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The N-Terminal Heptad Repeat Region of Reovirus Cell Attachment Protein α1 Is Responsible for α1 Oligomer Stability and Possesses Intrinsic Oligomerization Function

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The oligomerization domain of the reovirus cell attachment protein (α1) was probed using the type 3 reovirus α1 synthesized in vitro. Trypsin cleaved the α1 protein (49K molecular weight) approximately in the middle and yielded a 26K N-terminal fragment and a 23K C-terminal fragment. Under conditions which allowed for the identification of intact α1 in the oligomeric form (~200K) by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the N-terminal 26K fragment was found to exist as stable trimers (80K) and, to a less extent, as dimers (54K), whereas the C-terminal fragment remained in the monomeric form. A polypeptide (161 amino acids) containing the N-terminal heptad repeat region synthesized in vitro was capable of forming stable dimers and trimers. Using various criteria, we demonstrated that the stability of the intact α1 oligomer is conferred mainly by the N-terminal heptad repeat region. Our results are summarized in a model in which individual heptad repeats are held together in a three-stranded α-helical coiled-coil structure via both hydrophobic and electrostatic interactions.

INTRODUCTION

The reovirus cell attachment protein (protein α1) is strategically located at the twelve vertices of the outer capsid of the viral icosahedron (Lee et al., 1981; Furlong et al., 1988) and plays a pivotal role in viral infectivity and tissue tropism (Sharpe and Fields, 1985). In electron microscopy, this protein can sometimes be seen as lollipop-shaped structures with proximal fibrous tails and distal globular heads projecting from the surfaces of viral particles (Furlong et al., 1988). Protein α1 purified from reovirions or from a vaccinia expression system also has a similar morphology (Furlong et al., 1988; Banerjea et al., 1988; Fraser et al., 1990). The observations that the C-terminal half of α1 contains the receptor-binding domain (Nagata et al., 1987; Yeung et al., 1989) and the N-terminal one-quarter possesses intrinsic virion-anchoring property (Mah et al., 1990) suggest that the fibrous tail and the globular head represent the N- and C-terminal portions, respectively, of this protein. These findings concur with sequence analysis of the reovirus S1 gene (encoding α1), which predicts the existence of distinct structural domains in the α1 protein (Bassel-Duby et al., 1985; Duncan et al., 1990; Nibert et al., 1990). The N-terminal one-third of α1 is highly α-helical and contains a heptapeptide repeat of hydrophobic residues, suggestive of a coiled-coil structure. This is followed by a middle region composed largely of β-sheets. The C-terminal one-third of α1 does not possess any distinct patterns and is therefore predicted to assume a complex globular structure.

The oligomeric nature of α1 has also been examined (Bassel-Duby et al., 1987; Banerjea et al., 1988). When subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under dissociating conditions (boiled in SDS-containing sample buffer), the 455 amino acid long α1 migrates as a monomeric 44K molecular weight protein. However, if the boiling step is omitted, α1 migrates as an oligomer (~200K molecular weight). This observation, coupled with the identification of a total of four bands upon chemical cross-linking of purified α1 (with the largest species migrating at a position corresponding to approximately 200K), has led to the suggestion that α1 is a tetramer (Bassel-Duby et al., 1987). The oligomerization state of α1 is apparently closely linked to its function since of the two α1 species (monomeric and oligomeric) synthesized in an in vitro transcription and translation system, only the oligomeric form is capable of binding to cell receptors (G. Leone, R. Duncan, and P. W. K. Lee, unpublished data). This observation prompted us to examine the nature of α1 oligomerization, an understanding of which should lead to better definitions of structure–function relationships of this protein.
In this report, we used trypsin treatment as an initial step to identify regions that are important for maintaining and stabilizing the α1 oligomeric structure. Of the two fragments generated by such treatment (Yeung et al., 1989), only the N-terminal fragment, but not the C-terminal fragment, was found to exist as stable trimers (and dimers to a less extent) upon SDS-PAGE analysis. Subsequent in vitro transcription and translation experiments revealed that the N-terminal one-third of α1, which harbors the heptapeptide repeat region, possesses intrinsic dimerization and trimerization function. We further demonstrated that ionic interactions, in addition to hydrophobic interactions, within the heptapeptide repeat region are also responsible for stabilizing the α1 oligomer. These observations have led to a structural model of the oligomerization domain of protein α1.

