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Amphiphilic Properties of the Avian Myeloblastosis Virus Major Glycoprotein, gp 80

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The major glycoprotein (gp 80) from avian myeloblastosis virus (AMV) displays significant lipophilic properties, as shown by its strong interactions with acetylated uncharged decylamino agarose in hydrophobic chromatography. In effect, release from binding was achieved only by the added presence of a polarity reducing agent (ethylene glycol) and the strong anionic detergent sodium dodecyl sulfate. The hydrophobic behavior of the glycoprotein, coupled to the high content of hydrophilic carbohydrates, indicates its amphiphilic character. Confirmation of the amphiphilic nature of the AMV gp 80 was obtained by charge shift electrophoresis and crossed hydrophobic interaction immunoelectrophoresis. In both instances, the electrophoretic behavior of the glycoprotein was dependent on the presence of detergents. The AMV gp 80 displays the properties of integral membrane proteins.

Key words: amphiphilic, envelope, glycoprotein, membrane, virus

The envelope of avian oncornaviruses consists of a "unit" lipid membrane with knob-like projections on the surface [1]. The knobbed spikes are composed of two glycoproteins, a glycoprotein of apparent molecular weight 80,000 (gp 80), which forms the knob, and a glycoprotein of apparent molecular weight 35,000 (gp 37), which represents the spike portion [2]. Several studies have indicated that in the avian system the glycoprotein gp 80 has an essential role in the host range, virus infectivity, and several other properties (for review, see Bauer [3]).

The viral glycoprotein gp 37 shows self-aggregative properties even in denaturating agents such as 6 M guanidine hydrochloride and 8 M urea [4,5]. This property suggests an extremely hydrophobic nature for this glycoprotein. Based on this property and electron microscopy studies [2], it has been proposed that gp 37 represents the surface projection spike which is inserted into the lipid bilayer [6]. The nature of gp 80 is still unclear. It has been shown to be bound to gp 37 through disulfide bonds [5] and to constitute the knob-like portion of the surface projection [2]. Its high carbohydrate content, about 40% by weight [7,8], may be responsible for the water solubility of this glycoprotein.

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In general, glycoproteins associated with cell membranes or viruses show an amphiphatic molecular topography similar to that proposed for human erythrocyte glycophorin [9,10]. In this model the protein contains hydrophobic regions rich in amino acids with lipophilic side chains. These regions are removed from areas of more hydrophilic character which are usually glycosylated (for review, see Hughes [11]).

Several envelope viral glycoproteins [12,14] have been shown to possess such an amphiphatic structure. So far, all the envelope RNA viruses studied have surface glycoproteins that can be removed selectively from the virus by proteolytic enzymes [13]. These studies indicate that large regions of the glycoproteins are readily accessible to the aqueous environment, ie, that they are hydrophilic in character, as expected for an amphiphilic structure.

Recently we have reported that addition of purified avian myeloblastosis virus (AMV) gp 80 to chick embryo fibroblasts resulted in a stimulation of the hexose uptake and glycolysis [15]. The molecular mechanisms of the effects are still unknown. However, one of the possible mechanisms is that upon insertion in the cell membrane, an amphiphatic glycoprotein interacts specifically with some receptors and/or transport system, resulting in a stimulation of the metabolic functions mentioned above.

Here I present evidence that the AMV gp 80 shows an amphiphilic structure, with the capapcity for interaction with hydrophobic regions in the cellular membranes. These interactions could be involved in specific receptor binding, such as those present on the surface of target cells.

MATERIALS AND METHODS

Virus

Avian myeloblastosis virus (AMV) obtained from the plasma of leukemic chicks was prepared and concentrated according to published methods [16], and further purified with a discontinuous sucrose gradient centrifugation. The band containing the virus was collected and stored at −70°C until use.

Isolation of gp 80

The envelope glycoprotein of AMV was isolated by disruption of the virus with lithium diiodosalicylate and 2-mercaptoethanol, followed by partition with 25% phenol. The glycoprotein, gp 80, and viral RNA remained in the aqueous phase. After dialysis against buffer and addition of Triton X-100, gp 80 was separated by ion-exchange chromatography on DEAE-agarose in the presence of NaCl gradient with detergent [8,17]. The viral glycoprotein was found to be homogeneous by SDS-PAGE and by phenol-urea-acetic PAGE [18].

