three sets of 60 bonding domains mentioned above. Such an arrangement, which is at least formally equivalent to the 180-chain model of TYMV, satisfies the requirement for icosahedral symmetry and generates a model for ME virus, which is represented in Figure 2. Use of the numbers 1, 2, and 3 avoids the necessity of making specific assignments for \(\alpha\), \(\beta\), and \(\gamma\). The model is schematic and is not intended to represent the actual shape of the shell or its polypeptide elements. It implies that each polypeptide chain has specific neighbors but does not specify bonding contacts.

It is apparent that the polypeptide chains in such a structure may be clustered by certain strong bonding contacts into at least two basically different arrangements. One arrangement consists of 12 (one for each vertex of the icosahedron) identical chemical pentamers (Fig. 2, \(E_1\)) and 20 (one for each face) chemical hexamers (Fig. 2, \(E_2\)).

A second arrangement that can be described in terms of strong bonding contacts between 1, 2, and 3 leads to formation of 60 morphological subunits (Fig. 2a and b, A). In this case, the chemical, morphological, and crystallographic structure units are all identical. Such a subunit would contain each of the nonidentical polypeptide chains in equimolar amounts and would have a molecular weight of about 88,000. The 5S subunit fits this description almost exactly and, furthermore, is very similar to the structure unit postulated by Finch and Klug from crystallographic studies on poliovirus. Thus our basic model for ME virus is essentially the 60 identical protein subunit model of Finch and Klug with the added information that the subunit is composed of three physically associated nonidentical polypeptide chains. Such a model represents the simplest icosahedral surface lattice group\(^1\) \(T = 1\).

Some oligomers which might be expected through formation of additional bonding contacts with A are outlined in Figure 2a,b. Represented are the 2-mer (B), 3-mer (C), and 5-mer (D), which can be described as monomers bonded together by one specific contact between the 3–3, the 3–2, or the 1–1 sites, respectively. The expected sedimentation velocities calculated for such fragments are listed in Table 3.

The 14S fragment from ME virus has the physicochemical properties required by structure D (Fig. 2), i.e., a molecular weight five times that of A and equimolar proportions of each of the three nonidentical structural polypeptides. Such a structure has an axis of fivefold symmetry and can be visualized, in fact, as one of 12 interlocking rosettes from which the entire shell can be assembled by forming, for example, 3–3 bonds.

The minor components and intermediates in the assembly of poliovirus: Recent work on the biosynthesis of poliovirus suggests that maturation involves cleavage\(^{20–22}\) of a precursor polypeptide to form smaller chains. Examination of Table 1 (column 2) shows that the molecular weight of the \(\epsilon\) chain (41,000) is close to the sum of the molecular weights of the \(\beta\) (30,500) and \(\delta\) (10,000) chains. This observation suggests that the \(\beta\) and \(\delta\) chains may result from a single cleavage of the \(\epsilon\) chain. While verification of such a precursor-product relation-
A schematic model of the ME capsid consisting of 180 polypeptide chains (60 of each nonidentical type) arranged in an icosahedral surface lattice consisting of three different bonding domains.

(a) An icosahedron has 12 vertices and 20 faces. Only the fivefold vertex of each icosahedron is shown. Marked in bold outline is the set of possible morphological subunits generated by different sets of bonding contacts joining the chains. $E_1$ and $E_2$ represent the clustering pattern of a 32-capsomer shell consisting of 12 five-coordinated ($E_1$) and 20 three-coordinated ($E_2$) chain groupings. We have found no evidence supporting this structure.

$A$ represents one of 60 of the postulated structure units of ME virus. It depicts a protein consisting of three nonidentical polypeptide chains. Possible oligomers of this structure unit are the 2-mer ($B$), the 3-mer ($C$), and the 5-mer ($D$). Each is the consequence of a different bonding contact. Subunits satisfying the description of $A$ and $D$ are described in the text.

(b) Styrofoam-ball models of the subunits outlined in (a).

(c) A shell composed of 180 balls, 60 of each type as seen from the fivefold symmetry axis. The actual shapes of the subunits are unknown, but the three different polypeptide chains may be fused and intertwined to form a single protein subunit.
ship requires evidence of sequence homologies, the relative size and also the number of cysteine residues (Table 1, column 7) per chain \((2 + 0 = 2)\) are in accord with this notion.

This scheme implies that the mature virion should contain 60 each of the \(\beta\) and \(\delta\) chains (one each from the 60 \(\epsilon\) precursors), and no \(\epsilon\) chains. To account for the two \(\epsilon\) chains and the 30–35 \(\delta\) chains which were actually found, it might be assumed that not all the \(\epsilon\) chains are cleaved and that about half of the \(\delta\) chains can dissociate from the virion. Such a distribution might arise as a consequence of events involved in viral maturation. The location and possible function of the \(\delta\) and \(\epsilon\) chains remain to be determined.