MATERIALS AND METHODS

Plasmid construction

The plasmid (pG4T3) used in the present study was derived from our prokaryotic S1-gene expression vector pSP4 (Masri et al., 1986) in which the S1 gene contained additional sequences at the 3'-end derived from pBR322 (PstI–EcoRI) during the original subcloning procedure. This plasmid was cleaved with SstI, which cuts the S1 gene at position 1397, and the synthetic linker

5'-GGCACCTGGGGCGTATTCATCGGTAC-3'
3'-CGCCGTGACCCCGTAAAGTAGC-5'

was added which contained the authentic 3'-terminal S1 gene sequence from the SstI site to the 3'-end of the gene where a unique KpnI site was added. A BamHI–KpnI fragment was isolated (from the BamHI site at position 15), which cuts immediately after the ATG initiation codon of α1 to the newly introduced KpnI site at the 3'-end of the gene) and ligated to an EcoRI–BamHI translation initiation linker (Pharmacia):

5'-AATTCGGAGGAAAAATTATG3'-3'
3'-GCCTCCTTTTTAATACCTAG-5'.

The resultant EcoRI–KpnI fragment was cloned into the EcoRI–KpnI site of pGEM-4 (Promega Biotec) to produce pG4T3.

In vitro transcription–translation

The plasmid pG4T3 was linearized with either HindIII or EcoRV restriction endonucleases and used in in vitro transcription reactions to generate full-length or truncated S1 mRNAs. The mRNAs were then translated in vitro in rabbit reticulocyte lysates according to the manufacturer's specifications (Promega). A typical translation mixture contained 50–100 ng of RNA and 20 μCi of [35S]methionine in a total volume of 25 μl. After incubation at 37°C for 45 min, reactions were stopped by the addition of 200 μl phosphate-buffered saline (PBS). Protein sample buffer (5×) was then added (final 1× concentration was 50 mM Tris, pH 6.8, 1% SDS, 2% β-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue) and the mixtures were further incubated at 37°C for 30 min (nondissociating condition) or boiled for 5 min (dissociating condition) prior to SDS–PAGE.

Trypsin digestion

Upon completion of the in vitro translation reaction, TLCK-treated trypsin (Sigma) dissolved in PBS (0.05 mg/ml) was added to the translation mixtures to a final concentration of 0.005 mg/ml (unless otherwise stated). After incubation at 37°C for 30 min, trypsin inhibitors (soybean and egg white trypsin inhibitors, Sigma) were added and the mixtures were further incubated in protein sample buffer prior to SDS–PAGE (see above).

Immunoprecipitation

The monoclonal anti-α1 antibody G5 has been previously described (Burstin et al., 1982) and shown to interact with the C-terminal tryptic fragment of α1 (Yeung et al., 1989). The N-terminal specific polyclonal anti-α1 antiserum was prepared in rabbits using the SDS–PAGE-purified trpE–α1 fusion protein (containing trpE and residues 1–158 of α1) expressed in Escherichia coli using the pATH3 vector (Cashdollar et al., 1989). Aliquots of lysates or trypsin-treated lysates were mixed with an equal volume of appropriate dilutions of the antibodies and incubated at room temperature for 1 hr. Fixed Staphylococcus aureus (10% suspension) that had been preadsorbed with BSA (5 mg/ml) was then added to the mixture and incubated for an additional 30 min. Immunoprecipitates were washed three times with wash buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100), resuspended in 140 μl high-pH buffer (50 mM Tris, 0.1 M H3PO4, 2 mM DTT, 0.1% SDS, 6 M urea, pH to 11.6 with NaOH), and incubated at 37°C for 45 min. Suspensions were then pelleted and supernatants neutralized with 7 μl neutralizing solution (0.1 M H3PO4, 1 M Tris, pH 7.4). Protein sample buffer was then added to the samples and either incubated at 37°C for 30 min (nondissociating condition) or boiled for 5 min (dissociating condition) prior to SDS–PAGE.

