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Expression of the Major Viral Glycoprotein of Avian Tumor Virus in Cells of chf(+) Chicken Embryos

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The expression of gp85, the major viral glycoprotein of avian tumor virus, by certain chicken embryonic cells was studied by the use of sera directed to antigenic determinants of subgroup E viral gp85. As analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immune precipitates prepared with lysates of cells that had been labeled with [^{3}H]glucosamine, chf(+) chicken embryo cells synthesize molecules of gp85 which possess type and probably also group antigenic specificities. Under similar conditions of analysis, no gp85 or antigenically related components could be detected in lysates of chf(−) cells.

Uninfected chicken embryo cells possess a genetically integrated leukosis provirus (12, 20, 23). As a result of the partial expression of the provirus, group-specific (gs) antigenic determinants common to structural proteins of all avian tumor viruses are present in the cells from many embryos in the absence of virus production (3, 4). With certain of these embryos, the antigenic determinants characteristic of the viral envelope proteins of subgroup E virus (subgroup E type-specific determinants) are also expressed (9).

The existence of intracellular type-specific determinants was orginally inferred from the fact that certain chicken embryo cells were able to complement the defective Bryan high-titer strain of Rous sarcoma virus (BH-RSV) so that virions propagated in such cells become infectious for other chicken embryo cells (11, 22). The host range, interference, and neutralization properties of this infectious virus served to define a new virus subgroup, which was designated subgroup E (12). The complementation of BH-RSV was presumed to be a consequence of the acquisition by progeny virus of the envelope antigen that determines subgroup E type specificity. Cells that are able to complement BH-RSV are designated positive for chick helper factor [chf(+)] in contrast to chf(−) cells, which are unable to complement BH-RSV (11).

Direct evidence for the presence of subgroup E viral envelope antigen in uninfected chf(+) chicken embryo cells was obtained by Hanafusa et al. (9). They noted that the neutralizing activity of type-specific antisera prepared in chickens against BH-RSV(f), the infectious form of BH-RSV obtained after propagation of the defective virus on chf(+) cells, could be eliminated by absorption with extracts of uninfected chf(+) cells. Since the viral type-specific determinants are present on a subviral complex of gp85 and gp37, the two glycoproteins of avian tumor viruses (1, 5), the results of Hanafusa et al. (9) are consistent with the possibility that the glycoproteins themselves are synthesized by chf(+) cells. The observation of Scheele and Hanafusa (15) that after propagation of BH-RSV on chf(+) cells the progeny viruses acquire infectivity concomitantly with their acquistion of gp85 and gp37 is also consistent with this possibility. Alternatively, since uninfected chf(+) cells do not normally produce virus, it is possible that the type-specific determinants are present on some precursor form of gp85 and gp37 that is not processed into a structural and functional equivalent to the viral glycoproteins until virus infection occurs.

A method based on immune precipitation has recently been described for the isolation from lysates of radioactively labeled infected cells of gp85, the major viral glycoprotein (7). We report here the use of this method to isolate from uninfected chf(+) cells those molecules possessing the antigenic determinants that are present on viral gp85. Since these isolated molecules could be analyzed by gel electrophoresis, one could determine whether they have the same mobility on sodium dodecyl sulfate-polyacrylamide gel as viral gp85.

MATERIALS AND METHODS

Cells and viruses. Primary cultures were prepared from 10- to 12-day-old embryos of White Leghorn chickens or Japanese quails, as described by Vogt (18). Cells were grown in Ham F10 medium supplemented

with 10% tryptose phosphate, 10% calf serum, and 2% chicken serum (heat inactivated). Chicken embryo cells of types C/O, C/E, C/BE, and C/A were used in this study (18, 19). C/O cells are susceptible to avian sarcoma viruses of subgroups A, B, C, D, and E, whereas C/E, C/BE, and C/A cells are selectively resistant to viruses of subgroups E, B plus E, and A, respectively. Cell cultures were maintained at 35 C. Incubation was carried out in polyethylene bags as reported by Vogt and Harris (21).

Viruses used for infection of cells included a high-titer clone (no. 9) of avian sarcoma virus B_{77} and a Rous-associated virus type 7 pseudotype of BH-RSV [BH-RSV(RAV-7)], both of viral subgroup C, as well as a Rous-associated virus type 0 pseudotype of BH-RSV [BH-RSV(RAV-0)] of viral subgroup E.

Virus assays. Focus assays for avian sarcoma viruses were performed on secondary cultures (18). Cells were seeded at ca. 4×10^5 to 6×10^5 cells per 35-mm petri dish in Ham F10 media supplemented with 10% tryptose phosphate, 5% calf serum, and 2 μg of polybrene per ml, and infected the same day. The polybrene was added to enhance virus adsorption (17). Agar overlay medium (18) was added between 16 and 24 h after infection. The overlay medium for quail cells was supplemented with 1% dimethyl sulfoxide and 1% chicken serum (heat inactivated).

chf assay. The chf assay was performed on secondary cultures as described by Weiss et al. (24). The chicken embryo cells to be tested were seeded at 10^6 cells per 60-mm petri dish in primary growth medium supplemented with 2 μg of polybrene per ml and infected the same day with 5×10^4 focus-forming units of BH-RSV(RAV-7). The media of the infected cells was changed daily with Ham F10 medium supplemented with 10% tryptose phosphate, 5% calf serum, and 1% dimethyl sulfoxide. The culture fluids, harvested on day 5 postinfection, were frozen and thawed, sonically treated, and assayed for focus-forming units on C/E or C/BE chicken embryo cells as well as on quail cells. Quail cells are susceptible to viruses of subgroup E, but resistant to BH-RSV(RAV-7).