The cleavage hypothesis suggests that the postulated \((\alpha, \beta, \gamma)^{23}\) structure unit of the mature virus may derive from an “immature” \((\alpha, \epsilon, \gamma, \delta)^{23}\) subunit by cleavage of its \(\epsilon\) chain to generate \((\alpha, \beta, \gamma, \delta)^{23}\). Such a subunit would weigh about 100,000 daltons \((\alpha + \epsilon + \gamma = 33,000 + 41,000 + 25,000)\) and would be expected to sediment at about 5–6\(S\) (Table 3). A 5\(S\) fragment has, in fact, been observed in poliovirus-infected cells.\(^{24}\) Two other poliovirus-related fragments sedimenting at about 10\(S\) and 74\(S\) have also been described.\(^{24}\) The latter two, and probably also the 5\(S\) particle, contain three chains which are electrophoretically analogous to the \(\alpha\), \(\epsilon\), and \(\gamma\) chains of ME virus.

Phillips et al.,\(^{24}\) in a study on particles in poliovirus-infected cells, estimated the molecular weight of a 10\(S\) component to be between 250,000 and 350,000. They pointed out that the particle was too large to represent one of 32 capsomeres (Fig. 2, \(E_1, E_2\)). However, Table 3 shows that it is about the right size for an \((\alpha, \epsilon, \gamma)^{23}\) structure, that is, for a 3-mer of our hypothetical “immature” structure unit. These observations are consistent with the hypothesis that poliovirus may be assembled from such an immature structure unit by the following sequence:

\[
(\alpha, \epsilon, \gamma)^{5S} \rightarrow (\alpha, \epsilon, \gamma)^{10S} \rightarrow (\alpha, \epsilon, \gamma)^{74S} \rightarrow (\alpha, \beta, \gamma, \delta)^{74S} \rightarrow (\alpha, \beta, \gamma, \delta)^{105S}.
\]

Such an assembly sequence could be generated by the successive formation of just two different types of bonding contacts such as a 2–3 bond (Fig. 2a) to form the 3-mer followed by either a 1–1 or 3–3 bond, either of which, if used repeatedly, could complete assembly of the shell.

Ordering of a two-step bonding contact sequence might be controlled by a

Table 3. Calculated sedimentation coefficients of oligomeric structure units.

<table>
<thead>
<tr>
<th>Oligomeric index*</th>
<th>Molecular weight</th>
<th>(S_m)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86,000</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>172,000</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>258,000</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>430,000</td>
<td>14</td>
</tr>
<tr>
<td>60</td>
<td>(5.2 \times 10^4)</td>
<td>75</td>
</tr>
</tbody>
</table>

* The oligomeric index represents the number of structure units of which an oligomer is composed. For example, the oligomer \((\alpha, \beta, \gamma)^{5}\) has an index of 5 and is called a “5-mer.” Each oligomer of this type necessarily contains equimolar ratios of each nonidentical chain.
† The sedimentation coefficient was calculated from the molecular weight according to the relation \(S_m = (M/M_0)^{1/2}\) which applies to globular or almost globular proteins with similar partial specific volumes.\(^{30}\) For this calculation, the 14\(S\) subunit was assigned a molecular weight of 430,000 (see Table 2).
delay in formation of the stereospecific complementary surface configurations required for the second bonding step until the product of the first step is complete. Such modifications of surface configuration might be accomplished enzymatically or by shifts in the folding pattern of the polypeptide chains during the subassembly process. That such surface changes actually occur in poliovirus synthesis is indicated by differences in the antigenic determinant of the 5S and/or 10S (s-antigen),\(^{25, 26}\) the 74S empty shell (C-antigen),\(^{27, 28}\) and the intact virion (D-antigen).\(^{27, 28}\)

Finally, we wish to point out that reversing the order of the bonding sequence just described would not yield the same 3-mer intermediate but rather a 5-mer (Fig. 2, D), which could then condense to form the shell with just one additional type of bond. Furthermore, it is easy to see that a 2-mer could also be an intermediate in synthesis (for example, a 3-3 bond followed by a 1-1 bond). A particle probably corresponding to a 5-mer (14S, \(\sim 420,000\) daltons)\(^{29}\) has been found in cells infected with encephalomyocarditis virus, but it is uncertain whether this fragment represents a precursor or breakdown product. It should be evident by this time that the generation of the 14S and 5S fragments of ME virus could be explained by the sequential disruption of just two types of bonds, one by mild acid treatment and the second by 2 \(M\) urea. These considerations suggest a search for 2-mers, 3-mers, and 5-mers as possible intermediates in the biosynthesis or breakdown of other viruses with icosahedral symmetry.

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§ Recipient of predoctoral training grant 1-F1-GM-40,785 from the National Institute of General Medical Sciences.
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11 Smithies, O., Science, 150, 1595 (1965).
22 Holland, J., and E. D. Kiehn, these PROCEEDINGS, 60, 1015 (1968).
23 An oligomer consisting of chains with unspecified bonding contacts is written with commas (1, 2, 3); specific bonding partners are connected with a dash, e.g. (1–2).