Alteration of Capsid Proteins of Coxsackievirus A13 by Low Ionic Concentrations

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Several group A coxsackieviruses (A13, 15, 18, and 21), but not polioviruses or group B coxsackieviruses, are rapidly inactivated in low ionic strength solutions at neutral pH. The extent of inactivation is dependent upon temperature and molarity. Virions inactivated in this manner contain a normal complement of infectious RNA which remains in a state resistant to the action of ribonuclease. However, more than 95% of the virus particles are unable to attach to susceptible cells. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis reveals that coxsackievirus A13 virions contain five structural polypeptides (VP1, VP2a, VP2b, VP3, and VP4). Electrophoretic analysis indicates that inactivation of coxsackievirus A13 in low ionic strength solutions is due to the specific loss of the smallest polypeptide VP4 from the virus particle. These results suggest that adsorption of coxsackievirus A13 to receptors on susceptible cells is dependent upon the presence of the capsid protein VP4.

The inactivation of various picornaviruses by heat (2), alkaline pH (1, 11, 14), acid pH (12, 17), and diethylpyrocarbonate (3) has been widely studied. Furthermore, picornavirus particles that spontaneously elute from susceptible cells are also found to be noninfectious (5, 10). It has been established that under certain of these conditions inactivation is the result of structural alterations in the viral capsid which lead to the loss of the viral genome (1, 2, 11, 12, 14). Under other conditions, however, loss or inactivation of viral RNA does not occur. In the latter instances, it has been clearly shown that inactivation is due to the dissociation of the smallest structural polypeptide (VP4) from the capsid and that virions devoid of this polypeptide are non-infectious because they are unable to attach to susceptible cells (2, 3, 5, 12, 17).

In a previous report from this laboratory, techniques were described for the isolation in solubilized form of receptor activity for several picornaviruses from plasma membranes of susceptible tissues and cells (16). During the course of this study, it was noted that the hypotonic buffer used in the procedure for assaying the viral binding activity of solubilized receptor rapidly inactivated several serotypes of group A coxsackieviruses. The present communication describes the specific structural alteration in the coxsackievirus capsid which is induced under conditions of low ionic strength and demonstrates that the loss of infectivity of viral particles altered in this manner is not due to the loss or inactivation of viral RNA but is the result of their failure to adsorb to susceptible cells.

MATERIALS AND METHODS

Viruses and cells. The type 1 poliovirus (Mahoney strain) and coxsackievirus A13 (Flores strain) used in this study were propagated in HeLa cell monolayers. The tissue culture medium (MEM-CaS-TPB) consisted of Eagle minimal essential medium (MEM) containing 10% newborn calf serum (CaS), 10% tryptose phosphate broth (TPB), and 50 μg of aureomycin, 75 μg of neomycin, and 25 μg of mycostatin per ml.

Virus growth and purification. Viruses were adsorbed to cells for 1 h at 37°C with an input multiplicity of 10 to 50 PFU/cell. Medium with or without radioactive isotopes was added, and the cultures were incubated at 37°C for 12 to 18 h. Viruses labeled with 14C-labeled protein hydrolysate were grown in MEM with 0.1 amino acid concentration, 10% dialyzed newborn CaS, no TPB, and 5 μCi of 14C-labeled protein hydrolysate per ml. The medium for labeling viruses with 3H-labeled amino acids consisted of MEM (minus phenylalanine, tryptophan, and valine), 1.7 μCi of each of the corresponding 3H-labeled amino acids per ml, 2% dialyzed fetal CaS, and no TPB. Complete tissue culture medium (MEM-CaS-TPB) containing 5 μCi of 3H-labeled uridine per ml was used to label viral RNA. All media containing radioactive isotopes were supplemented with 5 μg of actinomycin D per ml. After incubation, the monolayers were frozen and thawed five times, and cellular debris was separated from the culture fluid by centrifugation at 900 × g for 10 min. The debris was suspended in a small volume of MEM, sonically
treated on ice for 1 min, and then added back to the original culture fluid. Particulate cellular material was removed by a second low-speed centrifugation, and the virus was pelleted by centrifugation in a Spino 30 rotor at 75,000 × g for 2 h. Virus was suspended in 2 ml of phosphate buffered saline (PBS) and chromatographed on a Sephadex G-75 (Pharmacia) column (25 by 1.5 cm) equilibrated with PBS. The column fractions containing virus were pooled, adjusted to a density of 1.34 g/ml with CaCl2, and centrifuged at 179,000 × g for 24 h in a Spino SW65 rotor. The banded virus was removed through the side of the tube with a syringe and needle, adjusted to a volume of 2 ml with PBS, and rechromatographed on Sephadex G-75. The fractions containing virus were again pooled and constituted the “purified virus preparations” referred to in subsequent experiments.

