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Evidence That a Precursor Glycoprotein Is Cleaved to Yield the Major Glycoprotein of Avian Tumor Virus

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A glycoprotein designated pr90, which is recognized by anti-gp85 serum, is present in lysates of pulse-labeled transformed cells. Under chase conditions, a reduction in the level of labeled pr90 is observed concomitant with the appearance of labeled, cell-associated viral glycoprotein.

The major and minor glycoproteins of avian tumor virus, designated gp85 and gp37, respectively, are disulfide-linked subunits of a virion substructure that is designated VGP (4). The experiments described in this report were undertaken to analyze the pathway of VGP biosynthesis. For this purpose, immune precipitation with an antiserum raised to purified gp85 (1) was used as a probe to detect intermediates in the synthesis of viral glycoprotein.

Figure 1a shows a sodium dodecyl sulfate (SDS)-gel electropherogram of an anti-gp85 immune precipitate formed with a lysate of chicken embryo cells transformed with the B_{77} strain (subgroup C) of avian sarcoma virus. The cells had been pulse labeled for 10 min with [^{35}S]methionine and immediately lysed. Under reducing conditions, a major peak of ^{35}S label (designated pr90) was resolved that migrated with a lower electrophoretic mobility than the peak of [^{3}H]fucose-labeled viral gp85. pr90 was not detected, however, when normal rabbit sera were substituted for anti-gp85 serum (Fig. 1b) or when the immune serum was reacted with lysates of [^{35}S]methionine pulse-labeled noninfected cells (data not shown). As measured by coelectrophoresis, reduced and alkylated pr90 had a slightly greater electrophoretic mobility than the VGP of nonreduced [^{3}H]fucose-labeled B_{77} virus (Fig. 1c).

To ascertain whether pr90 is glycosylated, anti-gp85 immune precipitates, prepared with lysates of transformed cells that had been pulse labeled for 10 min with [^{3}H]fucose or [^{3}H]-glucosamine, were analyzed. Approximately three times as much [^{3}H]glucosamine label was detectable in the cell-associated pr90 as compared with the cell-associated gp85 (the position of gp85 was defined by the coelectrophoresis with [^{14}C]glucosamine-labeled B_{77} virus) (Fig. 2a). By contrast, the majority of the [^{3}H]fucose label was detectable in gp85 rather than in pr90 (Fig. 2b). Immune precipitates prepared with both lysates and normal rabbit sera yielded only background levels of radioactivity in the region of pr90 and gp85 (data not shown). These results, taken together, indicate that pr90 is a glycoprotein that is significantly less fucosylated than gp85. As detailed in Fig. 2, the pattern of carbohydrate labeling of pr90 is qualitatively similar to that of the 110,000-molecular-weight glycoprotein that represents a nonfucosylated precursor to the gp69/71 glycoprotein of murine leukemia virus (5).

The possibility that pr90 represents a precursor to one or both of the glycoproteins of B_{77} virus was then tested by analyzing anti-gp85 immune precipitates of pulse-chased B_{77}-transformed cells. These immune precipitates were prepared using volumes of lysates adjusted to contain equal amounts of trichloroacetic acid-precipitable radioactivity. When a 10-min pulse label with [^{35}S]methionine was followed by a 15-min chase with medium containing unlabeled methionine, pr90, but not gp85 or gp37, was detected (Fig. 3a). After a 1-h chase, a minor fraction of the specifically precipitable radioactive counts per minute was detectable in gp85 and gp37 (Fig. 3b), whereas after a 3-h chase, the majority of the specifically precipitable radioactivity (defined by the normal rabbit sera control shown in Fig. 3d) was detectable in gp85 and gp37 (Fig. 3c; the ratio of ^{35}S label in cell-associated gp85 and gp37 was similar to that observed for the gp85 and gp37 of the labeled virus present in the culture medium).

The observation that the electrophoretic mobility on SDS-gel of pr90 was lower than that of gp85 is consistent with the interpretation that gp85 is derived from pr90 by proteolytic cleavage. This interpretation implies that the polypeptide moiety of pr90 is larger than that of