SDS–PAGE

Discontinuous SDS–PAGE was performed using the protocol of Laemmli (1970). Both 10 and 12.5% acryl-
Fig. 1. Detection of oligomeric forms of in vitro translated α1 and soluble α1 from reovirus-infected cells. [35S]Methionine-labeled reovirus-infected cell lysates (S-45) prepared as previously described (Lee et al., 1981), and [35S]methionine-labeled in vitro translation products of the reovirus S1 mRNA in rabbit reticulocyte lysates (Sp6), were precipitated with an anti-α1 monoclonal antibody (G5). After being released from the immunoadsorbent, precipitated proteins were mixed with protein sample buffer and were either boiled for 5 min (6) or incubated at 37° for 30 min (37) prior to SDS-PAGE. R (lane 1) represents purified [35S]methionine-labeled reovirus.

RESULTS

Generation of α1 oligomers in vitro

Since some of the following studies on α1 oligomerization involved the use of genetically truncated protein α1, it was necessary to express α1 in an in vitro system and to ascertain that oligomeric α1 was indeed generated in such a system. To this end, T3 reovirus S1 mRNA was prepared in vitro using Sp6 RNA polymerase and translated in a rabbit reticulocyte lysate. Translation products were then immunoprecipitated with an anti-α1 antibody (G5) and analyzed by SDS-PAGE under conditions that would not cause the disruption of α1 oligomers (see Materials and Methods). The results, illustrated in Fig. 1, show that stable α1 oligomers were indeed produced in vitro (lanes 4 and 5) and their migration rate was identical to that of authentic α1 from T3 reovirus-infected cells (lanes 2 and 3). Like the authentic protein, α1 oligomers synthesized in vitro were capable of binding to cell receptors (R. Duncan, G. Leone, and P. W. K. Lee, unpublished observations), and were cleaved by trypsin to yield a well-defined pattern (see below). Some α1 monomers (44K) were also produced in this system, but they were not precipitable by the anti-α1 antibody, did not manifest cell-binding function, and were highly susceptible to degradation by trypsin or chymotrypsin even when these proteases were used at very low concentrations (data not shown). Subsequent analysis exclusively of the oligomeric form of α1 was therefore possible.

Trypsin digestion of α1 oligomers

Previously it was found that trypsin cleaves the α1 oligomer near the middle to generate two fragments of approximately equal size (Banerjea et al., 1988; Yeung et al., 1989). The cleavage site has now been determined to be between Arg245 and Ile246 (Duncan and Lee, unpublished data). Thus the monomeric forms of the N-terminal fragment (245 amino acids) and the C-terminal fragment (210 amino acids) have molecular weights of approximately 26K and 23K, respectively, corresponding to their migration rates in SDS–PAGE under dissociating conditions (Yeung et al., 1989). Such a cleavage pattern was also obtained with α1 synthesized in vitro (Fig. 2, lane 3). An additional minor band of approximately 21K molecular weight was also observed sometimes. Using N- and C-terminal-specific amide gels were used. Gels containing 35S-labeled proteins were fixed and then treated with DMSO-PPO, dried under vacuum, and exposed to Kodak XAR-5 film at -70°.