Plaque assays. Infectious virus was assayed by the method of Holland and McLaren (8) except the overlay medium (0.5% inocar Difco) in MEM-Ca-TPB was supplemented with 30 mM MgCl2, to enhance plaque development. A modification of the method of Pagano et al. (18) was used to assay phenol extracts of infectious viral RNA. HeLa cell monolayers were rinsed five times with PBS and pretreated at 25 C for 5 min with 2 ml of PBS containing 500 µg of DEAE-dextran (Pharmacia) per ml. This solution was removed and 0.2-ml samples of infectious RNA diluted in PBS containing 500 µg of DEAE-dextran per ml were adsorbed to the monolayers for 15 min at 25 C. Inoculum was removed by rinsing three times with PBS, the final rinse being allowed to remain on the monolayers for 15 min. Overlay medium was added, and the cells were incubated at 37 C for 48 h (poliovirus RNA assays) or 96 h (coxsackievirus RNA assays) before staining with 0.5% crystal violet in 20% ethanol.

Inactivation in hypotonic buffer. The standard procedure for inactivation of coxsackievirus A13 in low ionic strength solutions was to dilute purified preparations of virus 1:50 into PBS (control) or into Robert hypotonic buffer (RHB, reference 20) which contains 0.01 M NaCl, 0.01 M Tris (pH 7.2), and 0.001 M MgCl2. Incubation of the RHB dilutions at 37 C for 90 min normally resulted in a 96% or greater loss of viral infectivity, whereas there was essentially no reduction in infectivity in the PBS controls. The PBS- and RHB-treated virus was recovered by centrifugation of these dilutions in a Spino SW65 rotor at 179,000 × g for 2.5 h and the virus pellets were resuspended in 0.5 ml of PBS. These suspensions constitute the “RHB-treated” and “PBS-treated” virus.

Polyacrylamide gel electrophoresis. The procedures used for electrophoresis of viral proteins were essentially the same as those described by Maizel (13). Samples to be electrophoresed were first exhaustively dialyzed at 4 C against 0.01 M sodium phosphate buffer (pH 7.2). Sodium dodecyl sulfate (SDS), glacial acetic acid, and urea were added to final concentrations of 1%, 10%, and 0.5 M, respectively, and the samples were heated at 37 C for 1 h. The samples were dialyzed for 24 h at 25 C in 0.01 M sodium phosphate buffer containing 0.1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol. Immediately before electrophoresis, the samples were placed in a boiling water bath for 2 min, adjusted to 10% and 0.002% with respect to sucrose and bromphenol blue, and 0.1- to 0.2-ml aliquots were immediately applied to gels. The gels (20 × 0.6 cm) contained 10% acrylamide, 0.23% bisacrylamide, 0.1% SDS, and 0.5 M urea in 0.1 M sodium phosphate buffer (pH 7.2). Tray buffer consisted of 0.1% SDS and 0.5 M urea in 0.1 M sodium phosphate buffer (pH 7.2) which was continuously recirculated during the runs. Electrophoresis was conducted at 3 mA/gel for 20 min, then at 4.5 V/cm for an additional 16 h. The gels were fractionated (about 2 mm of gel/fraction) with a Savant autogeldivider directly into scintillation vials containing 0.5 ml of H2O. The vials were held overnight at 25 C, then 10 ml of scintillation fluid was added to each, and radioactivity was counted in a Beckman LS220 scintillation spectrometer. The scintillation fluid contained 5 g of 2,5-diphenyloxazole and 0.15 g of dimethyl-1,4-bis-2-(5-phenyloxazolyl)benzene in 1.5 liters of a Triton X-100–toluene mixture (1:2, vol/vol). Recovery of radioactivity was greater than 90% in all experiments. In dual-label experiments, the spectrometer settings allowed approximately 10% spillover of 14C counts into the 3H channel and 1% spillover of 3H counts into the 14C channel.

