Structural Proteins of Rabies Virus

FRANTISEK SOKOL, DANIEL STANČEK, AND HILARY KOPROWSKI

The Wistar Institute of Anatomy and Biology, The World Health Organization International Reference Center for Rabies at The Wistar Institute, and The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104

Purified rabies virions, unlabeled or labeled with radioactive amino acids or D-glucosamine, were dissociated into their polypeptides by treatment with sodium dodecyl sulfate in a reducing environment and fractionated by electrophoresis in sodium dodecyl sulfate-containing polyacrylamide gel. The molecular weights of individual polypeptides were estimated by comparison of their rate of migration with that of protein markers of known molecular weight. Purified viral nucleocapsid and a mixture of envelope components, isolated from virions disrupted by sodium deoxycholate, were analyzed by the same procedure. The number of molecules per virion of each polypeptide was estimated from the proportions of the separated components, the known molecular weight of the viral ribonucleic acid, and the chemical composition of the nucleocapsid. The protein moiety of the nucleocapsid particle was estimated to consist of 1,713 molecules of a major polypeptide (molecular weight, 62,000 daltons) and 76 molecules of a minor polypeptide (molecular weight, 55,000 daltons). In addition to 1,783 molecules of a glycoprotein component (molecular weight, 80,000 daltons), the viral envelope contains 789 and 1,661 molecules, respectively, of two other polypeptides (molecular weight, 40,000 and 25,000 daltons).

The structural complexity of rabies virus (9) indicates that it is composed of several distinctive proteins. It was reported recently that treatment with sodium deoxycholate (DOC) causes disruption of the protein-lipid envelope of rabies virions and release of the helical nucleocapsid, a nucleoprotein containing the viral ribonucleic acid (RNA; reference 22). The disrupted virions were fractionated into nucleocapsid and a mixture of coat components on the basis of markedly different rates of sedimentation and buoyant density. Several properties of purified nucleocapsid preparations have been described, but properties of the individual coat components remain largely unknown.

In the present study, purified rabies virions or their components, dissociated by treatment with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (2-ME), were analyzed by electrophoresis in polyacrylamide gel to determine the number, the molecular weight, and the structural identity of the polypeptides of rabies virions. Of the four major polypeptides of different molecular weights, the second largest in molecular size is associated with the viral RNA in the nucleocapsid, whereas the remaining three form the viral envelope. The envelope polypeptide with the largest molecular weight is a glycoprotein.

MATERIALS AND METHODS

Virus. The 1210/A clone of the high-egg-passage (HEP) Flury strain (21) and the W clone of the ERA strain (14) of rabies virus, isolated by T. J. Wiktor, were used in all experiments.

Tissue culture. BHK/13S cells were propagated in roller cultures. The 2-liter bottles were seeded with 4×10^7 cells in 100 ml of BHK cell growth medium (17) supplemented with 10% fetal calf serum and were incubated at 37 C. The cell cultures became confluent in about 3 days and contained, at the time of infection, approximately 2.4×10^8 cells per bottle. BHK-21 cells were grown in 1-liter Blake bottles as described previously (21).

Infection of the cells and labeling of the virus. Cultures of BHK/13S or BHK-21 cells were infected with 1 plaque-forming unit of virus per cell, as described previously (21). After 1 hr of adsorption at 35 C, the inoculum was removed and the cells were refed with 50 ml of Eagle's minimum essential medium supplemented with 0.2% bovine serum albumin (BSA). For labeling of the virus, the medium contained 2.5 µCi of a mixture of 16 ^{3}H-amino acids per ml (average specific activity, 17.6 Ci/mmole; Schwarz/Mann mixture no. 3130-08; Schwarz BioResearch, Orangeburg, N.Y.), 0.5 µCi of a mixture of 13 ^{14}C-amino acids per ml (average specific activity, 250 mCi/mmole; Schwarz/Mann mixture no. 3122-08, Schwarz BioResearch), or 1 µCi of ^{3}H-D-glucosamine per ml (specific activity, 850 mCi/mmole; New England Nuclear Corp., Boston, Mass.).

Also for protein labeling, the concentration of the cold amino acids in the medium was reduced to one-fifth of the regular level. The cultures were incubated at 33 C. Extracellular virus was harvested approximately 72 hr after infection.

Purification of the virus and isolation of viral components. The virus purification scheme included precipitation by zinc acetate, desalting on a Sephadex column, treatment with ribonuclease and deoxyribonuclease, pelleting by high-speed centrifugation, and banding of the virus by centrifugation in a sucrose density gradient (21). Procedures for the fractionation by velocity centrifugation in a sucrose density gradient of DOC-disrupted virions into nucleocapsid and a mixture of coat components, as well as the purification of the nucleocapsid by isopycnic centrifugation in CsCl solution, have been previously described (22).