The complement fixation assays for avian leukosis (16), which detects the gs antigens of avian tumor virus, were performed on samples of sonically treated viscera prepared from the embryos employed in this study. An anti-gs serum was obtained from pigeons with regressing tumors (14, 16). The wing web tumors had been induced by 10^6 focus-forming units of the Schmidt-Ruppin strain of RSV of subgroup D.

Radioactive labeling. Labeling of cells with ^{3}H- and ^{14}C-labeled amino acids or [^{3}H]glucosamine (7.3 Ci/mmol) (all from New England Nuclear, Boston, Mass.) was as described previously (7). Secondary or tertiary cultures were seeded at 4×10^6 cells per 100-mm petri dish in primary growth media. Label was added 24 h after seeding of the cells. Fourteen to eighteen h after the addition of label, the culture fluid was removed and the cells were harvested. The purification of ^{14}C-labeled amino acid-labeled virus was carried out as described previously (8).

Immune precipitation. Two antisera were used for the isolation of gp85 from lysates of cells labeled with [^{3}H]glucosamine: (i) anti-gp85 serum, a rabbit antiserum prepared against the gp85 purified from the subgroup C strain of Prague RSV by gel filtration on Sephadex G-200 equilibrated with 6 M guanidine-hydrochloride by ion exchange chromatography on DEAE-cellulose (Rohrschneider et al., in press); and (ii) anti-RAV-0 serum, a rabbit antiserum prepared against gradient-purified BH-RSV (RAV-0) (6). Prior to its use in immune precipitation, 0.2 ml of the anti-RAV-0 serum was diluted to 2.0 ml with phosphate-buffered saline and then absorbed with the gp85 of subgroup B virus by passage over an immunoadsorbent column. The latter was prepared by coupling 2 mg of gp85 purified from subgroup B avian myeloblastosis virus to cyanogen bromide-activated sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) using the procedure for the linkage of protein described by Cuatrecasas (2) and Bolognesi et al. (in press). This serum was then absorbed with antigens of chf(−) cells by consecutive 4-h incubation periods at 37 C on two separate monolayers of primary cultures of chf(−) cells (10^7 cells on 100-mm petri dishes) that had been washed three times with phosphate-buffered saline. After removal of the serum from the second tissue culture plate, a sonic extract of 10^7 chf(−) chicken embryo cells was added to the serum, which was further incubated for 1 h at 37 C and overnight at 4 C. Finally, the serum was clarified by centrifugation with a Beckman 40 rotor at 30,000 rpm for 1 h.

The use of the nonionic detergent Nonidet P-40 for the preparation of cellular lysates and the method of indirect immune precipitation have been described (7). For the latter, 0.02 ml of the undiluted anti-gp85 serum, 0.02 ml of normal rabbit sera, or 0.2 ml of the anti-RAV-0 serum, which had been diluted 10-fold, were used. Precipitation of the gamma globulin fraction of the rabbit sera was effected with 0.2 ml of goat anti-rabbit gamma globulin sera (Antibodies Incorporated Davis, Calif.). The method of electrophoresis of the solublized immune precipitate and marker viral protein was as described previously (7).

In certain experiments, solid-phase immunoadsorbents prepared by coupling 1.0 ml of the absorbed anti-RAV-0 serum or 0.1 ml of normal rabbit sera to 8 ml of cyanogen bromide-activated sepharose (2; Bolognesi et al., in press) were employed. Volumes of sepharose to which 0.02 ml of undiluted sera had been linked were added to lysates of cells and incubated for 1 h at 37 C and overnight in the cold. The sepharose beads were then removed by low-speed centrifugation and washed once with 1 ml of phosphate-buffered saline. After removal of the beads from the buffer, the wash buffer was added to the original volume of lysate which was saved for further analysis.

Materials. C/E gs(−) and gs(+) embryos as well as C/O and C/BE gs(−) embryos were obtained from Heisdorf and Nelson Laboratories, Redmond, Wash.; Japanese quail embryos were obtained from Truslow Farms, Inc., Chestertown, Md. The viruses were the gift of Peter K. Vogt.

RESULTS

Recognition by the anti-gp85 serum of the gp85 of subgroup E virus. The subgroup E type-specific antigenic determinants in chf(+) cells serve to block the neutralizing capacity of antisera to subgroup E virus (9). Since the anti-gp85 serum used in this study neutralized subgroup C virus but not subgroup E virus (Rohrschneider et al., in press), preliminary experiments were undertaken to determine whether a serum, prepared against the purified gp85 of a subgroup C virus, would recognize determinants on the gp85 of a subgroup E virus. If the anti-gp85 serum did react with subgroup E gp85, a compelling reason for the use of this serum would derive from the fact the chf(−) cells do not express determinants that block the neutralizing capacity of type E-specific antisera (9). The anti-gp85 serum would make it possible, therefore, to assess whether chf(−) cells synthesize a form of gp85 that lacks such determinants.

To test for activity against subgroup E gp85, the anti-gp85 serum was reacted with a cellular lysate prepared from glucosamine-labeled quail embryo cells infected with BH-RSV(RAV-0), a subgroup E pseudotype of BH-RSV. A peak comigrating with viral gp85 was readily scored in the electropherogram of the resultant immune precipitate (Fig. 1A). When, however, normal rabbit sera were substituted for the immune serum or precipitation with the immune serum was effected with lysates of noninfected quail cells, no such peak was detected (Fig. 1B and C). These latter results serve to identify the peak in Fig. 1A as gp85, and we therefore conclude that the anti-gp85 serum employed here does recognize the gp85 of subgroup E virus. This was confirmed further by an experiment in which the antiserum was shown to specifically precipitate the gp85 of purified amino acid-labeled BH-RSV(RAV-0) that had been first disrupted with Nonidet P-40 to solubilize the viral envelope glycoprotein (data not shown).