Reagents. 14C-labeled protein hydrolysate (52 µCi/mM atom of carbon) and 3H-labeled L-phenylalanine, L-tryptophan, and L-valine (1 M, purchased from New England Nuclear, Boston, Mass. Crystalline ribonuclease was obtained from Worthington Biochemicals Corp., Freehold, N.J.; actinomycin D from Calbiochem, La Jolla, Calif.; and powdered Eagle MEM from Grand Island Biological Co., Grand Island, N.Y.

RESULTS

Loss of infectivity of coxsackievirus A13 in hypotonic buffer. The kinetics of inactivation of coxsackievirus A13 in low ionic strength solutions was determined by diluting the virus 1:50 into RHB. A similar dilution in PBS served as the control. The diluted viruses were incubated at 37 C or at 0 C, and at various intervals, samples were removed and assayed for infectious virus by the plaque technique. The results of this experiment (Fig. 1) show that coxsackievirus A13 is rapidly inactivated in RHB at 37 C, but not at 0 C. Maximum reduction in infectivity of greater than 99% occurs within 30 to 40 min. The residual (<1%) infectious virus found at this time appears to be stable since incubation at 37 C for an additional 3 h had no further effect. However, virus not inactivated by RHB treatment does not represent a population of genetically resistant viruses, because after recultivation they exhibit the same inactivation kinetics as the original preparation. The
sackie-virus A13. It has been previously shown that several picornaviruses can be inactivated with heat (2), alkaline pH (1, 11, 14), or acid pH (12). These conditions lead to the production of a class of subviral particles which are noninfectious because they lack RNA. To determine whether inactivation of coxsackievirus A13 in RHB was the result of a similar mechanism, samples of PBS- and RHB-treated coxsackievirus A13 were divided into four aliquots, and pairs of these aliquots were treated by one of the following experimental procedures (see Table 1): (A) one pair was incubated at 37 C for 15 min, and infectious virus was assayed by the plaque technique; (B) one pair was incubated at 37 C for 15 min in the presence of 100 µg of RNAse per ml, and then infectious virus was assayed; (C) one pair was incubated at 37 C for 15 min, the entire contents of the tube extracted with phenol, and infectious viral RNA assayed by the plaque technique; (D) the final pair was incubated at 37 C for 15 min in the presence of 100 µg of RNAse per ml, and then extracted with phenol and assayed for infectious viral RNA. Table 1 presents the results of this experiment. The control (experimental procedure A) shows the typical 95% or greater loss of viral infectivity due to RHB treatment. As expected, RNAse pretreatment of the fully infectious PBS-treated control virus (procedure B) has no effect on its infectivity. The residual 3.2% infectivity detectable after RHB treatment is also relatively insensitive to RNAse, which shows that the surviving fraction of infectious virus in the RHB-treated preparation also contains viral RNA in a state protected from the action of RNAse. The amount of infectious viral RNA extractable from the PBS-treated coxsackievirus A13 control (procedure C) represents approximately 0.8% of the infectivity of the

![Graph](image-url)

**Fig. 1.** Inactivation of coxsackievirus A13 at low ionic strength. Virus was diluted 50-fold into PBS (control) or RHB, incubated at 37 C or 4 C, and assayed for infectivity (PFU) at various intervals. Symbols: O, PBS at 37 C; ●, RHB at 37 C; and Δ, RHB at 0 C.

presence of 2% newborn CaS during incubation in RHB does not prevent inactivation of the virus.