Protein determination. Protein was assayed as described by Lowry et al. (16) by using BSA as standard.

Standard procedure for the preparation and electrophoresis of polypeptides. Proteins from diluted solutions were precipitated at 0 C with 10% trichloroacetic acid. The precipitate collected by low-speed centrifugation was washed at 0 C with 2 ml of 2% trichloroacetic acid and then with the same volume of ice-cold acetone. The sediment was then dried in a stream of warm air and dissolved in 0.01 M phosphate buffer (pH 7.2) containing 3.33% (w/v) SDS and 0.67% (v/v) 2-ME. If necessary, the pH of the solution was adjusted to 7.2 by addition of a few microliters of 1 N NH$_4$OH. In all instances, more than 94% of the original amino acid or glucosamine label was recovered in the dissolved precipitate. Sufficiently concentrated samples were directly adjusted to 3.33% in SDS and dialyzed at room temperature overnight against the SDS- and 2-ME-containing phosphate buffer. The polypeptide samples were stored at 4 C. Sucrose was then added to make a final concentration of 15%. Samples not exceeding 0.2 ml in volume and containing 20 to 400 μg of polypeptides were heated at 100 C for 1 min (18) and analyzed.

Nine volumes of a solution containing 7.5% acrylamide, 0.27% N,N'-methylenebisacrylamide, 0.1% SDS, and 0.05% N,N,N',N'-tetramethylethylenediamine in 0.1 M sodium phosphate buffer (pH 7.2), were mixed with 1 volume of 1% ammonium persulfate and polymerized to form gels 20 cm in length and 0.6 cm in thickness. The gels were prerun in the electrophoresis buffer (0.1 M phosphate buffer, pH 7.2, containing 0.1% SDS) for 4 hr at 2 ma per gel. The sample was then placed on the top of the gel and overlaid with the electrophoresis buffer. Electrophoresis was carried out at 2 ma per gel for 20 min and then at 5 ma per gel for 11.2 to 16.5 hr. Some gels were stained for 2 hr at 20 C with 1% Amido Black B-10 in 7% acetic acid and then destained in several changes of 7% acetic acid. All gels were cut into 1-mm slices, unless otherwise stated, and solubilized in 0.5 ml of 15% hydrogen peroxide at 90 C for 30 min. The radioactivity of the fractions was determined, after the addition of 10 ml of Aquasol

(New England Nuclear), in a Packard liquid scintillation spectrometer.

Protein standards for determination of molecular weight. The following proteins, purchased from Mann Research Laboratories, New York, N.Y., were used as markers for molecular weight determination: human gamma globulin (160,000 daltons), BSA (67,000 daltons), ovalbumin (45,000 daltons), whale sperm myoglobin (17,800 daltons), and horse heart cytochrome c (12,400 daltons).

RESULTS

Polypeptides of rabies virions. When purified rabies virions, labeled with radioactive amino acids, were disrupted by treatment with SDS and 2-ME and analyzed by electrophoresis in polyacrylamide gel, four major peaks of radioactivity were repeatedly observed (Fig. 1a). Similarly, the optical density tracing of virus polypeptides, fractionated by the same procedure and stained by Amido Black, showed four main bands of absorbance (Fig. 1b). The peaks of the stainable bands coincided with those of the radioactive bands (Fig. 1a, inset). The radioactivity or the absorbance recovered in the four main bands represented 85 to 90% of the total radioactivity or absorbance in the gel. A variable amount (5 to 12% of the total) of polypeptides of a molecular size larger than the slowest of the four major components was detected in each preparation of disassembled virus. For reasons which will be given later, we believe that most of these components represent aggregates (dimers) of the major polypeptides. Peaks of radioactivity observed in electrophoretograms of dissociated cellular proteins obtained from cell-free fractions of mock-infected cultures and subjected to the same sequence of fractionation procedures used for the purification of the virus did not coincide with the peaks of viral polypeptide bands (Fig. 2). Therefore, contaminating cellular proteins would be easily detected in electrophoretograms of insufficiently purified preparations of dissociated virions. Although the material not contained in the four large bands probably represents either aggregates of the major viral polypeptides or cellular contaminants, it is possible that minor structural proteins of the virus were not detected by the fractionation procedure used.