A second distinct peak of label (e.g., fraction 53 in the electropherogram of Fig. 1A) was detected in the immune precipitate of the lysates prepared from uninfected and infected cells labeled with radioactive carbohydrate. This peak was also detected (though in much reduced amounts) in the precipitate made with normal rabbit sera (Fig. 1B). This latter result would suggest that at least some of the label detected in the peak is due to the presence of a cellular glycoprotein that either aggregates out of solution or has an affinity for an antigen-antibody lattice. Some of the radioactive counts per minute in the peak from infected cells may also represent gp37, the minor glycoprotein of avian tumor viruses, which has a similar mobility on sodium dodecyl sulfate-gel to the component detected here, and, in addition, is known to complex with gp85 even in the presence of nonionic detergent (1, 5). The heterogeneous distribution of radioactivity observed in the leading edge of the gp85 peak (e.g., peak fraction 34 of the electropherogram in Fig. 1A) is also invariably detected with anti-gp85 immune precipitates of infected avian cells labeled under the conditions employed here. This region of the gel corresponds to the position of p70 which, on the basis of pulse labeling experiments, has been tentatively identified as incompletely glycosylated precursor to gp85 (7). It is possible that the high level of [^{3}H]glucosamine used to label these cells would also allow the detection of the p70 fraction.

Expression of gp85 as determined by immune precipitation. Having determined that the anti-gp85 serum would recognize the gp85 of subgroup E virus, we then carried out similar experiments with cells from both gs(−) and gs(+) chicken embryos. The results of the chf tests used to classify these embryos are shown in Table 1. As is usually, but not invariably, observed (10), the gs(−) embryos tested as chf(−), and the gs(+) embryos tested as chf(+). Analysis by equilibrium sedimentation on sucrose gradients of the supernatant fluid obtained from uninfected [^{3}H]glucosamine-labeled secondary cultures prepared from chf(−) cells (Fig. 2A) and from chf(+) cells (Fig. 2B) indicated that no virus synthesis occurred under conditions where particle production was readily detected in B_{77}-transformed sister cultures.

The electropherograms obtained with the immune precipitates prepared from the Nonidet P-40 lysates of uninfected cultures are shown in Fig. 3. A small but significant peak of label that comigrated with the gp85 of subgroup E virus was detected in the lysate of the chf(+) culture when the anti-gp85 serum was used for immune precipitation (Fig. 3C) but not when normal rabbit sera were employed (Fig. 3D). On the basis of the coelectrophoresis and the specificity of the immune precipitation, we identify this peak as gp85. In contrast, immune precipitation with a lysate of chf(−) cells using anti-gp85 serum did not result in the appearance of any component not also detectable by using normal rabbit sera (Fig. 3A and B; the peak of label at frac-

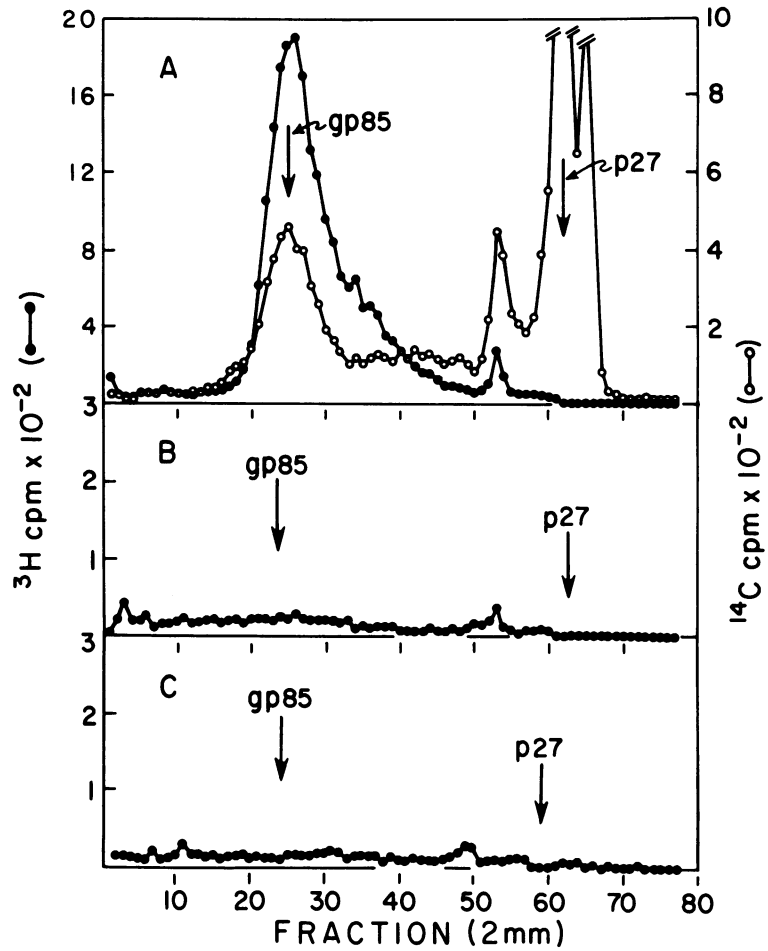

FIG. 1. *Electrophoreses on 5% sodium dodecyl sulfate-polyacrylamide gels of (A) an anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable counts/min of a Nonidet P-40 lysate of BH-RSV(RAV-0)-transformed quail embryo cells labeled with 60 μCi of [^{3}H]glucosamine per ml. (B) The same as (A), except that normal rabbit sera were substituted for the anti-gp85 serum. (C) An anti-gp85 immune precipitate prepared with 10^6 acid-precipitable counts/min of a Nonidet P-40 lysate of noninfected quail embryo cells. ^{14}C-labeled amino acid-labeled viral protein from BH-RSV(RAV-0) grown on quail embryo cells was co-electrophoresed on each gel (the pattern is shown in A), and the positions of viral gp85 and p27, the major gs protein, are indicated.*

