Conformational Changes in Sindbis Virus E1 Glycoprotein Induced by Monoclonal Antibody Binding

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SUMMARY

A monoclonal antibody (30.2) raised against Sindbis virus is able to precipitate both E1 and PE2 from [35S]methionine-labelled infected cells solubilized with non-ionic detergent. Addition of SDS to the lysate abolishes the precipitation of PE2 without affecting that of E1, thus demonstrating that the antibody is specific for E1. Other Sindbis E1-specific monoclonal antibodies (30.11 and 30.12) precipitate only E1, even from lysates containing only non-ionic detergent, and their presence in such a lysate prevents precipitation of PE2 by antibody 30.2. These data indicate that E1–PE2 complexes stable in the presence of non-ionic detergent can be precipitated as such by one antibody, but that binding of the other antibodies induces dissociation of E1 and PE2. Competition experiments using 125I-labelled antibodies indicate that all three antibodies bind to distinct antigenic sites on the E1 molecule. Antibodies 30.11 and 30.12 stimulate each other’s binding in such experiments, which suggests that binding of either of these antibodies alters the conformation of E1 in such a way as to increase its affinity for the other, and at the same time to release PE2. Antibody 30.2 also enhances binding of the other two antibodies, but this stimulation is only weakly reciprocated.

INTRODUCTION

The envelope proteins E1 and E2 of the alphaviruses Semliki Forest and Sindbis are organized into surface structures from basic heterodimeric units (Ziemiecki & Garoff, 1978; Rice & Strauss, 1982). These E1–E2 heterodimers survive disruption of the virus by non-ionic detergents and the analogous complexes of E1 and PE2 which exist in infected cells (Bracha & Schlesinger, 1976; Jones et al., 1977) also survive lysis of the cells in non-ionic detergent (Ziemiecki et al., 1980). Despite this stability, the Sindbis virus monoclonal antibodies which have been described so far (Roehrig et al., 1980, 1982; Chanas et al., 1982a) have been capable of precipitating only one of the envelope glycoproteins from lysates of virus or infected cells containing non-ionic detergent. This behaviour is reminiscent of the observation that the heterodimers are partially dissociated during immunoprecipitation by conventional monospecific antisera (Ziemiecki & Garoff, 1978). However, we describe here an E1-specific monoclonal antibody capable of precipitating the E1–PE2 complex from infected cells, thus providing an opportunity to investigate directly dissociation of the heterodimers by other monoclonal antibodies.

Antibody-induced conformational changes in viral proteins have been invoked to explain the phenomenon of enhancement of binding of one monoclonal antibody in the presence of another (Lubeck & Gerhard, 1982; Lefrancois & Lyles, 1982). We present evidence here that such conformational changes take place in Sindbis virus E1 glycoprotein on binding of monoclonal antibodies and may result in dissociation of the E1–PE2 heterodimer, enhanced binding of antibody at other antigenic sites on the molecule, or both, depending on the antibodies involved.
These data may be relevant to the potentiation of the neutralizing capability of one monoclonal antibody by another, non-neutralizing antibody, which has previously been observed in this system (Chanas et al., 1982a).

METHODS

Monoclonal antibody production and characterization. Antibody 30.2 resulted from the same fusion as those previously described. It was characterized in assays for virus neutralization, infectivity enhancement, haemagglutination inhibition, haemolysis inhibition, immunofluorescence and immunoglobulin class specificity as previously described (Chanas et al., 1982a). Mouse ascitic fluid containing the antibody was used in this study.

Radioimmunoprecipitation. BHK cells were infected with Sindbis virus and labelled with $[^{35}S]$methionine for 10 min as described previously (Chanas et al., 1982a). Cell extracts were made by lysing the cells in phosphate-buffered saline (PBS) containing 1% Triton X-100, and centrifuging at 12,000 g for 2 min; the supernatant was stored in aliquots at $-70^\circ$C. Immediately before immunoprecipitation, extracts were clarified by centrifugation at 12,000 g for 2 min, 50 μl samples mixed with 1 to 10 μl amounts of monoclonal ascitic fluids, and incubated at room temperature overnight. In some experiments, SDS was added to final concentrations of 0·1 to 1% for the incubation with antibody. Immune complexes were recovered by addition of 40 μl of a 50% (v/v) suspension of Protein A-Sepharose CL4B (Pharmacia) and analysed by SDS-polyacrylamide gel electrophoresis as described by Chanas et al. (1982a).