SDS–Polyacrylamide Gel Electrophoresis (PAGE)

Protein or peptide samples were analyzed by SDS-PAGE using cylindrical or slab gels according to the method of Laemmli [19]. Gels were stained for carbohydrate using the PAS reaction [20] and scanned or photographed. The gels were then stained for all proteins in 25% isopropanol–10% acetic acid–0.12% Coomassie blue and destained following the original procedure [20].

Hydrophobic Chromatography

Decyl amino agarose was acetylated following the procedure of Wilchek and Miron [21]. The acetylated alkyl amino agarose is devoid of charge, eliminating ionic interactions between the polypeptides and the absorbent matrix. The decyl amino agarose was resuspended in 0.05 M sodium phosphate pH 6.8 and poured into glass columns to yield a bed volume of 2 ml. Chromatography on decyl agarose was performed at room temperature using a flow rate of 10 ml/hr. The effluent was continuously monitored at 250 and 280 nm with an LKB Uvicord III unit, and 1 ml fractions were collected. Protein concentrations in each fraction were determined by the method of Lowry [22] using bovine serum albumin as a standard.

Virus, 2.0–2.3 mg protein, was resuspended in 0.6 ml of lysis buffer (0.4 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mg/ml dithiothreitol (DTT) and 25 mM Tris-HCl pH 8.0) and disrupted by sonicating the suspension three times for 20 sec every 7 min. The mixture was centrifuged at 10,000g for 15 min at 20°C. The clear supernatant was diluted 10 times with 0.05 M sodium phosphate buffer pH 6.8 and applied to the column. The column was washed with 0.05 M sodium phosphate buffer followed by stepwise elution with 0.05 M phosphate-50% v/v ethylene glycol and 0.05 M phosphate-50% v/v ethylene glycol-1% SDS. All the buffers contained 0.1 mg/ml DTT. Fractions from each peak were pooled, dialyzed against 0.05% SDS, and lyophilized. Purified AMV gp 80, 1 mg, was applied in 2 ml of 0.05 M sodium phosphate pH 6.8 (ionic strength, (μ = 0.30) and the column washed with the same buffer. Sequentially the column was eluted stepwise with 0.1 M sodium phosphate buffer (μ = 0.60), 0.05 M phosphate–20% ethylene glycol, 0.05 M phosphate–50% ethylene glycol, and 0.05 M phosphate–50% ethylene glycol–1% SDS. All the buffers contained 0.1 mg/ml DTT. Fractions from each peak were pooled, dialyzed against 0.05% SDS and lyophilized.

Charge Shift Electrophoresis

The agarose electrophoresis experiments were performed at 25°C, as described by Helenius and Simons [23], using a water-cooled horizontal gel system (LKB Multiphor). Samples containing 20–30 μg of viral glycoprotein were applied and electrophoresed following the original procedure. After electrophoresis gels were immediately dried under warm air and stained for protein (20–30 min in 0.1% Coomassie blue, 0.1% cupric acetate, 45% methanol, and 9% acetic acid; rinsed for 20 min in 7.5% acetic acid, 5% methanol).

Crossed Hydrophobic Interaction Immunoelectrophoresis

The electrophoresis experiments were performed at 15°C following the procedures described by Bjerrum [24]. The first dimension was performed in a 1.5-mm thick gel containing 1% (w/v) agarose (BRL low electroendosmosis $-M_r = <0.10$) and 40% (v/v) amphiphilic gel matrix, octyl agarose. Electrophoresis was performed at 10 V/cm for 1 hr. Second dimension electrophoresis was performed at 2 V/cm for 18 hr. The 1-mm thick gels contained 1% agarose standard low $-M_r = 0.13$, and 5.4 μl/cm^2 rabbit antiserum against the purified AMV gp 80. The antiserum-containing gels were prepared with and without 1% non-ionic detergent Triton X-100 and run under parallel conditions. Subsequently the gels were washed twice for 20 min each in phosphate buffered saline, once for 15 min in water, and dried under warm air.

After drying the gels were stained for 15 min in 0.5% Coomassie blue, 45% ethanol, 10% acetic acid, and destained in 45% ethanol, 10% acetic acid.