tion 12 presumably represents a cellular protein that aggregates out of solution). The profile of the electropherogram of the anti-gp85 immune precipitate prepared from the lysate of the B_{77}-infected chf(−) cells (Fig. 4A) was indistinguishable from the one prepared with the lysate of B_{77}-infected chf(+) cells. The lysates from infected cells contained approximately 30 times as much gp85 as the lysate of the uninfected chf(+) cells.

The amounts of glucosamine-labeled gp85 detected with anti-gp85 serum in all the noninfected cultures examined are tabulated in Table 1. Results similar to those diagrammed in Fig. 3 were obtained with cultures from four other chf(+) embryos and seven other chf(−) embryos; in no case did a chf(+) culture lack detectable glucosamine-labeled gp85 or did a chf(−) culture possess it even when immune precipitates were made with lysates from chf(−) cultures that contained 10 times more trichloroacetic acid-precipitable radioactive counts per minute than the amount needed for clear resolution of gp85 with a lysate from a chf(+) culture. Also included in Table 1 are data obtained with line 7 C/A chicken embryo cells. These cells spontaneously release small amounts of RAV-0 (20) and, as determined in

TABLE 1. *Results of COFAL, chf, and gp85 assays*

Embryo[a] no.	COFAL[b]	chf[c]		gp85	
		FFU (quail)/FFU (chicken)	Classification	Trichloroacetic acid-precipitable counts/min subject to immune precipitation[d]	Levels of gp85 (%)[e]
1	+	5×10^{-3}	+	1×10^{6}	0.10
2	−	$<4 \times 10^{-6}$	−	1×10^{6}	
3	−	ca. 1×10^{-6}	−	4×10^{5}	
4	−	$<3 \times 10^{-6}$	−	4×10^{5}	
5	+	1.7×10^{-2}	+	2×10^{5}	0.11
6	+	1.6×10^{-2}	+	4×10^{5}	0.05
7	−	ca. 2×10^{-6}	−	2×10^{6}	
8	−	ca. 4×10^{-7}	−	3×10^{6}	
9	−	$<3 \times 10^{-6}$	−	2×10^{5}	
10	+	7×10^{-2}	+	1×10^{6}	0.06
11	+	7×10^{-3}	+	1×10^{6}	0.08
12	−	ca. 8×10^{-7}	−	2×10^{6}	
13	−	$<1 \times 10^{-6}$	−	1×10^{6}	
14	NT[f]	NT[f]		3×10^{5}	0.31

[a] Embryos 1 through 13 were type C/E; embryo 14 was a C/A embryo (line 7) spontaneously producing the subgroup E avian leukosis virus, RAV-O (20). Embryos 9 through 13 were derived from different mating pairs. The data on the mating pairs giving rise to embryos 1 to 8 were unavailable.

[b] Complement fixation assays for avian leukosis (COFAL) were performed as described by Sarma et al. (14).

[c] Results are expressed as the ratio of focus-forming units (FFU) detected on quail cells to FFU detected on C/E or C/BE chicken embryo cells. In no instance were more than 10 FFU/ml detectable on quail embryo cells in BH-RSV(RAV-7) harvests from C/E gs(−) chicken embryo cells. As defined by Weiss et al. (24), these cells were scored as chf(−). We did not determine whether this low level of focus formation detected on quail embryo cells was due to low levels of chf expression, to a breakdown of resistance of the quail embryo cells to BH-RSV(RAV-7), or to the presence of transformed chicken embryo cells that had resisted freezing-thawing and sonic treatment. Cells classified as chf(+) yielded harvests upon infection with BH-RSV(RAV-7) which contained more than 1,000 FFU/ml as assayed on quail embryo cells (24).

[d] Each sample represents a lysate of chicken embryo cells that had been labeled with [^{3}H]glucosamine.

[e] The percentages of labeled gp85 were calculated from the electropherograms of the anti-gp85 immune precipitates. For the chf(+) cultures, the sum of the counts per minute in the fractions of the gel corresponding to gp85 (defined by the ^{14}C-labeled amino acid-labeled viral gp85 marker, e.g., fractions 20 throuth 35 in Fig. 3C) was computed after the background counts per minute had been subtracted from the counts per minute at each fraction (background counts per minute were defined as the average of the counts per minute at fractions 15 and 40). The actual percentage was obtained by dividing this sum by the total trichloroacetic acid-precipitable counts per minute which had been subject to immune precipitation. There was no discernible peak of label in the same region of the electropherogram with anti-gp85 immune precipitates prepared from any of the chf(−) cultures.

[f] Not tested.

this study, possess an intracellular gp85 that migrates with the same mobility as the gp85 isolated from chf(+) cells.