Purification and iodination of monoclonal antibodies. Antibodies were purified from ascitic fluid by affinity chromatography on Protein A-Sepharose (Ey et al., 1978). Protein concentrations were estimated using the dye-binding assay of Bradford (1976). Purified immunoglobulins (30 to 50 μg) were iodinated (Fraker & Speck, 1978) in tubes containing 10 to 20 μg 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril (Iodogen; Pierce and Warriner, Ltd.) and 150 μCi Na $^{125}$I (Amersham International), and freed of unreacted iodine by passage over a 3 ml column of Sephadex G-50. These preparations were 80 to 95% trichloroacetic acid (TCA)-precipitable and had specific activities between 1.4 and 2.5 μCi/μg.

Competitive antibody binding assay. The antigen used in the binding assay was purified Sindbis virus prepared from the culture medium of infected BHK cells by polyethylene glycol concentration and banding on glycerol-tartrate isopycnic density gradients (Obijeski et al., 1974). Polystyrene 96-well plates (M129B, Dynatech) were coated with purified Sindbis virus in 50 mM-sodium carbonate buffer pH 9.6 at 4°C overnight. In preliminary experiments the concentration of coating virus required to give suboptimal binding of each $^{125}$I-labelled antibody was determined, and was used in the competition assays. It varied from 0·25 to 1 μg virus per well with different iodinated antibodies. The coated plates were washed in PBS, 0·05% Triton X-100 and incubated with PBS containing 0·5% bovine serum albumin for 60 min to block remaining protein-binding sites. After washing again, duplicate wells received serial twofold dilutions of ascitic fluid containing competing antibody followed immediately by a fixed quantity of $^{125}$I-labelled antibody. The diluent was PBS, 0·05% Triton X-100, 0·5% foetal calf serum. The plates were incubated at room temperature for 2 h, washed, and the bound labelled antibody eluted into 150 μl 1M-NaOH per well and transferred to clean tubes for counting.

RESULTS

Characteristics of monoclonal antibody 30.2

Double diffusion against type- and subtype-specific immunoglobulins indicated this antibody was of the IgG1 class. Strong perinuclear staining was revealed by immunofluorescence of fixed infected cells and the surface of unfixed infected cells was also stained. The antibody failed to neutralize virus, even in the presence of anti-mouse globulin, or complement and was devoid of haemagglutination-inhibiting activity. There was a greater than fivefold enhancement of infectivity in the P388D1 macrophage-like cell line at suitable antibody dilutions (Peiris & Porterfield, 1979).

Radioimmunoprecipitation

When Sindbis virus-infected cells containing p120, PE2, E1 and C as major labelled species (Fig. 1, lane i) were lysed in PBS containing 1% Triton X-100 and incubated with antibody 30.2, both E1 and PE2 were immunoprecipitated (lanes b and d). The antibody appeared to be sufficiently reactive with Protein A-Sepharose for the immune complexes to be recovered, despite the fact that it belongs to the IgG1 subtype which generally does not bind Protein A strongly (Goding, 1980). Precipitation of PE2 appeared somewhat less efficient than that of E1. If SDS was present during the incubation with the antibody at concentrations of 0·1, 0·5 or 1%,
**Antibody-induced conformational changes**

Labelling of infected cells, immunoprecipitation and analysis by SDS–polyacrylamide gel electrophoresis were carried out as described in Methods. Lane (i) contains total cell extract showing virus-specific polypeptides as markers. Lanes (a) to (d) and (h) contain material immunoprecipitated in the absence of SDS by (a) no antibody, (b) and (d) antibody 30.2, (c) mixture of antibodies 30.11 and 30.2, (h) antibody 30.11. Lanes (e) to (g) show material precipitated by antibody 30.2 in the presence of (e) 0.1% SDS, (f) 0.5% SDS and (g) 1% SDS.