Other methods of releasing rabies virus polypeptides, such as treatment with SDS and 2-ME at 37 C with or without previous precipitation of the virions by trichloroacetic acid, replacement of 2-ME with dithiothreitol, additional treatment of SDS- and 2-ME-dissociated virions with 6 M guanidine or 10 M urea, were also tried. In all cases, the electrophoretic patterns obtained were
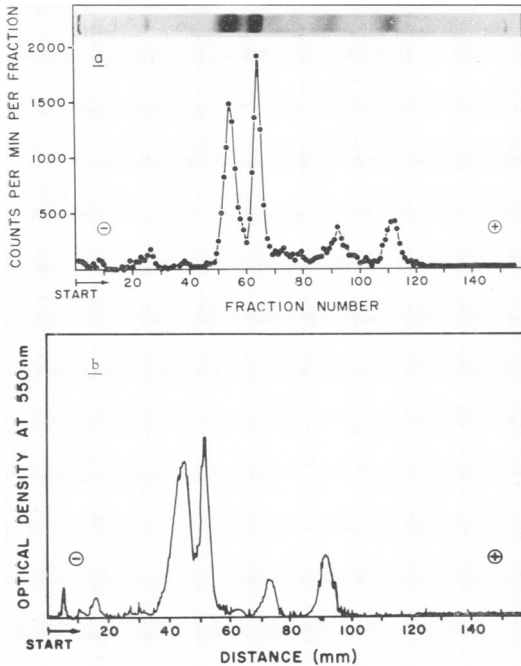
FIG. 1. *Fractionation of rabies virus polypeptides by electrophoresis in polyacrylamide gel. Purified rabies virus [high-egg-passage (HEP) Flury strain], labeled with a mixture of ³H-amino acids, was dissociated with sodium dodecyl sulfate in a reducing environment and electrophoresed in 7.0% gel for 13.5 hr. The ³H-amino acid-labeled polypeptides were stained before the gel was sliced (inset, panel a). The optical density profile of the viral polypeptides (HEP Flury strain) electrophoresed for 11.2 hr was obtained by scanning the stained gel in a Gilford spectrophotometer at 550 nm (b).*

similar to those observed after fractionation of polypeptides released from the virions by the standard procedure, i.e., treatment with SDS and 2-ME followed by heating at 100 C for 1 min.

The electrophoretic pattern of disassembled ERA virions (Fig. 3) was similar to that of dissociated HEP Flury virus (*see* also Table 1).

Estimation of molecular weights of rabies virus structural proteins. In the presence of excess SDS, the differences in the original net charge of various polypeptides are suppressed. Thus by electrophoresis in an SDS-containing polyacrylamide gel, polypeptides are separated largely on the basis of molecular size, the distance migrated being linearly related to the logarithm of the molecular weight (20). With proteins of known molecular weight as standards, the molecular weights of the four major HEP Flury virion polypeptides were

found to be 80,000, 62,000, 40,000 and 25,000 daltons, respectively (Fig. 4).

Identification of the nucleocapsid and envelope proteins of rabies virions. Purified rabies virions, labeled with a mixture of ³H-amino acids, were disrupted by treatment with DOC and subjected to velocity centrifugation in a sucrose density gradient so that the slowly sedimenting envelope components were separated from the 200S nucleocapsid (22). The nucleocapsid was then further purified by isopycnic centrifugation in a CsCl solution (22), treated with SDS and 2-ME, and analyzed by electrophoretic separation of the released polypeptides (Fig. 5). One major and two minor bands were repeatedly observed. On the average, 90.4% of the total radioactivity was associated with the polypeptide of intermediate size, having a molecular weight of about 62,000 daltons. The molecular weight of the heavy component, corresponding to 6.2% of the total radioactivity, was about 130,000 daltons, indicating that it could be a dimer of the major nucleocapsid polypeptide. The identity of the major nucleocapsid polypeptide with the virion polypeptide having a molecular weight of 62,000 daltons was
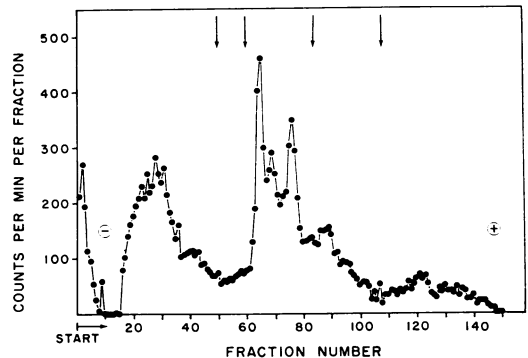