When immune precipitates, formed with lysates of chf(+) and chf(−) cells, were analyzed by sodium dodecyl sulfate-gel electrophoresis, a background of radioactive counts per minute was invariably present in the gel (e.g., fractions 20 through 50, Fig. 3B). This background may represent cellular glycoprotein nonspecifically bound to the antigen-antibody lattice. In general, the peak fraction on the gel of the labeled gp85 in chf(+) cells was of the order of fivefold above background. Therefore, if, relative to chf(+) cells, the gp85 in chf(−) cells is reduced by more than five- to ten-fold, this gp85 would have been obscured by the background radioactivity in the gel.

Presence of subgroup E type-specific determinants on the gp85 in chf(+) cells. Since the anti-gp85 serum was prepared against a gp85 of a subgroup C virus, the use of this serum could not establish that the gp85 detected in chf(+) cells actually possessed type E specificity. To isolate the molecules possessing subgroup E type-specific determinants, immune precipitation was effected by using a small volume of a rabbit antiserum that had been prepared against purified RAV-0 virus and initially characterized by Friis (6).

Although the anti-RAV-0 serum specifically neutralized subgroup E virus, immune precipi-

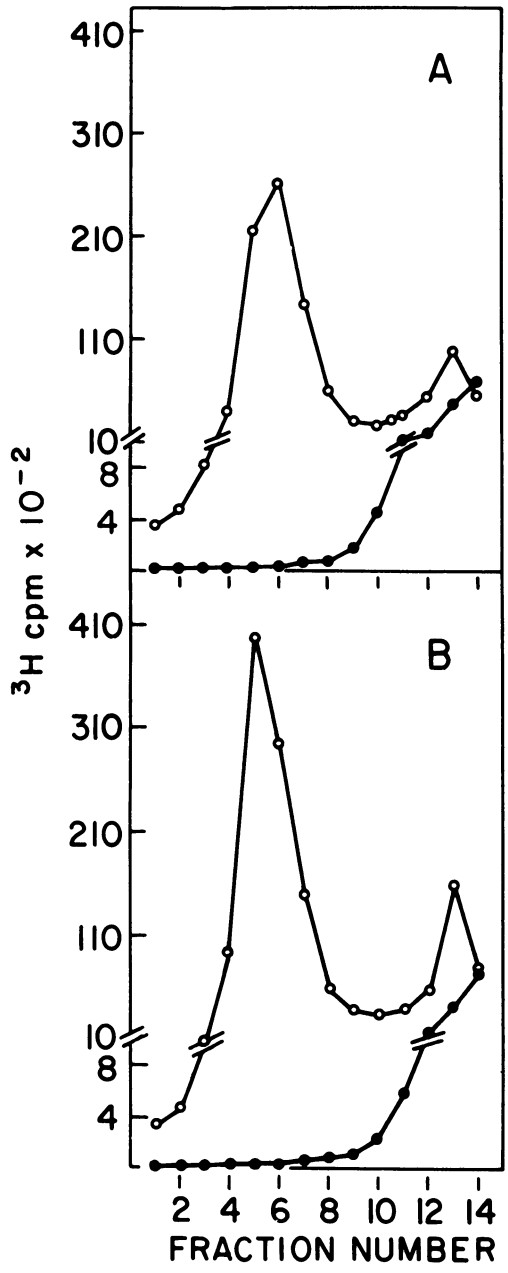

FIG. 2. *Equilibrium sedimentation on 24 to 48% sucrose gradients of the supernatant fluids of chicken embryo cells labeled with 60 μCi of [^{3}H]glucosamine per ml. (A) chf(−) chicken embryo cells (embryo no. 2, Table 1), (B) chf(+) chicken embryo cells (embryo no. 1, Table 1). Symbols: ○, B_{77}-infected cells; ●, uninfected cells. The peak fraction in each gradient corresponded to a density of 1.16 g/cm^3.*

tation with lysates of glucosamine-labeled uninfected and B_{77}-infected chicken embryo cells indicated that the serum reacted with chick cell components unrelated to viral structural protein as well as with the gp85 of the subgroup C virus. The former reactivity must reflect the fact that the purification procedure for the virus used for immunization did not eliminate all contaminating host cell protein. The latter reactivity is presumed to be due to the presence of antibodies to group-specific determinants that are shared by the gp85s of viruses from all subgroups (see Discussion). To remove these reactivities, the serum was absorbed both with the gp85 of subgroup B avian myeloblastosis virus and with intact chf(−) cells and cell extracts. After absorption, the serum recognized less than 1% of the amount of labeled gp85 in a lysate of B_{77}-infected chf(−) cells that was recognized by the anti-gp85 serum (Fig. 4B, comparison with Fig. 4A). Since, however, the serum still neutralized subgroup E virus and was able to recognize the gp85 in the lysate of the BH-RSV(RAV-0)-infected quail culture (Fig. 4C), we concluded that the absorbed serum was reactive essentially only to subgroup E type-specific determinants.

As would be expected from the regimen of absorption, use of the serum in immune precipitation with a lysate of glucosamine-labeled chf(−) cells did not result in the specific precipitation of any component (Fig. 5A). Use of the serum in immune precipitation with lysates of glucosamine-labeled chf(+) cells resulted in the precipitation of gp85 (Fig. 5B), thereby establishing that the gp85 of these cells possesses subgroup E type-specific determinants. The recovery of labeled gp85 with the anti-RAV-0 serum was about two- to threefold greater than with the anti-gp85 serum. Since the latter was used under conditions of antibody excess (as verified by the fact that increasing amounts of serum did not result in the precipitation of more labeled gp85), this result suggests that the molecules of gp85 that possess the determinants recognized by the anti-gp85 serum represent only a subset of the total pool of intracellular gp85.