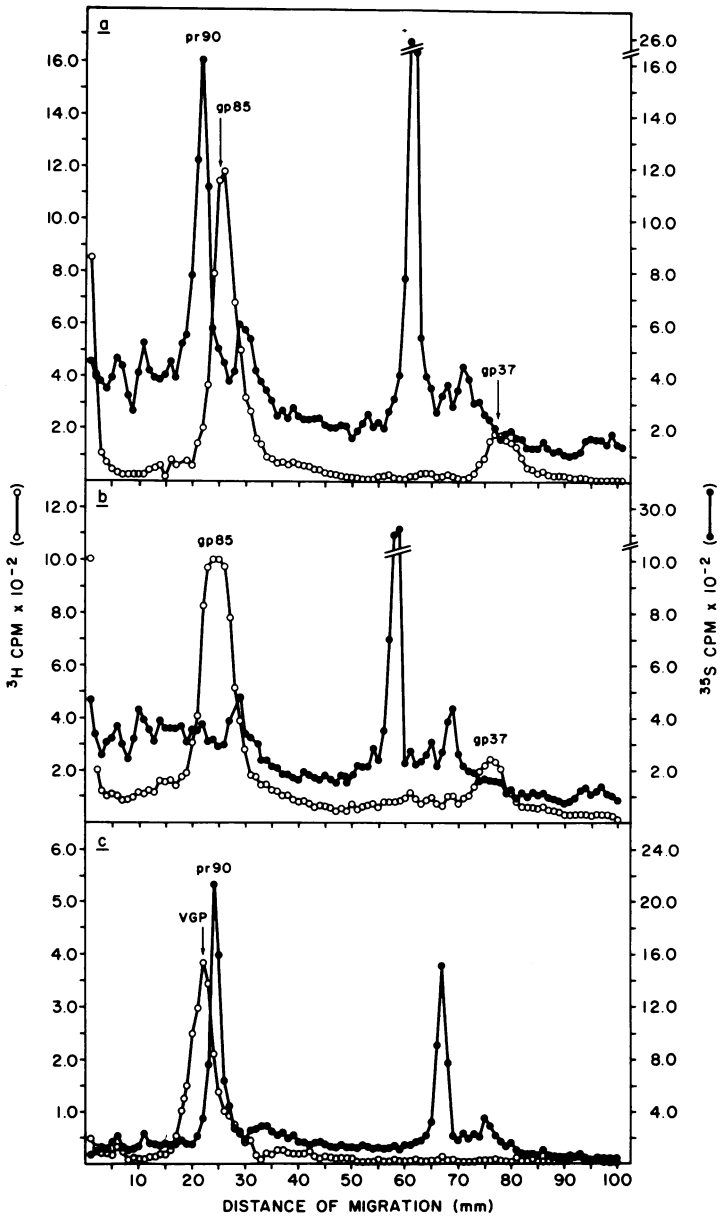

FIG. 1. *Electropherograms on 10% SDS-polyacrylamide gels (prepared according to the method of Laemmli [3]). (a) An anti-gp85 immune precipitate prepared with 10^6 trichloroacetic acid-precipitable cpm of a lysate of B_{77}-transformed chicken embryo cells. The infected cells were cultured on 100-mm tissue culture dishes. The methods of tissue culture were as described by Vogt (7). Primary cultures were prepared from 10-day-old embryos of the inbred SC line (Hyline Farms, Dallas Center, Iowa) which test as C/O, chf(−). After confluent transformation of secondary cultures, cells were pulse labeled for 10 min in 2 ml of Earle salt solution supplemented with 100 μCi of [^{35}S]methionine per ml (>100 Ci/mmol; purchased from New England Nuclear Corp., Boston, Mass.). Cytoplasmic lysates were prepared as described (4) except that 300 μg of phenylmethylsulfonyl fluoride per ml and 0.05 M iodoacetemide were added to the lysing buffer. Immune precipitation was effected by the indirect procedure (2). The immune precipitate was reduced with 0.1 M dithiothreitol before electrophoresis. (b) The same as (a) except that normal rabbit sera were substituted for the anti-gp85 serum. (c) The same as (a) except that the anti-gp85 immune precipitate was reduced and alkylated before electrophoresis. Reduced [^{3}H]fucose-labeled B_{77} virus was coelectrophoresed on the gels in (a) and (b), and nonreduced [^{3}H]fucose-labeled B_{77} virus was coelectrophoresed in (c). The positions of viral gp85, gp37, and VGP are indicated.*