Gels to be used for band excision were dried under vacuum without prior treatment, and then exposed to film. Developed x-ray film was superimposed on dried gels and bands to be excised were marked with a pouncer. Marked protein bands were excised from gels and rehydrated in Laemmli running buffer. Proteins from excised bands were electroeluted into electroelution cups in a volume of 200 μl. Protein sample buffer was added to electroeluted proteins and boiled for 5 min prior to SDS–PAGE.
FIG. 3. SDS-PAGE of oligomeric tryptic fragments of σ1. Protein σ1 tryptic digests prepared as described under Materials and Methods were either incubated at 37° for 15 min (37) or boiled for 5 min (B) in protein sample buffer and then subjected to SDS-PAGE. The 80K, 54K, and 48K fragments from the unboiled (37) sample were excised from the gel, eluted, boiled in protein sample buffer, and then reanalyzed by SDS-PAGE (lanes 4, 5, and 6, respectively). R (lane 1) represents purified [35S]methionine-labeled reovirus. Molecular weights of individual protein bands are indicated at right.

sera, we have identified the 26K and 21K tryptic fragments to be of N-terminus origin, and the 23K fragment to be of C-terminus origin (Fig. 4).

N-terminal half of σ1 is a trimer

To see whether any of the tryptic fragments could be identified in the oligomeric state, trypsin-treated σ1 in SDS-containing sample buffer was incubated at 37° (rather than boiled) prior to SDS-PAGE. It was found that the 26K N-terminal fragment was absent; instead two faint bands of approximately 48K and 54K molecular weight appeared. Protein bands of 80K, 54K, and 48K molecular weight were excised from a gel, eluted, boiled in sample buffer, and subjected to SDS-PAGE (Fig. 3). Both the 80K and 54K proteins were found to be converted to the 26K N-terminal fragment, whereas the 48K protein was converted to the 21K N-terminal fragment.

The most reasonable interpretation of these results would be that the 80K and 54K bands represent the trimer and dimer forms, respectively, of the 26K N-terminal fragment, and the 48K band represents the dimer of the 21K N-terminal fragment. The region in the 26K N-terminal fragment that is absent in the 21K N-terminal fragment may therefore be involved in stabilizing the third subunit of the 80K N-terminal trimer. The identity of this region is presently unknown.

Titration of trypsin digestion of σ1 oligomers

It is important to note that under conditions where the N-terminal tryptic fragment exists as stable trimers (and dimers), the C-terminal fragment was consistently found to be in the monomeric state. This is in contradiction to the findings of Banerjea et al. (1988) who reported that both the N- and C-terminal tryptic fragments exist as stable oligomers (tetramers) in SDS at 37°. To reconcile such differences, we decided to examine the effects of varying the trypsin concentration on σ1 cleavage pattern. At the lowest concentration (0.005 mg/ml) of trypsin that cleaves σ1 oligomers completely, an identical pattern to that shown in Fig. 2 was observed (Fig. 4A, lane 2). Increasing the concentration of trypsin to 0.05, 0.1, and 0.5 mg/ml, and subsequently boiling the samples in sample buffer, resulted in the gradual disappearance of the 26K N-terminal fragment with the concomitant appearance, in almost stoichiometric amounts, of a 25K molecular weight band (Fig. 4A, lanes 3–5). Thus the 25K band is a cleavage product of the 26K band. The 23K C-terminal fragment remained unchanged.

When identical samples were instead incubated at 37° in sample buffer prior to SDS-PAGE, both the 26K and 25K bands were replaced by bands with molecular weights of approximately 80K and 75K, respectively (Fig. 4A, lanes 6–9). Again, the mobility of the 23K C-terminal fragment remained unchanged. Radioimmunoprecipitation of the samples with the N- and C-terminus-specific antibodies confirmed that the 25K and 75K proteins, like the 26K and 80K proteins, were of N-terminus origin (Fig. 4B), and that the 23K protein was of C-terminal origin (Fig. 4C).