Experiments were then undertaken to determine whether the molecules of gp85 that possessed determinants reactive with the anti-gp85 serum also possessed the type E determinants. An immunoadsorbent was prepared by coupling the absorbed anti-RAV-0 serum to sepharose beads. To remove the gp85 that possessed type E determinants from a lysate of glucosamine-labeled chf(+) cells, a volume of the immunoadsorbent to which had been linked 0.02 ml of undiluted serum was added to the lysate. After incubation and subsequent removal of the sepharose beads, anti-gp85 serum was reacted

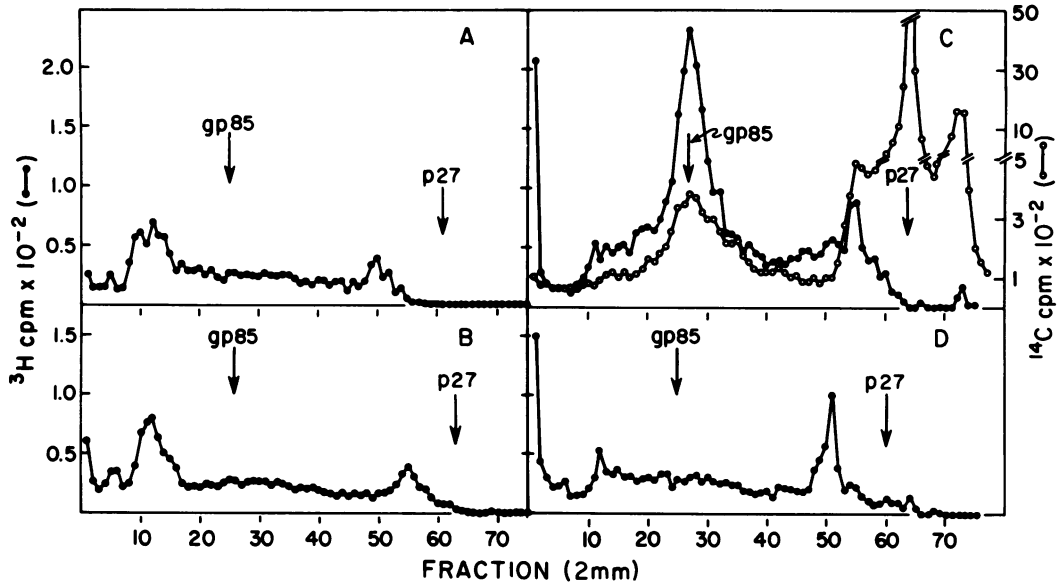

FIG. 3. *Electrophoreses on 5% sodium dodecyl sulfate-polyacrylamide gels of (A) an anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable counts/min of a Nonidet P-40 lysate of uninfected chf(−) chicken embryo cells (embryo no. 2, Table 1) labeled with 60 μCi of [^{3}H]glucosamine per ml. (B) The same as (A), except that normal rabbit sera were substituted for the anti-gp85 serum. (C) An anti-gp85 immune precipitate prepared with 10^6 acid-precipitable counts/min of a Nonidet P-40 lysate of chf(+) chicken embryo cells (embryo no. 1, Table 1). (D) The same as (C) except that normal rabbit sera were substituted for the anti-gp85 serum. ^{14}C-labeled amino acid-labeled viral protein from BH-RSV(RAV-0) grown on C/O chicken embryo cells was co-electrophoresed on each gel (the pattern is shown in C), and the positions of viral gp85 and p27 are indicated.*

with the lysate and immune precipitation was carried out in the standard manner.

When the resultant anti-gp85 immune precipitate was analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gel, the amount of labeled gp85 detected corresponded to less than 10% of the amount detected when an anti-gp85 immune precipitate was prepared with an equal volume of lysate that had not been reacted with the sepharose-anti-RAV(0) (Fig. 6B, comparison with 6A). A control experiment in which an equal volume of lysate was reacted with an immunoadsorbent prepared with normal rabbit sera was also carried out. In this case, when the anti-gp85 immune precipitate was prepared after removal of the sepharose beads, the amount of labeled gp85 detected corresponded to more than 85% of the amount detected when an anti-gp85 immune precipitate was prepared with an equal volume of lysate that had not been reacted with the sepharose-normal rabbit sera (Fig. 6C, comparison with Fig. 6A). Therefore, since specific removal of the population of molecules of gp85 that contained type E determinants also served to remove most of the molecules that contained the determinants recognized by the anti-gp85 serum, we conclude that the latter set of determinants must be present on molecules that possessed the type-specific determinants. Similar results to these were obtained in analyses of two other lysates of chf(+) cells.

DISCUSSION

By the use of immune precipitation, we have found that gp85 is synthesized by uninfected chf(+) cells. In addition, we have shown that this gp85 possesses type E-specific determinants. These results suggest that the absence of virus particle production normally observed with uninfected chf(+) cells is not due to the inability of these cells to synthesize a functional gp85.