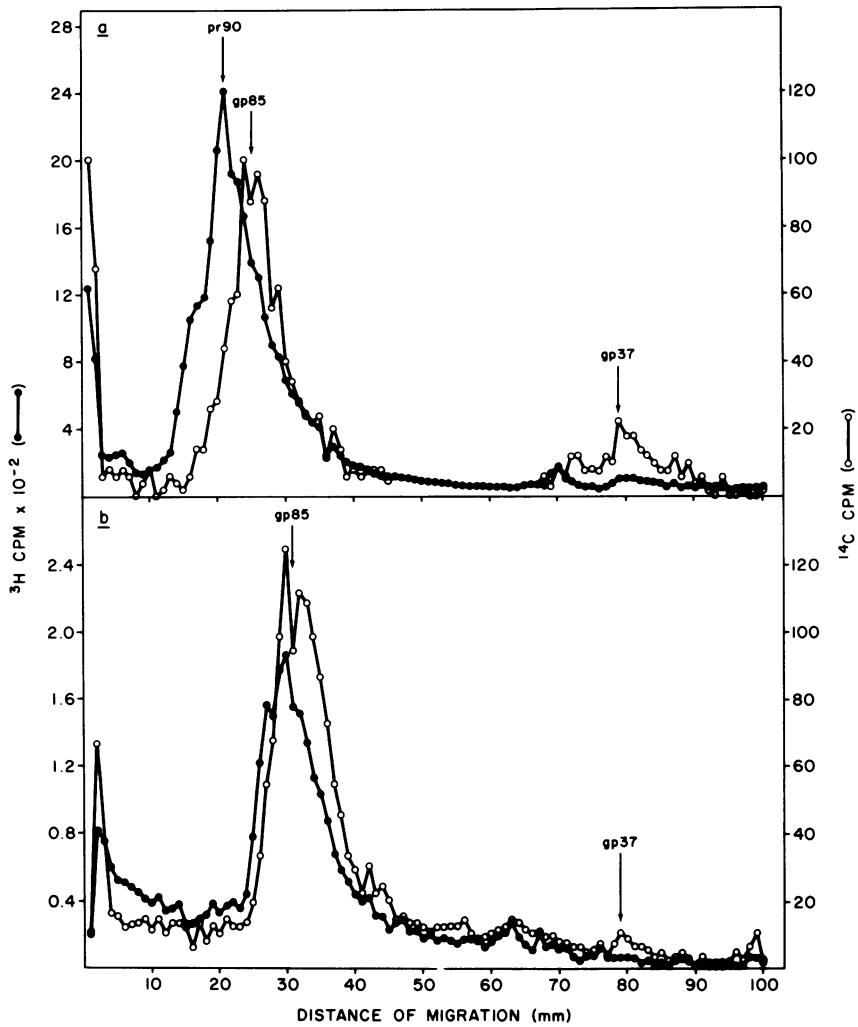

FIG. 2. *Electropherograms on 10% SDS-polyacrylamide gels. (a) An anti-gp85 immune precipitate prepared with 2.5 × 10^5 trichloroacetic acid-precipitable cpm of a lysate of B_{77}-transformed cells that had been pulse labeled for 10 min in 2 ml of culture medium supplemented with 500 μCi of* D-*[6-^{3}H]glucosamine-hydrochloride per ml (11 Ci/mmol; New England Nuclear Corp.). (b) An anti-gp85 immune precipitate prepared with 3 × 10^4 acid-precipitable cpm of a lysate of B_{77}-transformed cells that had been pulse labeled for 10 min in 2 ml of culture medium supplemented with 500 μCi of* D-*[6-^{3}H]fucose per ml (12 Ci/mmol; New England Nuclear Corp.). The immune precipitates were reduced before electrophoresis. Reduced [^{14}C]glucosamine-labeled B_{77} virus was coelectrophoresed on both gels, and the positions of viral gp85 and gp37 are indicated.*

gp85. The observed mobilities of pr90 and gp85 can also be rationalized, however, by assuming that the polypeptide of pr90 is either the same size as or smaller than that of gp85, but that the mass of the carbohydrate moiety of pr90 is greater. To distinguish between these possibilities, therefore, both pr90 and gp85 were exhaustively digested with Pronase, and the carbohydrate side chains of each glycoprotein were sized on a Bio-Gel P-6 column (6); these experiments indicated that the majority of the carbohydrate side chains of pr90 were significantly smaller than those of gp85, although a minor complement was apparently the same size as the carbohydrate side chains of gp85 (data not shown). These results cannot exclude the formal possibility that the lower mobility of pr90 relative to gp85 is due solely to a significantly greater number of carbohydrate side chains on pr90 that are smaller than or the same size as