Precipitation of PE2 was reduced at the lowest concentration and abolished at the higher concentration, while recovery of E1 was only partially affected by 1% SDS (lanes e to g). Antibody 30.2 is thus clearly specific for E1. Another E1-specific monoclonal antibody, 30.11 (Chanas et al., 1982a), precipitated only E1 even in the absence of SDS (lane h). Moreover, when a mixture of antibodies 30.2 and 30.11 was present, only E1 was precipitated (lane c). When the gel was stained to visualize the immunoglobulin polypeptides, light chains with mobilities characteristic of both antibodies were shown to be present in this immunoprecipitate (data not shown), so the possibility that antibody 30.11 competitively excluded antibody 30.2 from the Protein A–Sepharose can be excluded. The other E1-specific monoclonal antibody, 30.12, previously identified in these laboratories (Chanas et al., 1982a), also precipitated E1 only and prevented PE2 precipitation by antibody 30.2, while a nucleocapsid protein-specific antibody had no effect on immunoprecipitation by antibody 30.2. Thus, it appears that E1–PE2 heterodimers can be precipitated as such by antibody 30.2, but that SDS in low concentrations, or the binding of 30.11 or 30.12 antibodies, induces release of PE2.

**Competitive antibody binding assays**

To investigate the possibility that antibody-mediated dissociation of E1–PE2 heterodimers takes place via conformational changes induced in E1, competitive binding assays using 125I-labelled antibodies were performed. Such assays not only provide information on the topographical distribution of epitopes on a protein (Yewdell & Gerhard, 1981), but also detect conformational changes induced by one antibody which lead to enhanced binding of antibodies at other antigenic sites (Lubeck & Gerhard, 1982; Lefrancois & Lyles, 1982). To maximize sensitivity, these assays were performed using a limiting quantity of antigen, which was separately determined for each labelled monoclonal antibody used. Samples of purified IgG from each E1-specific monoclonal antibody were radioiodinated and tested in the presence of...
Fig. 2. Binding of $^{125}$I-labelled antibodies to Sindbis virus in the presence of unlabelled competing antibodies. The conditions of the assay were arranged so that in the absence of competing antibody about 3000 cpm were bound to a limiting quantity of antigen. This reference level of binding is indicated by the dashed line at 100% on the ordinate. Competing antibody (as ascitic fluid) was titrated in serial twofold dilution steps from 1/50 (antibody 30.2, O) or 1/200 (antibody 30.11, ▲; antibody 30.12, ■). The quantity of labelled antibody bound (average of duplicate determinations) is expressed as a percentage of the reference level bound in the absence of competing antibody. (a) $^{125}$I-labelled antibody 30.2; (b) $^{125}$I-labelled antibody 30.11; (c) $^{125}$I-labelled antibody 30.12.

serial dilutions of each unlabelled competing antibody. It is apparent from the results in Fig. 2 that none of the antibodies is able to inhibit the binding of any of the others although they do, of course, inhibit the binding of homologous labelled antibody. All three therefore bind to distinct antigenic sites on the E1 molecule. Enhancement of labelled antibody binding occurs in several combinations. Thus, binding of antibody 30.11 is stimulated by the presence of antibody 30.12 and vice versa, and binding of either of these antibodies is enhanced by the presence of antibody 30.2. Binding of antibody 30.2 was, however, only mildly enhanced in the presence of antibody 30.11 and hardly at all in the presence of antibody 30.12. Whatever the reason for this non-reciprocity, it is evident that binding of each of the three monoclonal antibodies does induce measurable changes in the way E1 binds antibodies at other antigenic sites.

DISCUSSION

The molecular specificity of antibody 30.2 is clearly E1-directed, since only this protein is precipitated in the presence of SDS, but in its absence significant quantities of PE2 are also brought down. Although an unexpected immunological cross-reaction between E1 and PE2 cannot be ruled out, it seems improbable that the interactions between the antibody and the two proteins would be differentially sensitive to SDS to such a degree. Antigen–antibody interactions are not generally sensitive to SDS in the presence of excess non-ionic detergent, and the binding of several other monoclonal antibodies to Sindbis, Japanese encephalitis, yellow fever and Lassa virus proteins takes place in the presence of SDS and Triton X-100 (Chanas et al., 1982a; J. C. S. Clegg, unpublished observations). No such cross-reaction between the two proteins has been reported using conventional antisera. The documented existence of non-ionic
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detergent-resistant heterodimers in infected cells (Ziemiecki et al., 1980) provides both a necessary and sufficient explanation for the precipitation of PE2 by antibody 30.2 in the absence of SDS and its sensitivity to the presence of this detergent, which is known to dissociate the two proteins.