FIG. 2. *Electrophoretogram of polypeptides derived from host components which have been released spontaneously into the tissue culture fluid from mock-infected cells. Mock-infected cultures were labeled with ³H-amino acid mixture and incubated in the same way as infected cultures. The tissue culture fluid (290 ml) was subjected to the same procedures used for the purification of the virus. The host components were then mixed with an excess amount of nonlabeled purified virus, dissociated by sodium dodecyl sulfate in a reducing environment, and electrophoresed in 7.0% polyacrylamide gel for 12.7 hr. The gel was first stained for determination of the position of viral polypeptides (arrows). It was then cut into slices for the determination of radioactivity contained in the fractions. For comparison, the amount of viral polypeptides used in the experiment shown in Fig. 1a, was derived from 29 ml of infectious tissue culture fluid.*

confirmed by co-electrophoresis of disassembled ^{14}C-amino acid-labeled virions with dissociated
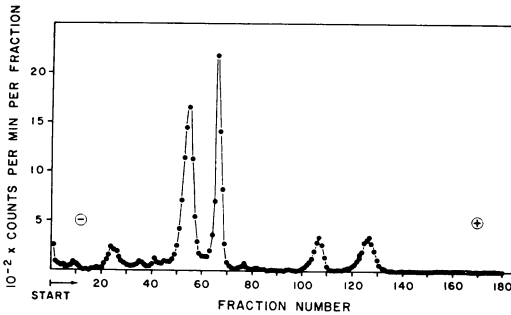

FIG. 3. *Electrophoresis of dissociated ERA virions in polyacrylamide gel. Purified rabies virus, labeled with ^{3}H-amino acids, was dissociated with sodium dodecyl sulfate and 2-mercaptoethanol and electrophoresed in a 7.0% polyacrylamide gel for 14 hr. Abbreviations: EP, envelope protein; BSA, bovine serum albumin; NCP, nucleocapsid protein; GlP, glycoprotein.*
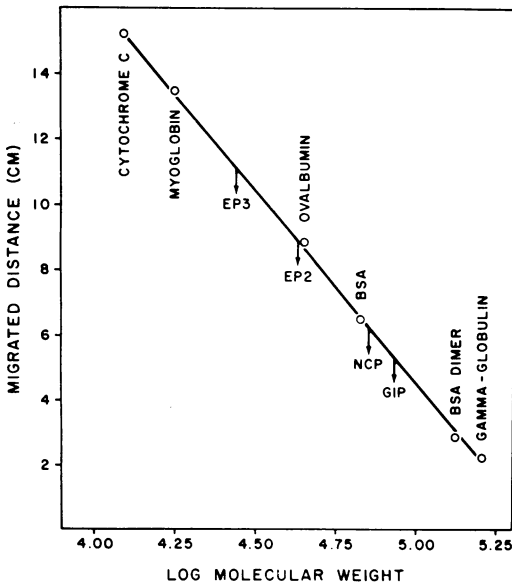

FIG. 4. *Estimation of the molecular weights of rabies virus polypeptides. Polypeptides released from purified rabies virions (high-egg-passage Flury strain) by treatment with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol were separated by electrophoresis in 7.0% polyacrylamide gel for 13.5 hr. Marker proteins of known molecular weight were treated with SDS and electrophoresed in parallel. The gels were then stained with Amido Black, and the distance from the origin of each band was plotted against the molecular weights of marker proteins. The four major components were designated GlP (glycoprotein), NCP (nucleocapsid protein), and EP (envelope protein) 2 and 3.*

^{3}H-amino acid-labeled nucleocapsid. The polypeptide with the lowest molecular weight, comprising 3.4% of the total radioactivity, is most likely a distinct structural component of rabies virus nucleocapsid, since it was present in all nucleocapsid preparations analyzed. Its molecular weight was estimated to be 55,000 daltons.

The remaining three major virion polypeptides were identified as structural parts of the viral envelope by electrophoresis of ^{3}H-amino acid-labeled envelope components, isolated from DOC-disrupted virions, with ^{14}C-amino acid-labeled virus (Fig. 6). All preparations of dissociated viral envelope were contaminated with a small amount of nucleocapsid protein. Viral RNA contained in the nucleocapsid molecules released from rabies virions by treatment with DOC was found to be partially sensitive to the degrading action of ribonuclease (22). This sensitivity is probably the result of the detachment of some protein subunits from the nucleocapsid particles during treatment with DOC. Thus when DOC-treated virions are fractionated by velocity centrifugation in a sucrose density gradient, the detached nucleocapsid protein is recovered in the fraction of the dissociated viral-envelope.