Rabbit antiserum against AMV gp 80 was prepared by injecting rabbits with 300 μg of purified product in Freund's complete adjuvant. After 15 days the animals were injected with 100 μg of gp following the same conditions. Fifteen days later, the animals were bled. After coagulation of the blood, the serum was removed and stored at -20°C.

RESULTS

Interactions of gp 80 With Acetylated Decylamino Agarose

In this work acetylated uncharged decylamino agarose was used. As a consequence, the absorption of polypeptides to the acetyl decylamino agarose must be due primarily to hydrophobic interactions [21]. The strength of these hydrophobic interactions was increased by the presence of phosphate anions in the elution buffers.

The elution pattern and polypeptide composition of the different fractions obtained from chromatography of disrupted AMV are shown in Figure 1. The viral polypeptides, p27, p12 and p15 (p10), were eluted with 0.05 M. sodium phosphate. Subsequently the column was eluted with buffer containing 50% ethylene glycol. Addition of this polarity reducing agent to the aqueous phase results in a weakening of the hydrophobic interactions between the polypeptides and the lipophilic matrix

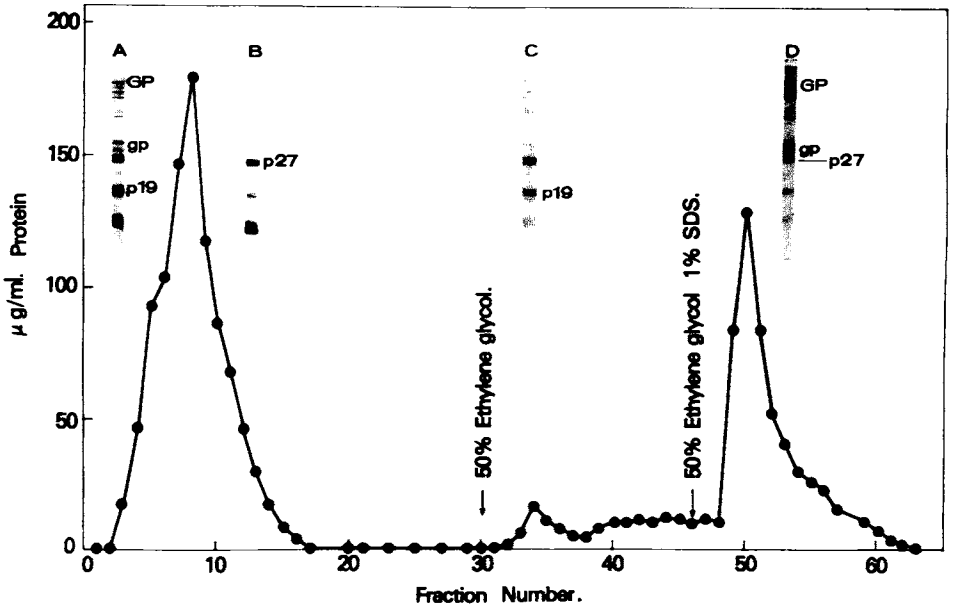

Fig. 1. Hydrophobic chromatography on acetyl decylamino agarose of AMV polypeptides. Disrupted virus (2 mg of protein) was applied to the column (2 ml bed volume) and chromatography was carried out as described under Materials and Methods. Arrows indicate the application of the different elution buffers. The inserts show the SDS-PAGE patterns of whole AMV (A); phosphate buffer elute fraction (B); 50% ethylene glycol elute fraction (C); and polypeptides in the SDS elute fraction (D). Gels were stained as described in the text.

[25]. A small fraction of the sample was displaced with this solvent. The polypeptide composition of this fraction seems to be mostly p19 and p27 (Fig. 1). A third fraction was obtained by eluting the column with buffer containing 50% ethylene glycol plus 1% SDS. This detergent interacts strongly with the alkyl chains of the gel and also with hydrophobic domains of the polypeptides, facilitating the desorption process. This fraction contained all the AMV gp 80 and gp 37 and polypeptides with molecular weights higher than 27,000 (Fig. 1). The viral glycoproteins gp 80 (Gp) and gp 37 (gp), were detected in the gel by specific staining with the periodic acid-schiff reaction. Subsequent treatment of the gel with Coomassie blue revealed only the non-glycosylated polypeptides. The multiple bands observed around the gp 80 and gp 37 regions are presumable polypeptides of the cellular membrane origin. The recovery of protein from the chromatographic step was 97%, indicating the absence of irreversible bindings between the polypeptides and the hydrophobic gel.