At present, we have no data bearing on the synthesis of gp37, the minor glycoprotein of avian tumor virus. By analogy with the results obtained here with gp85, however, it may well be that the subgroup E type-specific form of this molecule is also synthesized by chf(+) cells. If this is so, the helper function of chf(+) cells which allows the progeny of BH-RSV to become infectious can be most simply rationalized by postulating that the progeny viruses incorporate

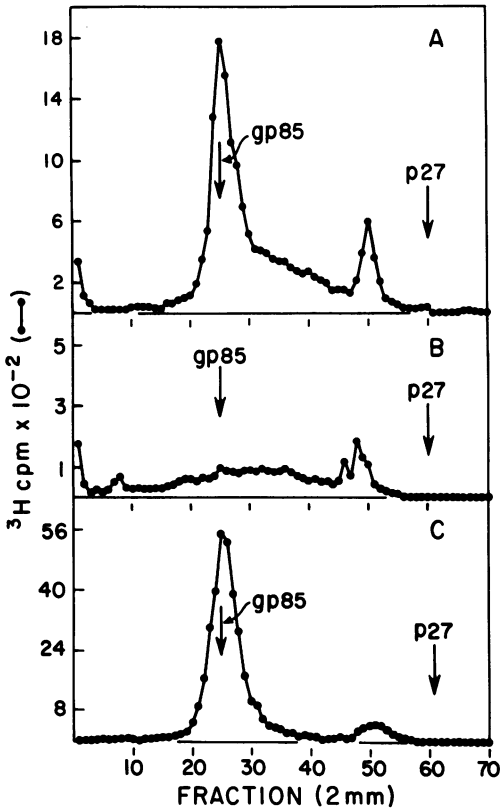

FIG. 4. *Electrophoreses on 5% sodium dodecyl sulfate-polyacrylamide gels of (A) an anti-gp85 immune precipitate prepared with 4 × 10^5 trichloroacetic acid-precipitable counts/min of a Nonidet P-40 lysate of B$_{77}$-infected chf(−) chicken embryo cells (embryo no. 2, Table 1) labeled with 60 μCi of [^{3}H]glucosamine per ml. (B) An immune precipitate prepared with the absorbed anti-RAV-0 serum and 10^6 acid-precipitable counts/min of the same lysate of B$_{77}$-infected chf(−) chicken embryo cells as used in (A). (C) An immune precipitate prepared with the absorbed anti-RAV-0 serum and 10^6 acid-precipitable counts/min of a Nonidet P-40 lysate of BH-RSV(RAV-0)-infected quail embryo cells. ^{14}C-labeled amino acid-labeled viral protein from B$_{77}$ grown on chf(−) chicken embryo cells was co-electrophoresed on the gels in (A) and (B), and the positions of viral gp85 and p27 are indicated. ^{14}C-labeled amino acid-labeled viral protein from BH-RSV(RAV-0) grown on quail embryo cells was used for the gel in (C).*

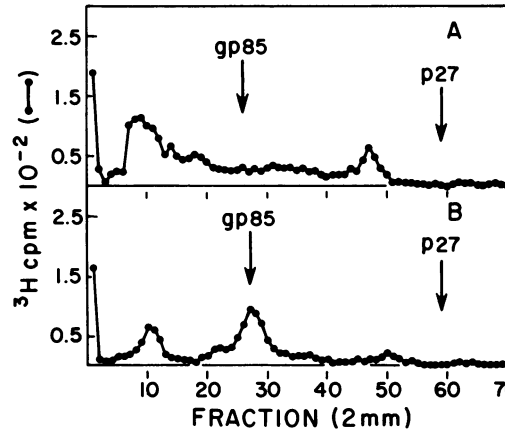

FIG. 5. *Electophoreses on 5% sodium dodecyl sulfate-polyacrylamide gels of (A) an immune precipitate prepared with the absorbed anti-RAV-0 serum and 10^6 trichloroacetic acid-precipitable counts/min of a Nonidet P-40 lysate of chf(−) chicken embryo cells (embryo no. 7, Table 1) labeled with 60 μCi of [^{3}H]glucosamine per ml. (B) An immune precipitate prepared with the absorbed anti-RAV-0 serum and 3 × 10^5 acid-precipitable counts/min of a Nonidet P-40 lysate of chf(+) chicken embryo cells (embryo no. 6, Table 1). ^{14}C-labeled amino acid-labeled viral protein from BH-RSV(RAV-0) grown on C/O chicken embryo cells was co-electrophoresed on each gel, and the positions of viral gp85 and p27 are indicated.*

the cellular gp85 and gp37 into their envelope at the time of particle maturation.

The nature of the determinants on subgroup E gp85 recognized by the anti-gp85 serum is not presently known. The two alternative possibilities are that either these determinants are group specific and therefore present on the gp85s of viruses from subgroups C and E or else they are type specific and present on gp85 of only subgroup E virus. Reactivity of the anti-gp85 serum to type E-specific determinants could have arisen if the preparation of PR-C virus used for purification of the gp85 immunogen had been contaminated with subgroup E endogenous virus that had been rescued by the infection with PR-C (12). It should be emphasized, however, that if the anti-gp85 serum is reactive to type E-specific determinants, these determinants cannot be identical to those involved in neutralization of virus since, unlike the anti-RAV-0 serum employed here, the anti-gp85 serum does not neutralize subgroup E virus.