Glycoprotein component of the viral envelope. Several enveloped viruses, such as myxovirus (5–7, 15, 25), arbovirus (24, 26), herpevirus (1, 13, 23), vesicular stomatitis virus (VSV; references 2, 12), poxvirus (8), and RNA-containing tumor (4) virus, have been shown to contain carbohydrates covalently bound to one or several of the envelope proteins. To determine whether carbo-hydrates are incorporated into rabies virions, and if so, whether they are covalently bound to some of the polypeptides, we labeled the virus with ^{3}H-glucosamine and fractionated the labeled components of dissociated virus by electrophoresis in polyacrylamide gel. Radioactive glucosamine was
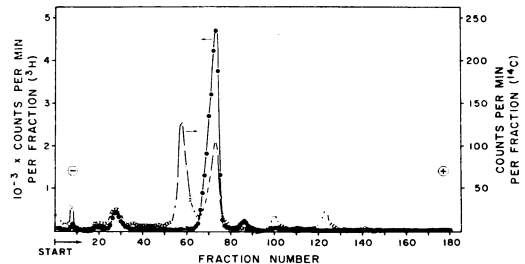

FIG. 5. *Fractionation of polypeptides of rabies virus nucleocapsid by electrophoresis in polyacrylamide gel. ^{3}H-amino acid-labeled purified nucleocapsid was mixed with ^{14}C-amino acid-labeled purified virions (high-egg-passage Flury strain), treated by sodium dodecyl sulfate in a reducing environment, and electrophoresed in a 7.0% polyacrylamide gel for 15 hr.*
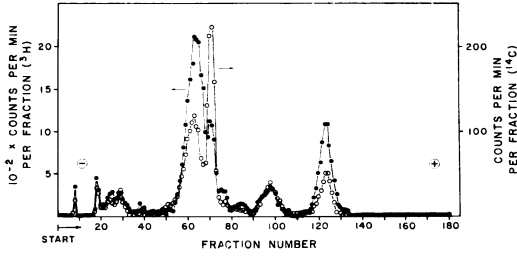
FIG. 6. *Fractionation of polypeptides of the rabies virus envelope by electrophoresis in polyacrylamide gel. ³H-amino acid-labeled envelope components (high-egg-passage Flury strain) were mixed with ¹⁴C-amino acid-labeled purified virions, treated by sodium dodecyl sulfate and 2-mercaptoethanol and electrophoresed in a 7.0% polyacrylamide gel for 15 hr.*

selected for labeling because its specificity as a label is very high, 85 to 97% of the radioactivity incorporated into the glycopeptide components of various viruses still being present in the original chemical form or in the form of N-acetylglucosamine (2, 3, 26). Moreover, the glycopeptides of Sindbis virus contain more glucosamine than any other sugar (3, 26).

When partially purified rabies virus, grown in tissue cultures labeled with ³H-glucosamine during infection, was centrifuged in a sucrose density gradient, the peak of the virus band coincided with that of the radioactivity (Fig. 7). Two components were seen after the purified ³H-glucosamine-labeled virus was dissociated by treatment with SDS and 2-ME and fractionated by electrophoresis in polyacrylamide gel (Fig. 8). The major component, comprising 73 to 79% of the total radioactivity contained in the gel, had the same molecular size as the largest of the four major virion polypeptides, i.e., a molecular weight of about 80,000 daltons. The minor component corresponding to 5 to 13% of the total radioactivity had a molecular weight of about 160,000 daltons and probably represented the dimer of the viral glycoprotein.

In almost all preparations, the band, corresponding to the viral glycoprotein obtained after electrophoretic separation of the components of dissociated virus, was broader than that of the major nucleocapsid polypeptide (e.g., Fig. 1), indicating that the glycoprotein component was heterogeneous in molecular size. As with VSV glycoprotein (2, 12), in preparations of decomposed rabies virions, which were labeled with ³H-carbohydrate and ¹⁴C-amino acids, the ratio of carbohydrate radioactivity to the amino acid radioactivity was significantly different in various fractions of the glycoprotein band (not shown here). Occasionally, the glycoprotein was distrib-
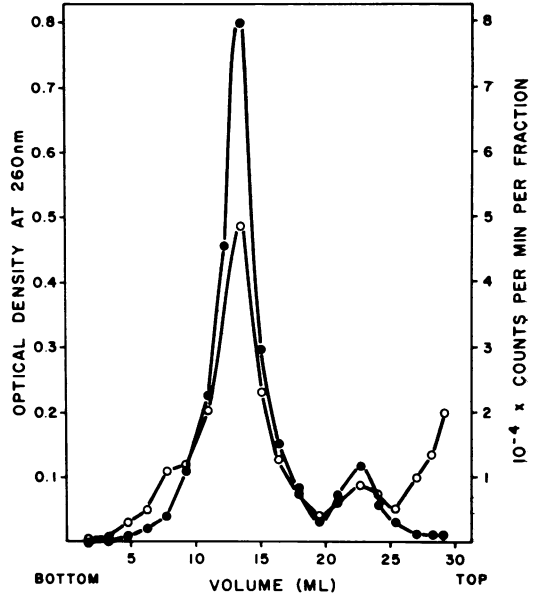

