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Heterocomplexes of tick-borne encephalitis structural and non-structural proteins

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The existence of the complex of structural and non-structural proteins in the tick-borne encephalitis (TBE) virus is shown. The complex was isolated from virus-containing cultural medium by immunoaffinity chromatography on monoclonal antibodies (MAbs). By enzyme immunoassay and immunoblotting with the use of appropriate MAbs it was demonstrated that this complex consists of structural (protein E), and non-structural (NS1) glycoproteins. Also, the trimer E–NS1–NS3 can be isolated. It is proposed that this trimer is the viral replicative complex.

Tick-borne encephalitis virus; Monoclonal antibody; Immunoaffinity chromatography; Complex structural and non-structural proteins

1. INTRODUCTION

One of the peculiarities of flaviviruses is the existence of glycosylated non-structural protein NS1 both in extracellular form [1] and on the surface of infected cells [2]. In studies of glycoproteins of viruses of Murrey Valley encephalitis [3], the natural heterodimer of E and NS1 was revealed, which confirms the role of NS1 in processes of maturation and/or transfer of virions from the cell. The possible existence of an extracellular form of the dimer E–NS1 was demonstrated in our previous paper [4]. The immunoaffinity purification of the extracellular protein complex, analysis and preliminary characterization of proteins composing the complex were carried out using the MAbs to TBE virus proteins.

2. MATERIALS AND METHODS

2.1. *Virus*

TBE virus (Sofyin strain) was proliferated in tissue cultures of human kidney cells (RH) [5].

2.2. *Monoclonal antibodies*

Production of MAbs against proteins E, NS1 and NS3 and their characteristics were as described elsewhere [6].

2.3. *Immunoaffinity chromatography*

MAbs specific to protein E (14D5) and NS1 (4C4) were purified by double ammonium sulfate precipitation and were dialyzed against 0.2 M $NaHCO_3$ pH 8.3, containing 0.5 M NaCl. Preparation of immunosorbents and chromatography were described in details in [4]. The virus-containing cultural medium was loaded onto the immunosorbent column with MAbs against NS1 (4C4) and after rinsing of the column with TNE (0.02 M Tris-HCl pH 8.2, 0.5 M NaCl, 0.005 M EDTA and 0.1% Na deoxycholate) the bound protein was eluted by 20 mM diethylamine (DEA) in TNE pH 11.5. The fractions containing NS1 were pooled, dialyzed against TNE without sodium deoxycholate and passed through an immunosorbent column with MAb to protein E. The fractions were analyzed for E and NS1.

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2.4. *Enzyme immunoassay (EIA)*

For EIA test-systems were used with solid phase sensibilized by specific to protein E immunoglobulins (antiserum was obtained to purified protein E [5] or to protein NS1 (antiserum was obtained to the expressed NS1 analog [4]. The antigen was detected by MAbs against protein E and NS1, respectively. EIA is described in detail in [4].

2.5. *Electrophoresis and immunoblotting*

The antigens were prepared according to [3] with or without the addition of 2-mercaptoethanol (2-ME). Moreover, the samples without 2-ME were not boiled for identification of the protein complex. Then the samples were exposed to electrophoresis in 7,5% PAG and immunoblotting [7] followed by the detection of antigens by MAbs.

3. RESULTS

Fig. 1 illustrates the typical immunoaffinity chromatography profiles. Both E and NS1 antigens were detected in each fraction of the first profile, the concentration of protein E was about 10% of that of NS1 (Fig. 1a). This number can be regarded as reliable since special experiments showed that the sensitivity of EIA methods for detection of these proteins was the same. The second step was affinity chromatography on the immunosorbent with MAbs specific to protein E. The antigens of both proteins (Fig. 1b) were detected in each fraction, but now they were found in equal concentrations.

And finally the results of electrophoresis followed by immunoblotting confirm the existence of protein E and NS1 complexes (Fig. 2). If the samples were not 2-ME-treated and not boiled, MAbs to NS1 and E demon-

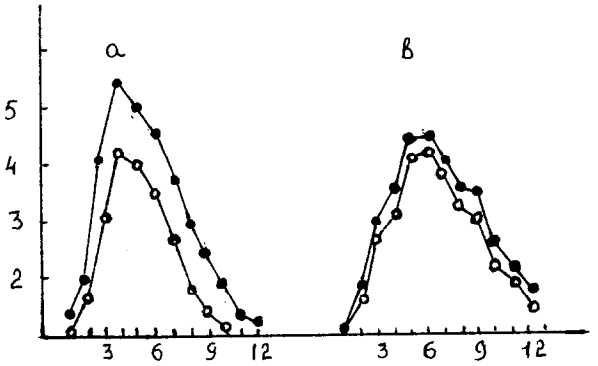

Fig. 1. Profiles of immunoaffinity chromatogramms. Protein contents are as titers E and NS1 antigens detected by EIA. (a) Chromatography on immunosorbent with MAbs (4C4) to NS1 (-●-●-); (b) Chromatography on immunosorbent with MAbs (14D5) to protein E (-○-○-).

strated antigens with M_r of about 100 kDa and 115 kDa and after the electrophoresis under denaturating conditions MAbs to NS1 indicated a protein with an M_r below 50 kDa, and MAbs against E revealed a protein with an M_r of about 55 kDa.