In this case, the precipitation of E1 alone in the absence of SDS by antibodies 30.11 and 30.12 or antibody clones 1 and 2 of Roehrig et al. (1980) requires that binding of these antibodies by E1 is accompanied by release of PE2. Such dissociation of E1–PE2 heterodimers has been observed to occur to a limited extent with conventional monospecific antisera (Ziemiecki & Garoff, 1978). Release of PE2 from E1 by E1-specific monoclonal antibodies might be mediated through competition by the antibodies for binding sites on E1 used by PE2, although it seems unlikely that antibodies would be directed at regions of a protein normally masked by interaction with another virus protein. It is more probable that release of PE2 occurs as a consequence of conformational changes in E1 induced by antibody binding. Such antibody-induced conformational changes have already been invoked to explain enhancement of binding of one monoclonal antibody by another in the influenza virus HA protein system (Lubeck & Gerhard, 1982) and the vesicular stomatitis virus glycoprotein system (Lefrancois & Lyles, 1982). Similar evidence is provided here for the induction of conformational changes in the Sindbis E1 glycoprotein by monoclonal antibody binding. Each of the three monoclonal antibodies used in this study binds to a separate antigenic site on E1, as indicated by their failure to inhibit each other’s binding in competition assays. This result is consonant with their different effects on biological functions of the virus. Antibody 30.11 inhibits viral haemagglutination and neutralizes infectivity, antibody 30.12 does neither of these things but does inhibit virus-mediated haemolysis (Chanas et al., 1982a), while antibody 30.2 has no effect on any of these functions. There is also evidence from immune electron microscopy that antibody 30.11 attaches to the apex of the spikes on the surface of the virions and antibody 30.12 binds at a topographically distinct site near the virus membrane (Chanas et al., 1982b).

The competitive binding assay also indicates that binding of any of the three monoclonal antibodies induces conformational changes in E1. These changes are shown in the binding assay as enhanced binding of 125I-labelled antibody in the presence of another non-labelled antibody, and we suggest that the conformational changes induced by antibodies 30.11 and 30.12 not only enhance each other’s binding, but also lead to dissociation of the E1–PE2 heterodimer. The conformational change induced by antibody 30.2 also stimulates the binding of antibodies 30.11 and 30.12, but without releasing PE2 from its association with E1.

It should be pointed out, however, that the somewhat similar enhancement of binding of monoclonal antibodies to human chorionic gonadotrophin was not attributed to allosteric changes in the conformation of the hormone, since it was not brought about by F(ab) fragments of antibody (Ehrlich et al., 1982). Results of such experiments are not yet available for any of the viral systems, but it is difficult to envisage mechanisms for the release of Sindbis PE2 from E1 which involve either the divalency of the binding antibody or its Fc region.

The possible biological significance of these antibody-induced conformational changes is indicated by the ability of antibody 30.12 to potentiate the neutralization of Sindbis virus infectivity mediated by antibody 30.11. This effect was apparent over a limited range of virus dilutions when infectivity was assayed in Vero cells, but was much more striking when P388D1 cells were used. It was not mirrored, however, by any effect on haemagglutination inhibition by antibody 30.11 (Chanas et al., 1982a), but this assay may be insufficiently sensitive to detect the enhancement (Lubeck & Gerhard, 1982). It would be expected from the binding assay data that a similar potentiation of antibody 30.11-mediated neutralization would be found using antibody 30.2; this possibility is at present under investigation. Antibody-induced dissociation of the envelope protein heterodimers does not itself appear to be correlated with neutralizing or other biological activity, since the two antibodies which have this property have very different effects on virus functions. The phenomena of potentiation of neutralization mentioned above and the synergistic binding of antibodies 30.11 and 30.12 to virus particles observed by electron microscopy (Chanas et al., 1982b) suggest that conformational changes in E1 can take place while it is incorporated into the virus membrane architecture, but it is not likely that dissociation of E1 and E2 can take place in such an environment.
REFERENCES


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