It is possible to explain the discrepancies between our present findings and those reported by Banerjea et al. (1988) on the basis of the methods used for protein σ1 detection. A polyclonal anti-T3 reovirus serum was used by the aforementioned investigators to identify σ1 tryptic fragments on Western blots as opposed to radioimmunoprecipitation with anti-σ1 N- and C-terminal-specific antibodies used in our studies. In our hands, polyclonal anti-native σ1 serum is incapable of recognizing the presumably denatured C-terminal tryptic fragment on a blot, although the N-terminal fragment is easily detectable. These observations, together with the fact that the trypsin concentration (1 mg/ml) used by Banerjea et al. was within the concentration range where we found both the 26K and the 25K N-terminal cleavage products, have led us to conclude that the two bands previously identified by these inves-
tigators as N-terminal and C-terminal oligomers correspond to our 80K and 75K bands, and are therefore in fact both N-terminal oligomers.

Role of the N-terminal portion in stabilizing σ1 oligomers

To determine the extent of involvement of the N-terminal portion in stabilizing the σ1 oligomer, native σ1 and trypsin-treated σ1 were subjected to various treatments prior to SDS–PAGE and the relative stability of native σ1 oligomers and N-terminal trimers (80K protein) was compared. Whereas dissociation of oligomers by heat was used as a general measure of oligomer stability, the effects of pH variation and presence or absence of urea and β-mercaptoethanol were also examined.

Under all conditions tested, native σ1 oligomers and N-terminal trimers behaved identically (Fig. 5). Both σ1 oligomers and N-terminal trimers were stable at 50°C in sample buffer but dissociated at 60°C (Fig. 5A). Both were stable under alkaline (pH 11.6) to mild acidic (pH 6.0) conditions, but dissociated at pH 5.5 (Fig. 5B). Urea (6 M) apparently had no effect on either oligomeric species (Fig. 5C). It was previously reported that σ1 oligomers were rendered less stable when the concentration of β-mercaptoethanol in the sample buffer was reduced (Bassel-Duby et al., 1987). Consistent with these findings, we observed that both native σ1 oligomers and N-terminal trimers were less stable in the total absence of β-mercaptoethanol (Fig. 5C).

In summary, the stability of the N-terminal trimer was found to be very similar to that of the native σ1 oligomer. These data suggest that interactions between σ1 subunits responsible for stabilizing the oligomeric structure occur mainly, although by no means solely, within the N-terminal half of the σ1 protein.

Involvement of ionic interactions within the N-terminal half of σ1

The absence of cysteine residues within the N-terminal tryptic fragment of σ1 suggests that the stabilizing effect of β-mercaptoethanol could not be directly due to its reducing properties. Indeed, enhanced oligomer stability was not observed when another reducing agent, dithiothreitol, was used in place of β-mercaptoethanol (Fig. 6A).

A less well-characterized property of β-mercaptoethanol is that of chelation (McMichael and Ou, 1977). If β-mercaptoethanol indeed stabilizes oligomers by chelating divalent cations, addition of divalent cations should destabilize oligomers. Indeed, MgCl₂ at concentrations above 1 mM was found to destabilize N-terminal trimers (Fig. 6B) as well as native σ1 oligomers (data not shown). Other divalent cations (zinc and calcium) had a similar effect (data not shown). The effects of divalent cations and β-mercaptoethanol on oligomer stability are clearly antagonistic. Thus the destabilizing effect of 5 mM and 20 mM MgCl₂ could be reversed by the inclusion of 1 and 5% β-mercaptoethanol, respectively, in the sample buffer (Fig. 6B). Similar results were obtained using EDTA as the chelator in place of β-mercaptoethanol, except that at EDTA concentrations above 5 mM, oligomer stability was inconsistent (data not shown).