The chromatographic behavior of the AMV gp 80 (GP) on acetylated decylamino agarose suggests a lipophilic protein with a relative degree of hydrophobicity similar to that of the minor glycoprotein (gp). However, earlier studies have shown that the disruption of reduced avian oncornaviruses with non-ionic detergents resulted in the recovery of a gp 80 and gp 37 complex [26,27]. The presence of this complex indicates the existence of non-covalent interactions between the two glycoproteins, interactions that can be disrupted only by agents such as SDS, urea, and guanidine hydrochloride [4,5,26]. This situation raises the possibility that in hydrophobic chromatography a glycoprotein complex is being bound to the hydrocarbon chains of the gel through lipophilic interactions with only gp 37. A direct evaluation of the hydrophobicity of gp 80 was obtained from hydrophobic chromatography of the purified glycoprotein. As shown in Figure 2, a small fraction of the preparation, ie, about 20%, was eluted with buffer containing 50% ethylene glycol. The major fraction of the preparation, 80% was released only when SDS was present in the eluent. Both fractions showed identical electrophoretic patterns in SDS-PAGE (Fig. 2). This chromatographic behavior shows that the major glycoprotein possesses highly hydrophobic domains in its structure.

Electrophoretic Studies

Charge shift electrophoresis. Figure 3 shows the electrophoretic patterns obtained for the AMV gp 80 in agarose gels containing Triton X-100 (TX), Triton X-100 plus sodium deoxycholate (TX-DOC) and Triton X-100 plus cetyltrimethylammonium bromide (TX-CETAB). The viral glycoprotein showed a more anodal migration when electrophoresis was performed in the presence of TX-DOC than in the presence of TX alone. The electrophoresis in the presence of TX-CETAB showed, on the other hand, a more cathodal migration than in the presence of TX alone. Well-known hydrophilic proteins, such as bovine serum albumin, ovalbumin, and chymotrypsinogen did not show differences in migration in the three different detergent systems (not shown). According to Helenius and Simons [24], only amphiphilic proteins capable of binding detergents as micelle-like clusters around their hydrophobic domains show alterations in their electrophoretic migrations. This detergent-induced shift in mobility is a function of the net charge of the detergent-protein complex. The alterated electrophoretic migration of the AMV gp 80 in the different systems is consistent with an amphiphilic nature.