In cases where the immunoaffinity column with sorbed MAb 4C4 was not washed with sodium deoxycholate the electrophoresis showed the presence of the other proteins in the complex. Thus, native electrophoresis showed a band with mobility of about 200 kDa and under denaturating conditions proteins appeared with an M_r of about 70 kDa. These proteins bound to MAbs (18B2) against non-structural protein NS3 and the protein with M_r 200 kDa to MAbs against protein E. EIA confirmed the presence of NS3 in isolated pro-

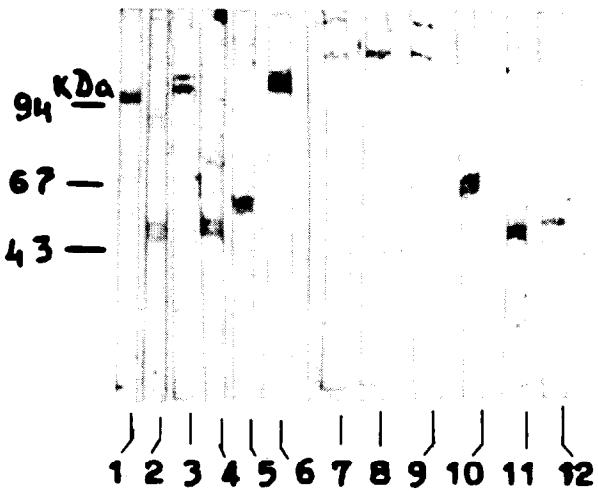

Fig. 2. Electrophoresis and immunoblotting of affinity-purified preparations of NS1 (1,2), E-NS1 (3–6) and E–NS1–NS3 (7–12). Samples 1, 3, 6–8 were treated with 2-ME and boiled before electrophoresis. Samples 2, 4, 5 and 10–12 untreated by 2-ME and unheated. Samples were then analyzed by SDS-PAGE and immunoblotting using monoclonal antibodies (1–4, 8,11 – MAb (4C4) to NS1; 5,6,9,12 – MAb (14D5) to E protein; 7 and 10 – MAb (18B2) to NS3).

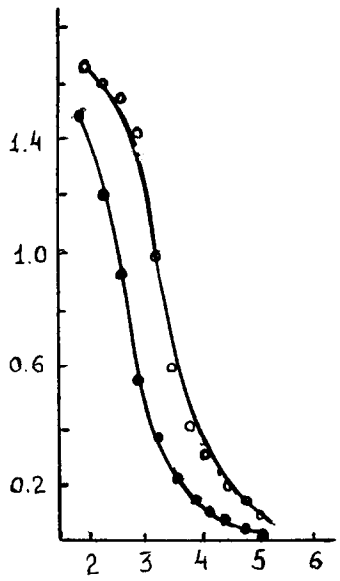

Fig. 3. The curves of antigenic titration of the protein complex by EIA. (a) Solid-phase sensibilized rabbit immunoglobulins specific to NS1; the titration was carried out by MAbs to NS3 (-○-, 18B2) and to E (-●- 2H3).

tein complex (Fig. 3). It is not clear, why the protein complex (200 kDa) was not detected by MAbs to NS1, although the complex was isolated by immunoaffinity chromatography with the use of these MAbs.

4. DISCUSSION

Schlesinger et al. [8,9] reported on NS1-protective properties of Yellow Fever and Denge viruses, although such activity has been commonly associated with structural virion components, in particular with protein E, appearing to be the main virus immunogen [10]. The recently obtained data for TBE virus [4] did not reveal any protection by NS1. In hyperimmunization experiments the high immunogenicity of the E–NS1 dimer was shown, accompanied by an increase in neutralizing activities of the protein E in complex with NS1.

As the main processes of TBE virus maturation take place in the cell endoplasmic reticulum, it would be reasonable to suppose that the release of the E–NS1–NS3 structure occurs due to the destruction of infected cells. However, immunoaffinity chromatography data show the concentration of the complex in virus-containing medium to be higher, than that expected due to the above reason. A special investigation is required to make the definite conclusion about the functional role of the trimer E–NS1–NS3, which is likely to be a form of a replicative complex.

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