The disruption of σ1 oligomers by divalent cations suggests that ionic interactions are involved in stabilizing the N-terminus trimer (and hence the σ1 oligomer). Indeed, the distribution of charged residues in the N-terminal coiled-coil region was found to highly favor the...
formation of salt bridges between \( \alpha 1 \) subunits (see Discussion). However, the presence of the extended heptad repeat in the same region suggests that hydrophobic interactions must play a major role in oligomer stabilization. In this regard, the aforementioned ionic interactions are presumed to serve an augmentative function. In agreement with the above hypothesis, temperature stability experiments indicated that in the presence of divalent cations (abolishing ionic interactions), oligomers were stable up to a temperature of 20\(^\circ\)C, but in the absence of divalent cations (maximizing ionic interactions), oligomers were stable up to a temperature of 50\(^\circ\)C (data not shown).

Intrinsic dimerization and trimerization properties of the heptad repeat region of \( \alpha 1 \)

It is believed that sequences with heptapeptide repeats of apolar residues are involved in stabilizing
coiled-coil structures through hydrophobic interactions between the α-helices. In the case of σ1, the heptad repeat region spans the N-terminal one-third of the protein (from residue 28 to 164). It was then of interest to see whether this region possesses intrinsic dimerization and, in view of the trimeric nature of the N-terminal tryptic fragment of σ1, trimerization functions.

To this end, the plasmid pG4T3 was treated with the restriction endonuclease EcoRV, which cuts the S1 gene at nucleotide 497 (encoding the N-terminal 161 amino acids). Run-off mRNA transcripts were then prepared and translated in vitro, and the ability of the translational products to oligomerize was then determined by SDS-PAGE. The results are shown in Fig. 7. When the samples were boiled in sample buffer prior to SDS–PAGE, closely migrating bands of approximately 18K molecular weight were found (Fig. 7, lane 2), as was predicted from the amino acid sequence. The lack of absolute homogeneity in size of the translational products was due to the absence of a translation stop codon in the mRNA transcripts, necessitating translation termination to occur by the falling off of ribosomes close to, but not precisely at, the 3′ ends of the mRNAs. When identical samples were instead incubated at 37° in sample buffer (Fig. 7, lane 3), there was a noticeable decrease in the intensities of the bands migrating at approximately 18K molecular weight were found (Fig. 7, lane 2), as was predicted from the amino acid sequence. The lack of absolute homogeneity in size of the translational products was due to the absence of a translation stop codon in the mRNA transcripts, necessitating translation termination to occur by the falling off of ribosomes close to, but not precisely at, the 3′ ends of the mRNAs. When identical samples were instead incubated at 37° in sample buffer (Fig. 7, lane 3), there was a noticeable decrease in the intensities of the bands migrating at approximately 18K. Concomitantly, two additional bands with estimated molecular weights of 36K and 54K appeared. These two bands corresponded to the dimeric (36K) and trimeric (54K) forms of the EcoRV translation products. Clearly, the dimer was the predominant oligomeric form identified. Whether trimer formation was inefficient or whether trimers were less stable in our SDS–PAGE system is not known. None-theless, for the first time, direct evidence is presented that demonstrates the intrinsic ability of a heptad repeat (18 repeats in this case) to form dimers and trimers, an ability that may very well depend on the number of repeats present in the polypeptide.

Heptad repeat imparts oligomer stability

To determine the extent to which the heptad repeat region is involved in stabilizing the N-terminal tryptic trimers, the EcoRV translational products were subjected to the various treatments previously applied to the N-terminal tryptic fragment and the native σ1 oligomer. The results are shown in Fig. 8. Both the dimeric and trimeric forms of the heptad repeat were unstable at 50°, a temperature at which the N-terminal tryptic trimer was found to be stable. However, like the N-terminal trimer, heptad dimers and trimers were both stable under alkaline (pH 11.6) to mild acidic (pH 6.0) conditions (but dissociated at pH 5.5), and in 6M urea, and were destabilized by the absence of β-mercaptoethanol or by the presence of divalent cations in the sample buffer. Again, as observed for the N-terminal tryptic trimer, the destabilizing effect of divalent cations could be neutralized by β-mercaptoethanol.

