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STRUCTURAL PROTEINS OF FIXED RABIES VIRUS IN THE SUCKLING MOUSE BRAIN

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Summary. — Structural proteins of fixed rabies virus grown in the suckling mouse brain were analysed by SDS-polyacrylamide gel electrophoresis (PAGE). The relative content of G protein to L, N and M_1 proteins of brain-grown virus was much lower when compared with that of the virus grown in chick embryo fibroblasts. The results indicate that nucleocapsid complexes, consisting of L, N and M_1 proteins were the predominant antigens accumulating in the brain.

Key words: *rabies virus*; *structural proteins*; *purification*; *radioimmunoprecipitation*; *suckling mouse brain*

Introduction

Morphogenesis of rabies virus in the mouse brain has been extensively studied by electron microscopy. Especially, in the mouse brain infected with fixed rabies viruses, electron microscopy revealed the budding as a final stage of viral maturation in neurons along with a great excess of viral nucleocapsid materials (Iwasaki *et al.*, 1975; Matsumoto, 1975; Murphy, 1975). Purification of the fixed rabbies virus from mouse brain by ECTEOLA-cellulose columm chromatography (Thomas *et al.*, 1965; Turner and Kaplan, 1967) or from sheep brain by CsCl density gradient centrifugation (Nikiel, 1974) has been reported, and, in the latter case, various virus forms were observed by electron microscopy. Since no data on polypeptide compositon of the rabies virus obtained from animal brains are available, we compared the relative content of structural proteins with that in chick embryo fibroblasts (CEF).

Materials and Methods

Preparation and purification of CEF-grown virus strain Flury HEP (CE-HEP virus) have been described previously (Arai *et al.*, 1976). As the mouse brain-grown virus, the CVS strain of fixed rabies virus was used; it was passed 12 times through adult mice and successively 2 to 4 times through suckling mice by intracerebral inoculation in our laboratory (brain-CVS virus). Emulsion of the infected brains, 10% (w/v), was prepared using NTE buffer (0.05 mol/l Tris-HCl, pH 7.9, 0.13 mol/l NaCl, 0.001 mol/l EDTA) and centrifuged at 10,000 × g for 20 min. Supernatants were centrifuged at 78,000 × g for 120 min to precipitate virus. The pellet was resuspended in NTE

buffer of 1/5 volume of the starting material and centrifuged at 10,000 × g for 20 min and used as partially purified virus.

Preparation and titration of antisera, infectivity titrations and determination of complement fixation (CF) activity were described previously (Arai *et al.*, 1976; Lodmell *et al.*, 1981).

Labelled virus was prepared by the chloramin-T method (Wiktor *et al.*, 1972). Viral proteins (500 μg) were labelled with 18.5 MBq of carrier free ^{125}I (Armersham Seale Co.) in cold bath. The iodinated samples were dialyzed against phosphate buffered saline (PBS) with several changes for 3—4 days at 4 °C.

The viruses and sera used for immunoprecipitation were centrifuged at 10,000 × g for 30 min to remove any precipitates in advance. To 100 μl of iodinated viral proteins, 100 μl of rabbit anti-rabies sera or normal rabbit serum (1 : 2 diluted) and 200 μl of 0.1% bovine serum albumin in PBS were added and incubated at 37 °C for 1 hr, followed by standing overnight at 4 °C. Precipitates were separated by centrifugation at 2,000 × g for 20 min in 4 °C and washed twice with PBS. Final pellets were heated in the resolving buffer containing 1% sodium dodecyl sulphate (SDS) and 1% 2-mercaptoethanol for 2 min at 100 °C, and subjected to 7.5% SDS-PAGE as described by Laemmli (1970). After electrophoresis the gels were stained with Coomassie brilliant blue and dried under vacuum on Whatman 3 mm filter papers. Autoradiography was done by exposing dried gels to Fuji X-ray films (Fuji Photo Film Co.).

Results and Discussion

The brain-CVS virus partially purified by centrifugation was further purified by a Sepharose 4 B gel filtration (Pharmacia, Uppsala, Sweden). A typical elution pattern with 0.01 M Tris-HCl buffer containing 0.5 mol/l NaCl (pH 7.9) is shown in Fig. 1. Two peaks in optical density at 280 nm were observed. The CF activity to anti-CE-HEP virus was found in both peaks, and infectivity was detected mainly in the first peak (void volume). Fractions of the first peak (numbers 16—19) were pooled. Infectivity of the pool was $10^{5.8}$ LD_{50} per 0.03 ml as assayed in adult mice. The pool was centrifuged in CsCl density gradient. As shown in Fig. 2, the first peak at OD 280 nm revealed infectivity. The peak (CsCl-purified brain-CVS virus fraction) had a broader range of density, 1.24—1.26 g/cm^3, compared with a sharp peak at 1.22 g/cm^3 observed with CE-HEP virus. Haemagglutination activity was not detected in all of these brain samples. SDS-PAGE of Sepharose 4 B or CsCl-purified brain-CVS virus fraction showed an intense band located at the position corresponding to N protein of CE-HEP virus. In contrast,