Several experiments were performed to rule out the possibility that aggregation of viral particles at low ionic strength was the cause of the apparent inactivation. RHB-treated suspensions of coxsackievirus A13 filtered through a series (0.3, 0.22, 0.1, and 0.05 µm) of membrane filters (Millipore Corp., Bedford, Mass.) pretreated with 10% fetal CaS (23) showed no additional loss of infectivity, and dilution of RHB-inactivated virus into 6 M LiCl, 1% SDS, or 0.01 M Tris (pH 8.6) did not result in any increase in infectivity. Therefore, the formation of viral aggregates does not appear to be the cause of the inactivation observed in RHB.

The effect of RHB was also determined on other members of the picornavirus group. Group A coxsackieviruses (type 15, 18, and 21) were inactivated in RHB with kinetics similar to that of coxsackievirus A13. However, RHB-treated type 1 poliovirus and several group B coxsackieviruses (types 1, 3, and 5) showed no significant loss of infectivity.

**Effect of RNAse on RHB-treated coxsackievirus A13.** It has been previously shown that several picornaviruses can be inactivated with heat (2), alkaline pH (1, 11, 14), or acid pH (12). These conditions lead to the production of a class of subviral particles which are noninfectious because they lack RNA. To determine whether inactivation of coxsackievirus A13 in RHB was the result of a similar mechanism, samples of PBS- and RHB-treated coxsackievirus A13 were divided into four aliquots, and pairs of these aliquots were treated by one of the following experimental procedures (see Table 1): (A) one pair was incubated at 37 C for 15 min, and infectious virus was assayed by the plaque technique; (B) one pair was incubated at 37 C for 15 min in the presence of 100 µg of RNAse per ml, and then infectious virus was assayed; (C) one pair was incubated at 37 C for 15 min, the entire contents of the tube extracted with phenol, and infectious viral RNA assayed by the plaque technique; (D) the final pair was incubated at 37 C for 15 min in the presence of 100 µg of RNAse per ml, and then extracted with phenol and assayed for infectious viral RNA. Table 1 presents the results of this experiment. The control (experimental procedure A) shows the typical 95% or greater loss of viral infectivity due to RHB treatment. As expected, RNAse pretreatment of the fully infectious PBS-treated control virus (procedure B) has no effect on its infectivity. The residual 3.2% infectivity detectable after RHB treatment is also relatively insensitive to RNAse, which shows that the surviving fraction of infectious virus in the RHB-treated preparation also contains viral RNA in a state protected from the action of RNAse. The amount of infectious viral RNA extractable from the PBS-treated coxsackievirus A13 control (procedure C) represents approximately 0.8% of the infectivity of the

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*PBS- or RHB-treated virus was incubated for 15 min at 37 C in the presence or absence of 100 µg of RNAse per ml, and then assayed for residual infectivity (PFU of infectious virus or infectious RNA per ml).

*Based on the titer (1.8 × 10⁶ PFU/ml) of the PBS-treated virus control.
preparation due to infectious virus particles. This reduction in infectivity simply reflects the normal inefficiency of the assay procedure for infectious RNA. The amount (0.5%) of infectious RNA that can be extracted from the RHB-treated preparation is comparable to that extractable from the PBS-treated control. This observation indicates that the inactivated virus, although not infectious, contains infectious RNA equivalent in amount to the total number of viral particles originally treated with RHB. To rule out the possibility that the extraction procedure is measuring infectious RNA released from virions into the medium, rather than encapsidated RNA, the suspensions were treated with RNase prior to extraction (procedure D). The results confirm that procedure C is indeed measuring RNA contained within virions. Collectively, the data in Table 1 indicate that loss of virion RNA does not entirely account for the effect of low ionic strength since over 50% of the RHB-inactivated coxsackievirus A13 virions contain infectious RNA in a state inaccessible to the action of RNase.