FIG. 7. *Fractionation of partially purified, ³H-glucosamine-labeled rabies virions (high-egg-passage Flury strain) by centrifugation in a sucrose density gradient. A suspension of labeled virions (0.6 ml), partially purified by precipitation with zinc acetate, filtration through Sephadex G-75 column, and pelleting by high-speed centrifugation, was layered over 28.5 ml of 10 to 50% (w/w) linear gradient of sucrose in NTE buffer (0.13 M NaCl, 0.05 M Tris-hydrochloride, 0.001 M ethylenediaminetetraacetate, pH 7.8) and centrifuged at 4 C and 61,000 × g for 90 min in the SW 25.1 rotor of a Spinco centrifuge. Absorbance and radioactivity of fractions collected from the bottom of the tube were then determined. The absorbing band located approximately in the middle of the tube coincides with those of various viral activities (20). Symbols: ○, optical density at 260 nm; ●, radioactivity of ³H-glucosamine.*
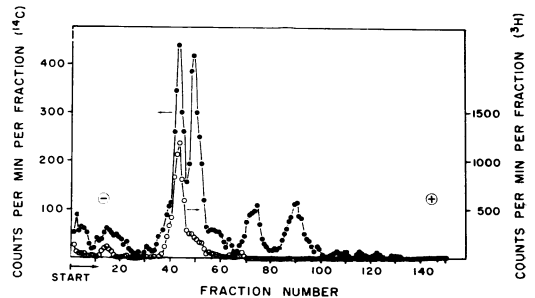

FIG. 8. *Electrophoresis in polyacrylamide gel of ³H-glucosamine-labeled rabies virus (HEP Flury strain) dissociated by treatment with sodium dodecyl sulfate and 2-mercaptoethanol. ³H-glucosamine-labeled virus was mixed with ¹⁴C-amino acid-labeled virions, treated with the detergent in a reducing environment, and electrophoresed in a 7.0% polyacrylamide gel for 16 hr. Each fraction corresponds to a 1.5-mm gel slice.*

uted in two adjacent bands (Fig. 9). Two observations indicated, however, that the latter type of extensive heterogeneity represents an artifact: (i) the proportion and the mobility of the separated glycoprotein "components" were variable in different preparations; and (ii) preparations of dissociated virus which originally showed only one broad glycoprotein band became extensively heterogeneous in the molecular size of their envelope glycoprotein after being stored for several months at 4 C. The reason for the lability of the viral glycoprotein is unknown. Precipitation of the viral proteins by trichloroacetic acid or heating of the viral polypeptides at 100 C for 1 min was not responsible for the degradation of the glycoprotein component, since virion preparations not treated with trichloroacetic acid and disassembled by SDS and 2-ME at room temperature or 37 C occasionally showed a similar extensive heterogeneity.

Relative proportions of rabies virus polypeptides. The relative proportion of the major rabies virus polypeptides was determined from the amount of dye bound by these four components, as well as from the amount of ^3H or ^{14}C radioactivity recovered from them (Table 1). The differences in the polypeptide composition of the virions, as determined by these three methods, reflect, in addition to experimental errors, the differences in the amino acid composition and the specific radioactivities of the two reconstituted protein hydrolysates used for labeling, as well as the difference between overall labeling with the dye and the labeling of specific amino acids with selected radioactive precursors. Moreover, it has to be assumed that the glycoprotein moiety of the glycoprotein component is not labeled, or only to a negligible extent, with the dye or the radioactive amino acids. Thus data presented in Table 1 do not include the contribution of the glycopeptide to the relative proportion of the viral glycoprotein.

Estimates of the molecular weights of the proteins of the viral nucleocapsid and envelope, as well as of the number of molecules of these components per one complete virion, are given in Table 2. A complete virion is defined here as a virus particle containing one nucleocapsid particle of full length (22). The estimates were derived from the data presented in Fig. 4 and Table 1, as well as from previous findings that the molecular weight of rabies virus RNA is 4.6 × 10⁶ daltons and that the viral nucleocapsid is composed of 96% protein and 4% RNA (22). They are also based on the assumption that the glycopeptides do not affect the mobility of the viral glycoprotein in an SDS-polyacrylamide gel to the extent that estimation of its molecular weight is unreliable.
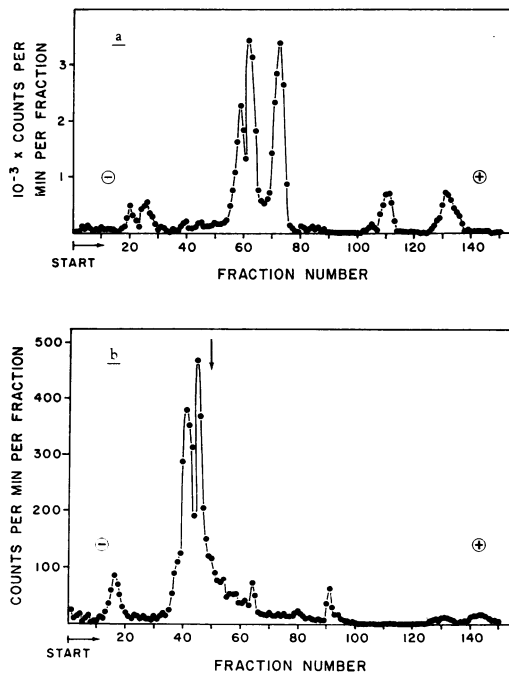