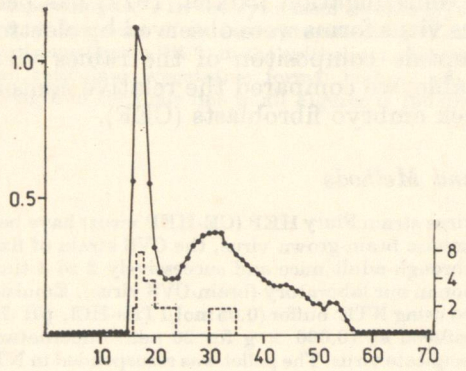

Fig. 1.

Elution profile of brain-CVS virus by Sepharose 4 B gel filtration
Abscissa: fraction number; ordinates: left — OD at 280 nm (●——●), right — CF titre (dashed line).

bands of G protein and M_2 protein of brain-CVS virus were much weaker than those of CE-HEP virus (Fig. 3). No significant difference in the relative mobilities of corresponding polypeptides was observed by SDS-PAGE between CE-HEP and brain-CVS viruses.

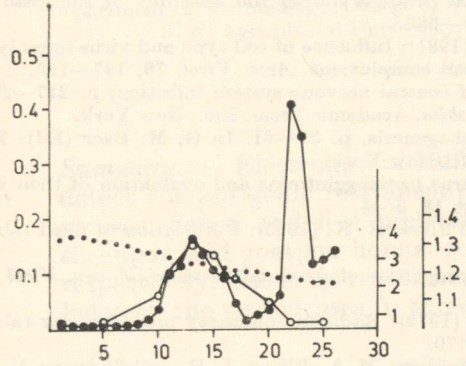

Fig. 2.

Equilibrium density gradient centrifugation of brain-CVS virus obtained after gel filtration
Virus was mixed with CsCl and centrifuged for 40 hr at 100,00 ×g. Infectivity was titrated by intracerebral inoculation into 4-week-old mice (ddY). LD_{50} was calculated to 5 mice for each dilution. Abscissa: fraction number; ordinates: left — OD at 280 nm (●), right — — $\log_{10}$ LD_{50}/0.03 ml (○) and density gradient (g/cm³, dotted line)

As shown in Fig. 4, the intensity of G protein band in the immunoprecipitates of brain-CVS virus was remarkably reduced as compared to N and M_1 proteins. M_2 protein was hardly detected. To examine the possibility that G protein was dissociated from the pellets during centrifugation at 78,000 × g for 2 hr, brain-HEP virus was obtained directly from a 50 % (w/v) emulsion of suckling mouse brain infected with brain-HEP virus, which was the CE-HEP virus passed 6 times through suckling mouse brain by means of precipitation with anti-CE-HEP virus, and the preparation was submitted to analysis by SDS-PAGE. Again, the band corresponding to G protein was as faint as observed in the case of brain-CVS virus (data not shown). These data showed that the reduced G protein was not dissociated artificially during preparations of the virus.

Electron microscopy of the CsCl-purified brain-CVS virus fraction revealed the presence of many nucleocapsid-like structures and irregular spheroidal particles bearing spikes as reported by Nikiel (1974) (data not shown).

These results indicate that, besides the presence of a little amount of complete virion, many subviral particles consisting of L, N and M_1, proteins as reported by Zaides and co-workers (1979) would actually accumulate in suckling mouse brain during infection with fixed rabies virus. Another possibility may be that, in the brain of suckling mouse, G protein-reduced or even G protein-deficient viral particles would have the ability to multiply and share the infectivity to mouse brain. Further studies will be necessary to determine the infectivity of these subviral particles in mouse brain.

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Explanation of Figures (Plates XIII—XIV):

Fig. 3. SDS-polyacrylamide gel electrophoresis.
CE-HEP virus (a); brain-CVS virus, the pooled fraction in void volume obtained after Sepharose gel filtration (b); the infectivity peak after CsCl gradient centrifugation (c); staining with Coomassie briliant blue.

Fig. 4. Autoradiography of SDS-polyacrylamide gel electrophoresis of immunoprecipitates from brain-CVS virus.
CE-HEP virus (a), brain-CVS virus mixed with anti-CE-HEP virus (b), with anti-brain-CVS virus (c), with anti-brain-CVS virus which was absorbed by acetone powder of normal mouse brain (d), or with normal rabbit serum (e); and CE-HEP virus mixed with anti-CE-HEP virus (f).