**Adsorption of RHB-treated coxsackievirus A13 to HeLa cells.** It has been demonstrated that under certain conditions inactivation of several picornaviruses results from the loss of their ability to adsorb to host cells. Polioviruses inactivated with heat (2) or diethylpyrocarbonate (3) and acid-inactivated rhinoviruses (12, 17) cannot adsorb to cells, and the fractions of poliovirus (10) and coxsackievirus (5) that spontaneously elute from cells after adsorption are incapable of reattachment and, therefore, are noninfectious. Accordingly, the following experiment was performed to determine the effect of RHB on the capacity of coxsackievirus A13 to attach to HeLa cells. Purified [H]uridine-labeled coxsackievirus A13 was treated with PBS or RHB as described above. Aliquots (0.1 ml) of the PBS- and RHB-treated virus were adsorbed to a series of three HeLa cell monolayers for 1 h at 0°C at a multiplicity of approximately 50 PFU/cell (with respect to the uninactivated PBS control). Unadsorbed virus was removed by thoroughly rinsing the monolayers five times with cold PBS, and the cells were scraped from the surface and resuspended in 1 ml of cold PBS. The 1-ml cell suspensions were added directly to 10 ml of Triton X-100 scintillation fluid, and the counts of triplicate monolayers were averaged to determine the amount of radioactive virus adsorbed. The results of this experiment (Table 2) show that greater than 95% of the RHB-treated virus is not adsorbed, suggesting that inactivation of coxsackievirus A13 in the presence of RHB results from a loss in the ability of these virus particles to attach to susceptible cells.

**Loss of VP4 from RHB-treated coxsackievirus A13.** Several investigators (2, 3, 5, 12, 17) demonstrated that specific removal of the smallest structural polypeptide (VP4) of certain picornaviruses, without concomitant release or inactivation of the viral genome, renders the viruses noninfectious because of their inability to attach to susceptible cells. Our finding that RHB inactivation is also due to a failure of the virus to adsorb lead us to investigate the polypeptide profiles of PBS- and RHB-treated coxsackievirus A13.

Although two groups (5, 7) have investigated the protein composition of several group B coxsackieviruses, we are unaware of any reports dealing specifically with coxsackieviruses belonging to group A. Therefore, as controls for further experiments, the polypeptide profile of coxsackievirus A13 was analyzed in comparison to type 1 poliovirus. Purified preparations of H-labeled amino acid-labeled coxsackievirus A13 and C-labeled protein hydrolysate-labeled type 1 poliovirus were mixed and subjected to disruption and SDS-polyacrylamide gel electrophoresis. The only significant difference noted (Fig. 2) between the two viruses was that the capsid protein of coxsackievirus A13 is routinely resolved into five distinct components, whereas separation of poliovirus into more than four polypeptides (VP1, VP2, VP3, and VP4) was never observed under these conditions of electrophoresis. We have tentatively designated the two coxsackievirus polypeptides, which migrate slightly faster than VP2 of poliovirus, as VP2a and VP2b. With the molecular weights of the polypeptides of poliovirus reported by Maizel and Summers (15) as internal standards, and by plotting the log of the molecular weight of the polypeptides against fraction number, the molecular weights of the coxsackie.
Fig. 2. Comparative SDS-acrylamide gel electropherogram of a mixture of \(^{3}H\)-labeled amino acid-labeled coxsackievirus A13 and \(^{14}C\)-labeled protein hydrolysate-labeled type I poliovirus. The anode is to the right in this and subsequent figures. Symbols: O, poliovirus; and ●, coxsackievirus.

Coxsackievirus polypeptides were calculated to be: VP1, 36,000; VP2a, 27,000; VP2b, 25,500; VP3, 21,500; and VP4, approximately 7,000.