FIG. 9. *Heterogeneity in molecular size of the glycoprotein component of rabies virus. (a) ^3H-amino acid-labeled virions (ERA strain), disassembled by the standard procedure, were electrophoresed in 7.0% polyacrylamide gel for 15.5 hr. (b) ^3H-glucosamine-labeled virions (high-egg-passage Flury strain) were dissociated by the standard procedure and electrophoresed in 7.0% polyacrylamide gel for 16 hr. The position of the major nucleocapsid polypeptide is indicated (arrow). Each fraction corresponds to a 1.5-mm gel slice. The occasional distribution of the viral glycoprotein in two partially overlapping bands could also be observed after staining of the gel with Amido Black (not shown here).*

DISCUSSION

The results of the present study have shown that the protein moiety of rabies virus is composed of at least four major and one minor polypeptide of different molecular sizes. The viral RNA is associated with one of the major and with the minor polypeptide to form the nucleocapsid of the virion. The remaining major polypeptides are contained in the viral envelope. One of them, the largest in size, was shown to be a glycoprotein. A relatively small amount of components of much higher molecular weight (from 100,000 to 160,000 daltons) than that of the envelope glycoprotein (80,000 daltons) was detected in all preparations of disassembled virions. Insufficiently purified virus preparations were shown to contain contaminating cellular proteins composed of polypeptides of such molecular size. On the other

TABLE 1. *Relative proportions of major rabies virus polypeptides[a]*

Method	Virus strain	No. of determinations	Per cent in component (average values)			
			GlP	NCP	EP2	EP3
Optical density at 550 nm after staining with Amido Black	ERA	2	48.0	32.0	9.5	10.5
	HEP Flury	2	49.0	31.0	7.5	12.5
Labeling with ³H-amino acid mixture	ERA	2	47.0	34.0	8.5	10.5
	HEP Flury	6	43.5	32.5	10.5	13.5
Labeling with ¹⁴C-amino acid mixture	HEP Flury	5	43.0	34.5	10.0	12.5

[a] Abbreviations: GlP, glycoprotein; NCP, nucleocapsid protein; EP2 and EP3, envelope proteins; HEP, high egg passage.

TABLE 2. *Protein composition of complete rabies virus[a]*

Polypeptide	Mol wt	No. of molecules per virion[b]
GlP	80,000	1,783
NCP	62,000	1,713
NCP-M[c]	55,000	76
EP2	40,000	789
EP3	25,000	1,661

[a] Abbreviations as in footnote [a], Table 1.

[b] Average proportions of the four major polypeptides of HEP Flury virus used for the calculation (from Table 1): 44.3% GlP, 33.0% NCP, 9.8% EP2, 12.9% EP3.

[c] NCP-M, minor nucleocapsid protein; the number of NCP-M molecules per virion was calculated by using a weight ratio of NCP/NCP-M = 26.6.

hand, with sufficiently purified virion preparations, they might represent aggregates (dimers) of the four major viral polypeptides because (i) the proportion of these in different preparations of disassembled virus varied widely; and (ii) preparations of disassembled viral nucleocapsid or of ³H-glucosamine-labeled virus contained minor components of approximately twice as high molecular weight as the nucleocapsid polypeptide and the viral glycoprotein, respectively. A similar conclusion is suggested from the comparison of the size of the viral RNA and the total molecular weight of the virion polypeptides. The single-stranded viral RNA having a molecular weight of 4.6×10^6 daltons (22) can code for distinct polypeptides with a total molecular weight no greater than 4.6×10^5 daltons. The molecular weights of the five structural polypeptides totals 2.6×10^5 daltons. If the viral genome would direct the synthesis of two additional polypeptides, each with molecular weight larger than 100,000 daltons, the viral RNA could not code for non-structural proteins.

