The Surface Nature of Proteins of a Bovine Enterovirus, Before and After Neutralization

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SUMMARY

The surface nature of the proteins of a bovine enterovirus have been determined by using $^{125}$I and pyridoxal phosphate-sodium borohydride labelling techniques. As found previously, $^{125}$I labels only VP$_1$ in intact capsid particles, whereas reaction with pyridoxal phosphate followed by reduction with tritiated sodium borohydride labels VP$_1$, VP$_2$ and VP$_3$. Only VP$_4$ is found to have no surface tyrosine, histidine or lysine available for reaction. After neutralization with homologous antisera, however, VP$_4$ becomes exposed and is then available for labelling with $^{125}$I. This must reflect a substantial conformational change in the virus particle after neutralization.

INTRODUCTION

The surface nature of the structural proteins of picornaviruses has been investigated previously using $^{125}$I labelling by either the chloramine T or lactoperoxidase methods. Both techniques have shown that for foot-and-mouth disease virus (Talbot et al. 1973) or bovine enterovirus (Carthew & Martin, 1974) only VP$_1$ has available tyrosine or histidine which can be iodinated. More recently the technique of reacting available lysine in proteins with pyridoxal phosphate, followed by reduction of the Schiff base formed by tritiated sodium borohydride, has become another useful probe to the surface nature of proteins in macromolecules, such as viruses (Rifkin, Compans & Reich, 1972). This technique has been used to determine whether VP$_2$, VP$_3$ and VP$_4$ are exposed on the surface of bovine enterovirus particles, but do not have any tyrosines in their exposed portions.

Work by Mandel (1971) has shown a distinct difference in the isoelectric properties of poliovirus, before and after neutralization with homologous antisera. In an attempt to corroborate this work by another technique, bovine enterovirus has been neutralized with antisera and then iodinated. Since, prior to neutralization, only one protein is iodinated, it was hoped that any conformational change in the surface proteins might reveal other tyrosine sites in other proteins which would then react with iodine.

METHODS

Cell cultures. Baby hamster kidney cells, BHK 21 (Macpherson & Stoker, 1962), were cultured in Eagle's medium obtained from Burroughs Wellcome Ltd, Beckenham, as previously described by Martin, Johnston & Clements (1970).

Virus production. Bovine enterovirus (serotype VG-5-27) was grown in BHK 21 cells and purified as previously described by Johnston & Martin (1971). The virus (165S component)

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was isolated from sucrose density gradients and re-cycled through a second gradient to remove a minor virus-specific 145S component found in virus harvests (Hoey & Martin, 1974).

Isotopic labelling of virus in vivo. Virus was grown in the presence of 1 μCi/ml 14C-amino acids (protein hydrolysate; sp. act. 57 mCi/mAtom). The cells were incubated with Earle's saline for 2 h prior to infection and the 14C-amino acids added 30 min after infection.

Production of antiserum to bovine enterovirus. Antiserum to bovine enterovirus was produced by injecting rabbits intramuscularly with purified virus in Freund's adjuvant. The injections were given three times at intervals of two weeks, before bleeding and assaying of the antisera by the plaque neutralization technique.

Bovine enterovirus neutralization by antisera. A concentrated suspension of purified bovine enterovirus (approx. 10⁹ p.f.u. as measured by plaque assay) was incubated with an equal vol. of various dilutions of antiserum for 2 h, prior to dilution and assayed by the standard plaque assay procedure.

Preparation of γ globulin fraction from antisera. The γ globulin fraction of the antisera was prepared by sodium sulphate precipitation as described by Breese & Hsu (1971).

Iodination of virus. The iodination of bovine enterovirus was performed using the chloramine T method, as previously described by Carthew & Martin (1974).

Iodination of neutralized bovine enterovirus capsid particles. A suspension of bovine enterovirus particles (10⁹ p.f.u./ml) was neutralized by the addition of an equal vol. of antiserum, (γ globulin fraction) diluted to give 80% neutralization of the virus particles. The iodination was carried out using chloramine T as described above. After iodination unreacted 125I was removed on Sephadex G 50.

Reaction of disrupted virus with ³H-dansyl chloride. Virus (500 μg) was disrupted as described below using SDS and urea and then reacted with 25 μCi ³H-dansyl chloride (sp. act. 5 Ci/mmol) as described by Talbot & Yphantis (1971).

Reaction of virus with pyridoxal phosphate and sodium borohydride. Virus (500 μg) was reacted with pyridoxal phosphate, followed by reduction with tritiated sodium borohydride (sp. act. 5·9 Ci/mmol) as described by Rifkin et al. (1972). The virus was then repurified on a sucrose gradient and pelleted prior to disruption for electrophoresis.

Degradation of virus and electrophoresis of virus proteins. Particles were dissolved in 1% (w/w) SDS + 0·8 m-urea in 0·04 m-phosphate buffer solution, pH 7·4, and heated at 100 °C for 2 min. Samples were applied to 12·5% acrylamide gels containing 0·375% bisacrylamide, 0·1% SDS and 0·5 m-urea. Electrophoresis was carried out according to Johnston & Martin (1971). Gel slices were heated in sealed vials at 60 °C with 0·2 ml of 0·5 m-NaOH for 14 h.