To determine the effect of low ionic strength on the structural polypeptide composition of coxsackievirus A13, purified \(^{14}C\)-labeled protein hydrolysate-labeled virus was diluted 1:50 into PBS or RHB and incubated at 37°C for 90 min. Five-milliliter samples of each were centrifuged in a Spinco SW65 rotor at 179,000 x g for 2.5 h. The pellets and supernatants were then separated by the following procedure for subsequent analysis on SDS-gels. The upper 4.5 ml of each supernatant was carefully removed and saved. The remaining 0.5 ml of the supernatants were discarded, and the pellets (virus pellets) were rinsed twice with cold 5% trichloroacetic acid, resuspended in 0.5 ml of 0.01 M sodium phosphate buffer (pH 7.2), and dialyzed overnight at 4°C against the same buffer. The virus pellets and trichloroacetic acid precipitates were then solubilized and subjected to electrophoresis.

The polypeptide profiles of the virus pellets from PBS- and RHB-treated preparations were determined on separate gels, but are plotted together in Fig. 3. These data reveal that the only detectable alteration in those virions that are sedimentable after RHB inactivation is the specific loss of the minor structural polypeptide VP4, suggesting that the inability of inactivated virus to attach to cells is directly correlated with the loss of VP4.

If VP4 is dissociated during RHB inactivation, it should be recoverable from the nonsedimentable material in the RHB supernatants. Accordingly, the trichloroacetic acid precipitates of PBS and RHB supernatants were electrophoresed on separate gels, and the combined data are presented in Fig. 4. The electrophoretic profiles show that the only virion polypeptide...
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present in significant amounts in the nonsedi-
mentable fraction of RHB-inactivated virus is
VP4, and that VP4 can be recovered by tri-
chloroacetic acid precipitation provided the in-
activated viral particles are first removed by
centrifugation. These results provide additional
evidence which supports the premise that RHB
induces the specific loss of VP4 from the capsid
of coxsackievirus A13.

DISCUSSION

Release of RNA from virions of group A
coxsackieviruses would account a priori for the
loss of infectivity that occurs in hypotonic RHB
at neutral pH (Fig. 1). However, a comparison
of the amount of infectious RNA extractable
from control and RHB-treated virus (Table 1)
indicates that more than one-half of the inacti-
vated particles contain RNA in an essentially
normal state, judged by the fact that infectivity
of the RNA remains insensitive to the action of
RNase. Therefore, it is unlikely that inactiva-
tion under these conditions is entirely due to
degradation or release of viral nucleic acid. In
fact, a preliminary experiment (unpublished
data) has shown that greater than 90% of
3H-labeled amino acid-labeled, RHB-inac-
tivated particles sediment in sucrose gradients
at a rate only slightly slower than that of intact
virions (150 S) and little or no radioactivity can
be detected in these gradients in the position
that empty capsids (80 S) would be expected to
band. These results suggest that most, if not all,
RHB-inactivated particles contain viral RNA.

RHB-inactivated virions are unable to adsorb
to susceptible cells (Table 2), and this finding
provides an explanation for the previous obser-
vation that RHB-treated virus is not infectious,
in spite of the fact that it contains a relatively
normal complement of infectious RNA. Fur-
thermore, the finding that VP4 is specifically
dissociated from coxsackievirions during inacti-
vation with RHB (Fig. 3 and 4) suggests this
polypeptide is involved in the adsorption of
the virus to receptor sites on susceptible cells.