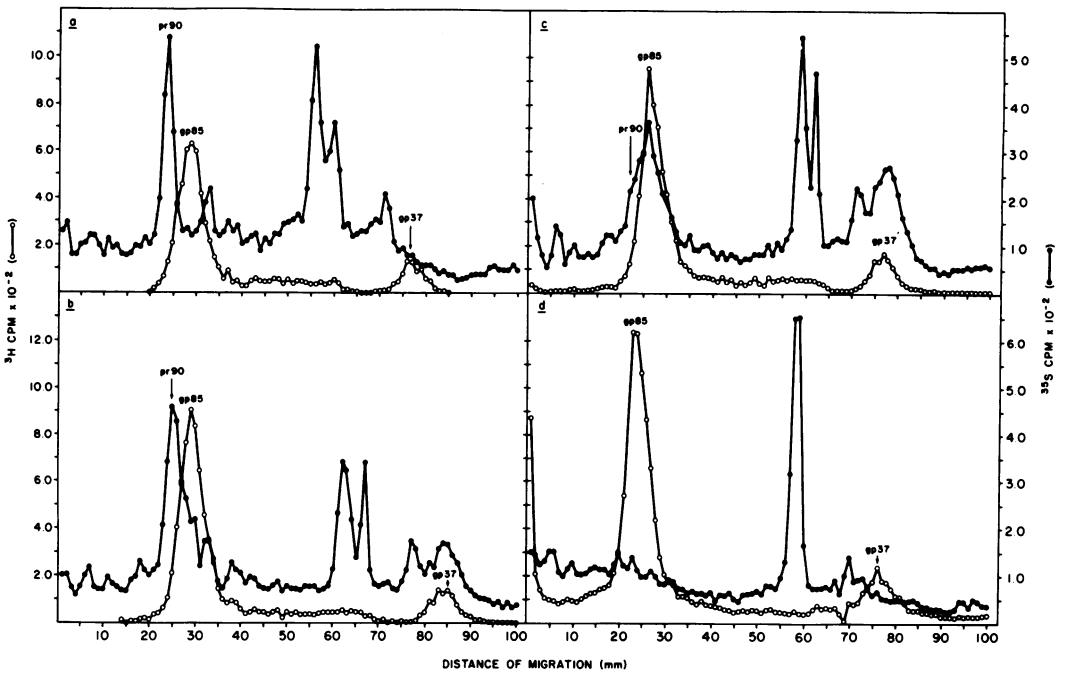

FIG. 3. *Electropherograms on 10% SDS-polyacrylamide gels. (a) An anti-gp85 immune precipitate prepared with 130 μl of a lysate of B_{77}-transformed cells, pulse labeled as in Fig. 1a, and chased for 15 min in complete medium 199 supplemented with 6.0 × 10^{-3} M unlabeled L-methionine. The immune precipitate was reduced before electrophoresis. (b) The same as (a) except that the chase was for 1 h, and 155 μl was subject to immune precipitation. (c) The same as (a) except that the chase was for 3 h, and 190 μl was subject to immune precipitation. (d) the same as (c) except that normal rabbit sera were substituted for the anti-gp85 serum. The total volume of each lysate was 4 ml, and the volumes of lysates utilized for immune precipitation all contained 3 × 10^5 acid-precipitable cpm. Reduced [^{3}H]fucose-labeled B_{77} virus was coelectrophoresed on all the gels, and the positions of viral gp85 and gp37 are indicated.*

those on gp85. Nevertheless, we feel that the data are more reasonably interpreted as indicating that pr90 has a larger polypeptide moiety than gp85. Accordingly, these data support the view that a proteolytic cleavage step is involved in the processing of pr90 to gp85.

It should be noted that no component corresponding to the previously described p70 moiety, which was specifically precipitable with anti-gp85 serum from lysates of ^{3}H-labeled amino acid or [^{3}H]glucosamine pulse-labeled transformed cells and migrated as a broad peak with a slightly greater electrophoretic mobility than viral gp85 (2), was detected in the present experiments wherein transformed cells were pulse labeled for 10 min with [^{35}S]methionine or [^{3}H]glucosamine (Fig. 1a and 2a). We have observed, however, that the p70 moiety, but not pr90, is specifically precipitable with anti-gp85 serum from lysates of transformed cells pulse labeled for 2 min with [^{3}H]mannose (data not shown). The sequential appearance of p70 and pr90 in anti-gp85 immune precipitates and the apparent heterogeneity of the p70 moiety, as judged from its diffuse pattern of migration on SDS-gels (2), suggest that the p70 moiety is incompletely processed pr90.

The finding that gp37 was detected concomitantly with gp85 on the chase represented in Fig. 3 is consistent with the possibility that pr90 is also a precursor to gp37. The polypeptide moiety of pr90 may, for example, correspond to the uncleaved form of the polypeptide of VGP with the increased mobility of pr90 relative to VGP (Fig. 1c) reflecting a lesser degree of glycosylation of the intracellular form of the glycoprotein. Alternatively, the detection of gp37 on the chase may simply reflect the fact that cell-associated gp85 and gp37 are linked by disulfide bonds (4). Tryptic mapping should serve, however, to clarify the relationship of pr90 to VGP and, in addition, p70 to pr90.

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