Radioactive counting. After the addition of 6 ml of Triton N101/toluene/PPO (1 vol.: 2 vol. + 4 g/l PPO) and 0·5 ml of distilled water the samples were counted in an Intertechnique Liquid Scintillation Spectrometer model SL 30. Tritium settings were used for counting ¹²⁵I.

RESULTS

Reaction of disrupted virus particles with ³H-dansyl chloride

Bovine enterovirus particles were disrupted and reacted with ³H-dansyl chloride by the method of Talbot & Yphantis (1971) prior to electrophoresis. The resulting polyacrylamide gel profile (Fig. 1) shows clearly that all four proteins incorporated tritiated dansyl chloride by reaction with the N terminal amino groups and ε-amino lyside side chains.

From quantitative N terminal analysis of the bovine enterovirus proteins (manuscript in preparation) using isotope-labelled dansyl chloride it has been found that large amounts of
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Fig. 1. Polyacrylamide gel electrophoresis profile of bovine enterovirus capsid particle proteins, after disruption and reaction with ^3H-dansyl chloride.

\( \epsilon \)-lysine, labelled with dansyl chloride, are found after chromatography and scintillation counting. The number of \( \epsilon \)-lysine counts found on chromatograms is at least five times that of the total product found for end groups. No reaction due to the reaction of histidine was observed. Hence, the proportion of counts found in VP\(_4\) (roughly 10\% of the total) is too great to be due to N terminal labelling only and means that VP\(_4\) contains lysine which is also dansylated.

Reaction of virus particles with pyridoxal phosphate and sodium borohydride

Intact virus particles labelled with \(^{14}\)C were reacted with pyridoxal phosphate as described in Methods, then treated with tritiated sodium borohydride to reduce the Schiff base and incorporate tritium. Purification was then carried out by sucrose density gradient sedimentation, which showed that no degradation of capsid particles occurred under these conditions (Fig. 2). After the virus was recovered from the sucrose gradient and pelleted, it was disrupted and subjected to polyacrylamide gel electrophoresis. Fig. 4 shows that proteins VP\(_1\), VP\(_2\) and VP\(_3\) contain tritium label, but that VP\(_4\) does not.

Neutralization of virus

The neutralization characteristics of concentrated solutions of virus, suitable for iodination were studied. Using a suspension of bovine enterovirus containing \(10^9\) p.f.u. a study of
neutralization with antisera was carried out in a similar manner to that used by Dulbecco, Vogt & Strickland (1956). From Table 1 it can be seen that the results obtained are similar to those obtained by these authors, with a 20 % residue of unneutralized virus particles, presumably due to aggregation in concentrated suspensions, as found by Wallis & Melnick (1967).

**Iodination of neutralized virus particles**

Suspensions containing approx. $10^8$ p.f.u. of bovine enterovirus were mixed with dilutions of rabbit antisera $\gamma$ globulin against bovine enterovirus (final dilution 1 in 100 in the reaction mixture) and a similarly diluted sample of normal rabbit sera $\gamma$ globulins used as a standard
for reference. After iodination the samples were subjected to polyacrylamide gel electrophoresis. Some heavy (H) and light (L) chains of γ globulin were found as well as intact rabbit γ globulin, due to disruption of disulphide bonds during the standard dissociating conditions. However, in the sample containing antiviral γ globulin, VP₁ and VP₄ were both iodinated (Fig. 4), whereas in the comparison reference standard of virus and γ globulin of normal serum only VP₁ was iodinated, as in the unneutralized bovine enterovirus capsid particles (Carthew & Martin, 1974).

DISCUSSION

The location of virus proteins in picornaviruses has been the subject of much discussion. Breindl (1971) has suggested that VP₄ is a surface protein in poliovirus and is the antigenic determinant. However, when particles of foot-and-mouth disease virus (Talbot et al. 1973) and bovine enterovirus (Carthew & Martin, 1974) are iodinated, only VP₁ becomes labelled. Experimentation with probes for other amino acids has been shown in this paper to be useful in studying the surface nature of other proteins in picornaviruses.

From the evidence presented in Fig. 2 it is apparent that VP₁, VP₂ and VP₃ all have some surface protein nature. In view of the failure of VP₄ to label either under iodinating conditions, or with pyridoxal phosphate NaB₃H₄ it seems unlikely that this polypeptide has a surface location.

The neutralization of virus particles in concentrated suspensions has received little attention. Dulbecco et al. (1956) discussed the residue of infectious particles found when
neutralizing suspensions of $10^7$ p.f.u. of Western equine encephalitis virus (WEE) in terms of these being different types of particles. Wallis & Melnick (1967) have shown that the existence of this residue is due to the formation of aggregates, which if removed will reduce the titre to zero. In the present experiments with bovine enterovirus, it has been shown that when the particles are neutralized VP$_4$ becomes accessible to iodination (Fig. 4). This provides evidence that a considerable change in conformation occurs when the virus particles are neutralized. This is in agreement with Mandel's suggestion for poliovirus. It is surprising to note that there seems to be little change, by comparison with the normal rabbit serum
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γ globulin control, in the iodination of VP₁; suggesting that the conformational change is highly specific in terms of rendering the virus particle non-infective and that VP₄ is implicated in this change for bovine enterovirus.

REFERENCES


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