Preparations of purified HEP Flury virus contain a small proportion of truncated virus particles (21). It was assumed, in interpreting the results of the present study, that their protein composition was identical with that of complete virions of full length. This assumption is supported by the finding that purified B and T virions of VSV do not exhibit significant differences in their protein composition (11, 27). Purified preparations of ERA strain of rabies virus contain only a negligible amount of truncated virions (K. Hummeler, F. Sokol, and H. F. Clark, *unpublished data*).

VSV, which could be structurally and morphologically compared to rabies virus, contains three major polypeptides (11, 27, 28). The nucleocapsid polypeptide and the glycoprotein (2, 12) of VSV have molecular weights similar to those of corresponding components of rabies virus (11, 27). On the other hand, it is uncertain which of the remaining two envelope polypeptides of rabies virus (EP2 and EP3) is comparable in molecular size to the second major envelope protein of VSV. Three laboratories reported appreciably different molecular weights for the latter VSV component (2, 11, 27). With sufficient time for electrophoretic separation, a fourth minor structural polypeptide was detected in preparations of dissociated VSV (19), the molecular weight of which is comparable to the EP2 polypeptide of rabies virus. Although the exact biological functions of the individual envelope proteins of rabies virus are unknown, it was found that the mixture of the three envelope proteins induces virus-neutralizing antibodies when injected into animals (T. J. Wiktor and F. Sokol, *unpublished data*). Since a glycoprotein isolated from cell- and virus-free lysates of VSV (12) and the glycoprotein components of Rous sarcoma virus (4) were shown to bind virus-neutralizing antibodies, it can be assumed that the glycoprotein of rabies virus might exhibit a similar activity.

In only one of seven preparations of dissociated rabies virus could the envelope glycoprotein be separated by electrophoresis in polyacrylamide gel into two adjacent components differing in molecular weight by 5,000 to 10,000 daltons. For reasons given above, we believe that this heterogeneity of the viral glycoprotein is an artifact caused by an unknown factor during the purification or dissociation of the virions. Examination of electrophoretograms of VSV polypeptides also revealed an occasional heterogeneity of the glycoprotein component (11, 27). Moreover, it was found that the molecular size of VSV-specific glycoprotein isolated from the virus-free fraction of infective tissue culture fluid was smaller and contained about twice as much glucosamine than the glycoprotein component of the virions (12). Most likely a part of the polypeptide chain can easily be split off the viral glycoprotein. Attempts to demonstrate the presence of polypeptides having a molecular weight of 5,000 to 10,000 daltons in preparations of disassembled rabies virions were, however, unsuccessful. This suggests that a portion of the polypeptide moiety of the viral glycoprotein, located at one end of the molecule, may have been degraded to amino acids or peptides, which escaped detection.

Another type of heterogeneity in the molecular size of the viral glycoprotein, detected in almost all preparations of dissociated rabies virus, was manifested as an extensive width of the band corresponding to this component in SDS-polyacrylamide gel. Moreover, the carbohydrate content of the glycoprotein was found to be variable. It was observed in the VSV glycoprotein band, obtained after electrophoretic separation of dissociated structural proteins of the virus, that the peak of glucosamine label did not coincide with that of the amino acid label (2, 12). This finding was interpreted as suggesting the presence in the band of a larger polypeptide and a smaller glycoprotein (2). Another interpretation would be the variability of the proportions of protein and glycopeptide in individual glycoprotein molecules.

There is some evidence that the glycopeptide moiety of viral glycoproteins may be of host cell origin (2, 7, 15, 26). If this is so, then viral glycoproteins represent a complex antigen, composed of both host- and virus-specific components. Several rhabdoviruses can be propagated in BHK cells in amounts sufficient to attempt isolation and immunological comparison of their envelope glycoproteins. It would be of interest to find out whether they indeed contain a common host-specified antigen.

Infection by rabies virus does not suppress the synthesis of host-specified macromolecules in BHK cells (F. Sokol, *unpublished data*). It would

be, therefore, difficult to investigate the dynamics of synthesis of structural and functional viral proteins by electrophoretic fractionation of components of infected cells synthesized during the infectious cycle. Electron microscopic and immunological studies on rabies virus-infected cells indicate, however, that this experimental approach might still be feasible. Relatively soon after infection, large inclusion bodies, composed mainly of viral nucleocapsid particles, are formed in the cytoplasm of BHK cells (10). On the other hand, the plasma membrane of infected cells contains virus-specific antigens demonstrable by various immunological techniques (29). Thus an attempt to investigate the kinetics of the synthesis of virus-specific proteins by electrophoretic analysis of polypeptides, derived from the cytoplasm or the plasma membrane of infected cells and labeled with radioactive precursors during the infectious cycle, is indicated.

LITERATURE CITED

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