![Fig. 3. Effect of low ionic strength on the polypeptide profile of coxsackievirus A13. Purified, 3H-labeled virus was diluted 50-fold into PBS (control) or RHB and incubated at 37 C for 90 min. Virus particles were sedimented by centrifugation, solubilized, and subjected to SDS-acrylamide electrophoresis on separate gels. Symbols: O, PBS-treated virus; ●, RHB-treated virus.](http://jvi.asm.org/.../3H-labeled virus was diluted 50-fold into PBS (control) or RHB and incubated at 37 C for 90 min. Virus particles were sedimented by centrifugation, solubilized, and subjected to SDS-acrylamide electrophoresis on separate gels. Symbols: O, PBS-treated virus; ●, RHB-treated virus.)
The possibility that the presence of VP4 is required for adsorption of picornaviruses to cells has been previously suggested by others (2, 5, 12); however, their results and the data presented in this paper do not provide any direct evidence that VP4 is the component which specifically interacts with receptors on cells. Attempts to directly confirm this hypothesis by demonstrating specific binding of VP4 isolated from rhinoviruses to HeLa cells have not been successful (17). Indeed, alternate hypotheses have been proposed in which VP4 is not believed to be directly involved in the adsorption process. For example, it has been suggested that VP2 of group B coxsackievirus carries the site that binds to cellular receptors (5), and there is indirect evidence that VP1 is necessary for the adsorption of foot-and-mouth disease virus to cells (24). Noble and Lonberg-Holm (17) have recently suggested that attachment of rhinoviruses to cells is dependent upon a specific native conformation of the entire surface of the virion rather than upon the presence or absence of any one of the individual structural polypeptides. Various conditions, including low ionic strength inactivation described in this report, can be used to produce subviral particles deficient in one or more of their individual virion polypeptides (2, 11, 12, 14, 17). It may be possible to determine whether adsorption "sites" are carried by one or more of the virion polypeptides by measuring the ability of these subviral particles or their isolated structural proteins to specifically attach to intact cells or to solubilized cell receptors (16). Experiments of this nature are currently in progress in our laboratory.

The finding that group A coxsackieviruses, but not poliovirus or group B coxsackieviruses, are rapidly inactivated in hypotonic solutions at neutral pH was somewhat unexpected, since enteroviruses are generally considered to be stable to all but the most rigorous conditions that inactivate members of other picornavirus subgroups (see reference 21). The specificity of this inactivation suggests that susceptibility to low ionic strength may be a useful characteristic for determining the taxonomic relationships between the enteroviruses, much in the same manner that acid lability has been used to characterize the rhinoviruses (21). Furthermore, by studying the dissociation of viruses under conditions such as these, it may be possible to determine the nature of the nonionic bonds which are involved in the assembly of isometric capsids from nonidentical polypeptides.

The results shown in Fig. 2 reveal that coxsackievirus A13 is composed of five structural polypeptides which have been designated VP1,
VP2a, VP2b, VP3, and VP4. To our knowledge, this is the first report dealing with the electrophoretic profile of group A coxsackieviruses. It is interesting to note that no more than four polypeptides have been detected in the closely related group B coxsackieviruses (5, 7).

Although picornaviruses are generally considered to contain four major size classes of structural proteins, recent evidence has shown that at least three of the capsid polypeptides (VP1, VP2, and VP3) of several poliovirus strains can be resolved into two or three subcomponents (4, 19, 22). Therefore, electrophoretic profiles of these viruses show from five to seven bands, depending upon the strain. Cooper et al. (4) have postulated that subcomponents of a given virion polypeptide are formed as a result of alternate cleavage sites in the larger proteins which serve as precursors to the final polypeptide products.

The nature of VP2a and VP2b of coxsackievirus A13 (Fig. 3) is not clear at the present time. First, the virus used in these studies had undergone several passages subsequent to plaque purification and, therefore, the data do not exclude the presence of a mutant containing a genetically altered VP2. Secondly, it is possible, due to alternating cleavages of VP0 (the precursor to VP2, reference 9) at two sites during maturation (4, 19), that two types of particles are formed, one containing VP2a, the other VP2b. Finally, if two sites on VP0 were cleaved simultaneously (19, 22), all virions produced would be composed of five polypeptides, including both VP2a and VP2b. The data do not distinguish between these three possibilities.

Although VP2a and VP2b of type 1 poliovirus differ in molecular weight by 2,500 to 3,000 (4, 19), we have been unable to resolve these polypeptides by using the same strain (Mahoney), even under electrophoretic conditions in which the two analogous components of coxsackievirus A13, which have an even smaller diversity in molecular weight, can be separated (Fig. 2). It is possible that the differences in the sizes and numbers of picornavirus structural polypeptides that have been reported by various laboratories may depend to a greater extent than previously expected on the passage history of the virus, the host cells, or on other conditions of the culture system being used. Further investigation into these possibilities would seem to be warranted.

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LITERATURE CITED