DISCUSSION

The reovirus cell attachment protein, σ1, is a homooligomer and has a lollipop morphology. The receptor-binding domain of σ1 resides in the C-terminal globular head and the virion-anchoring domain resides in the N-terminal fibrous tail. Sequence analysis of the S1 genes of all three reovirus serotypes concur with such structural and functional domain assignments. The ob-
were restabilized by the addition of a chelator. Indeed, an examination of the distribution of charged residues reveals a similar, albeit less perfect, distribution pattern of charge residues (data not shown).

It is important to point out that whereas the present study clearly indicates that the $\sigma_1$ oligomer is stabilized mainly via the N-terminal half of the protein, the implications of the intrinsic oligomerizing function of the heptad repeat region on the oligomerization process of the intact $\sigma_1$ oligomer need to be viewed with caution. Recent evidence suggests that intracellular protein folding and oligomerization are mediated by chaperones and that these events are ATP-dependent. In the case of $\sigma_1$, we have recently observed that whereas the oligomerization of the N-terminal one-third of $\sigma_1$ occurs spontaneously, that of full-length $\sigma_1$ is an ATP-dependent event (unpublished data), which further suggests that, as has been reported for a number of oligomeric proteins, the formation of the $\sigma_1$ oligomer is chaperone-mediated. This would in turn imply that the intrinsic oligomerizing function of the heptad repeat region of $\sigma_1$ is necessary, but not sufficient, for intact $\sigma_1$ oligomerization, and that a domain(s) downstream of this region must also be involved. We are currently probing the interactions between the three governing factors, namely, the heptad repeat region, a downstream domain(s), and chaperones, in the $\sigma_1$ oligomerization process, the revelation of which may have general and fundamental implications.

Our demonstration that the N-terminal tryptic fragment is a trimer and the N-terminal heptad repeat region possesses intrinsic trimerization function suggests that native protein $\sigma_1$ is most likely also a trimer, although the possibility of $\sigma_1$ being a multiple of a trimer (e.g., dimer of a trimer) cannot be ruled out based on the present data alone. Either model contradicts a recent suggestion, based on sequence analysis and computer-processed electron microscopy, that $\sigma_1$ is a tetramer (Fraser et al., 1990). Although the precise reasons for this discrepancy remain to be revealed, we have recently obtained data from biophysical studies and in vitro $\sigma_1$ assembly experiments that are compatible with a trimeric, but not a tetrameric (or a hexameric), model of $\sigma_1$ (unpublished data). A trimeric $\sigma_1$ would theoretically migrate at a position corresponding to 150K in SDS–PAGE, rather than at ~200K. How-
ever, our present studies suggest that under the conditions used for its detection (incubation at 37° in SDS), the N-terminal fibrous tail of the σ1 trimer would still be intact, whereas the C-terminal globular head would be totally unfolded. The resulting σ1 structure would accordingly resemble a "hydras," rather than a "lollipops," and would therefore manifest a somewhat retarded migration rate. Results from more refined experiments correlating incubation temperatures (in SDS) with migration rates of intact σ1 and σ1 tryptic fragments concur with such a rationale (unpublished observation).

It is noteworthy that, in terms of oligomerization status, σ1 is not unlike other viral cell-attachment proteins such as the influenza hemagglutinin (Wilson et al., 1981; Wiley et al., 1981), the VSV G protein (Doms et al., 1981; Kreis and Lodish, 1986), the adenovirus fiber (van Oostrum et al., 1987; Devaux et al., 1990), the envelope glycoprotein (gp120) of the human immunodeficiency virus (Weiss et al., 1990), and the coronavirus spike protein (Delmas and Laude, 1990), all of which appear to be trimeric, and the trimeric state may reflect a unifying concept, hitherto unidentified and perhaps pertinent to structural and/or functional requirements, in the virus attachment process.

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Note added in proof. Shortly after submission of our manuscript, Banerjea and Joklik (1990) reported the intrinsic oligomerizing property of the N-terminal heptad repeat region of α1.

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