Although the possiblity cannot be ruled out that the anti-gp85 serum recognized subgroup E gp85 because of a type E-specific reactivity, it seems to us more probable that the recognition was based upon reactivity to group-specific determinants on gp85. Rohrschneider et al. (in press) have shown, by a sensitive radioimmunoassay utilizing the same anti-gp85 serum as used here for immune precipitation, that the gp85s of viruses from subgroups A, B, and C share common, presumably group-specific, determinants; it seems reasonable to suppose that the gp85 of virus from subgroup E would also possess such determinants. Furthermore, the subgroup C strain of Prague RSV used for preparation of the gp85 immunogen was propagated on chicken embryo cells prepared from

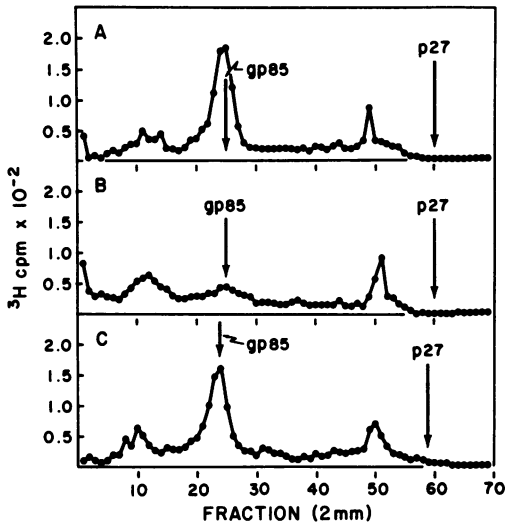

FIG. 6. *Electrophoreses on 5% sodium dodecyl sulfate-polyacrylamide gels of (A) an anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable counts/min of a Nonidet P-40 lysate of chf(+) chicken embryo cells (embryo no. 10, Table 1) labeled with 60 μCi of [^{3}H]glucosamine per ml. (B) The same as (A) except that an aliquot of lysate had been first absorbed with sepharose beads to which had been linked 0.02 ml of anti-RAV-0 serum. (C) The same as (A) except that an aliquot of lysate had been first absorbed with sepharose beads to which had been linked 0.02 ml of normal rabbit sera. ^{14}C-labeled amino acid-labeled viral protein from BH-RSV(RAV-0) grown on C/O chicken embryo cells was co-electrophoresed on each gel, and the positions of viral gp85 and p27 are indicated.*

gs(−) embryos, the great majority of which were also chf(−) (Rohrschneider et al., in press). The amount of subgroup E virus relative to subgroup C virus in the harvests of culture fluid from these cells should, therefore, have been extremely small. Finally, the anti-RAV-0 serum that had been elicited from a preparation of RAV-0 that could not have been contaminated with subgroup C virus (6) reacts with the gp85 of B_{77} (unpublished data). This latter result indicates that the gp85s of viruses of subgroups E and C do share common antigenic determinants.

In contrast with the results obtained with chf(+) cells, no labeled component antigenically related to gp85 could be specifically precipitated from lysates of chf(−) cells when immune precipitation was carried out with the anti-gp85 serum, nor did absorption with intact chf(−) cells and cell extracts prevent the anti-RAV-0 serum from reacting with type-specific determinants on the gp85 present in chf(+) cells. These observations, which suggest that gp85 is lacking in chf(−) cells, are consistent with others that have been made with chf(−) cells. Hanafusa et al. (9) had earlier noted that extracts of chf(−) cells could not absorb the neutralizing activity of a chicken antiserum to subgroup E virus. Hayward and Hanafusa (13) had also failed to detect significant quantities of cytoplasmic RNA in chf(−) cells that would anneal with a DNA transcript of viral RNA even though such RNA could be readily detected in chf(+) cells. These findings would suggest that the message for the synthesis of viral structural protein is absent from chf(−) cells.

Despite these considerations, we do not feel that the possibility that chf(−) cells express a component antigenically related to gp85 has been rigorously excluded. Immune precipitation, as employed here, would not have detected glucosamine-labeled gp85 were it present in chf(−) cells in amounts 5- to 10-fold lower than in chf(+) cells. In addition, chf(−) cells could conceivably contain a non- or incompletely glycosylated (i.e., non-glucosamine containing) form of gp85. Such a component could not have been detected in the experiments reported here which were carried out with metabolic labeling of cells with [^{3}H]glucosamine. The fact that absorption with extracts of chf(−) cells did not eliminate the reactivity of an anti-RAV-0 serum for the gp85 in chf(+) cells does not rule out the possibility of non-glycosylated precursor to gp85 in chf(−) cells. If the anti-RAV-0 serum was mainly elicited to determinants of gp85 dependent on carbohydrate for their antigenicity, then absorption of this serum with chf(−) cells might still leave its reactivity for the glycosylated form of gp85 intact.

An obvious test of the hypothesis that chf(−) cells possess non-glycosylated precursor to gp85 would be to use immune precipitation to isolate from lysates of amino acid-labeled chf(−) cells any component that would specifically react with the anti-gp85 serum. The structural relatedness to gp85 of the isolated component could then be analyzed. Such an experiment has been carried out in this study, but no peaks of label were detected in the electropherogram of the resultant immune precipitate in additon to those also obtained in control experiments with normal rabbit sera (unpublished data). Even with lysates of chf(+) cells, however, the level of amino acid-labeled gp85 is too low to permit its resolution from the background of labeled cellular protein detectable in the gel. The results with lysates of amino acid-labeled chf(−) cells do not, therefore, eliminate the possibility that chf(−) cells contain non-glycosylated precursor to gp85. Experiments in progress utilizing exogenous labeling of cellular proteins with radioac-

tive iodine may serve, however, to further define the state of gp85 expression in chf(−) cells.

At present, there is no compelling reason to suppose that chf(−) cells do possess gp85-related material. We have considered the possibility that chf(−) cells might contain non-glycosylated precursor to gp85 simply because the existence of such a moiety cannot be excluded by our data. It is clear, however, that a significant quantitative difference must exist between the levels of glycosylated gp85 present in chf(+) and chf(−) cells. It is likely that this difference reflects the levels of helper activity for the complementation of BH-RSV observed with these cells.

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