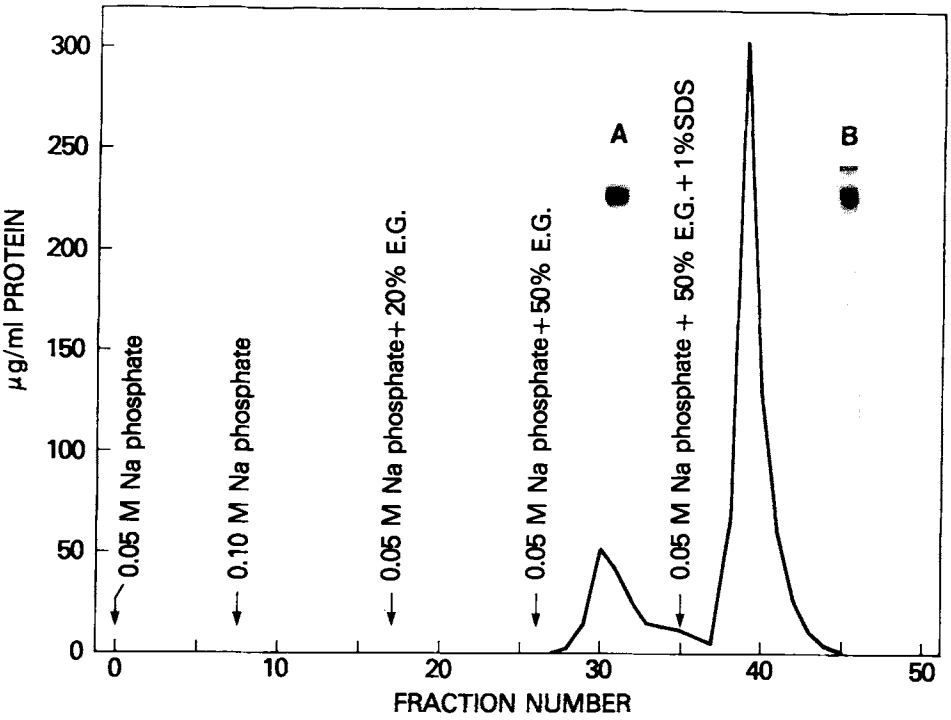

Fig. 2. Hydrophobic chromatography on acetyl decylamino agarose of purified AMV gp 80. Approximately 2 mg of glycoprotein were applied to the column (2 ml bed volume) and chromatography carried out as described under Materials and Methods. Arrows indicate the application of the different elution buffers. The inserts show the SDS-PAGE patterns of the 50% ethylene glycol elute fraction (A) and the glycoprotein in the SDS elute fraction (B). Gels were stained with the PAS reaction as described in the text.

Crossed hydrophobic interaction immunoelectrophoresis. The crossed hydrophobic immunoelectrophoresis of purified AMV gp 80 in the presence of Triton X-100 is shown in Figure 4A. A single immunoprecipitate showing total retardation was observed. This precipitate (gp) corresponds to the viral glycoprotein which is bound to the octyl agarose gel during the first dimension electrophoresis. Displacement from the amphiphilic matrix and immunochemical detection of the bound glycoprotein was made possible by addition to the second-dimension gel of Triton X-100. This detergent enters the amphiphilic gel by means of the electroendosmotic water flow in the agarose and displaces the glycoprotein from the octyl agarose [24]. In the absence of detergent the viral glycoprotein remains bound to the amphiphilic matrix, as is shown by the absence of the immunoprecipitate in Figure 4B. The behavior of this viral glycoprotein is typical of well characterized amphiphilic proteins [24].

DISCUSSION

The present data show that the AMV gp 80 is highly hydrophobic in spite of its water soluble properties. In effect, complete elution of the glycoprotein from the

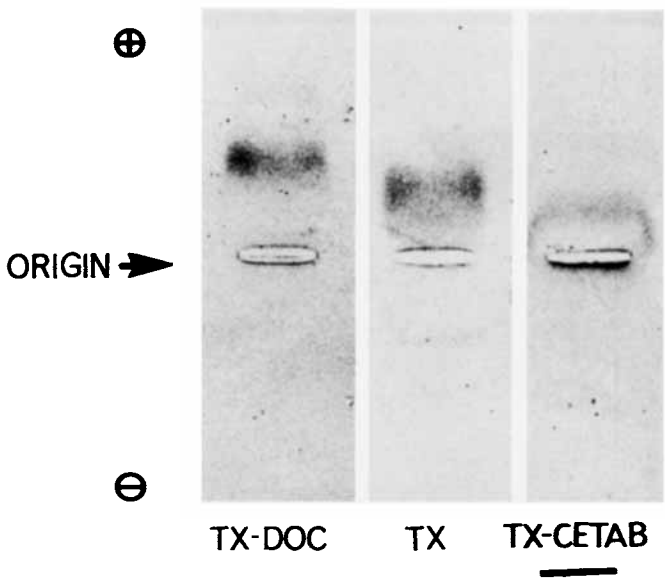

Fig. 3. Charge shift electrophoresis of AMV gp 80. Combined results from agarose gel electrophoresis of the viral glycoprotein in the presence of Triton X-100 and deoxycholate (TX-DOC), Triton X-100 alone (TX), and Triton X-100 and cetyltrimethylammonium bromide (TX-CETAB). The samples contained 20–30 μg of viral glycoprotein in 12 μl. Electrophoresis was performed for 2 hr at 4.5 V/cm. Gels were stained as described in the text. The bar represents 1 cm.

hydrophobic matrix was accomplished only by the use of the anionic detergent sodium dodecyl sulfate. Detergents interact strongly with the hydrophobic surfaces of the protein, making them more hydrophilic and facilitating desorption from the matrix. The fact that a small fraction of the purified glycoprotein was eluted by high concentrations of ethylene glycol can be explained by aggregation in the sample. The viral glycoprotein when exposed to high ionic strength may aggregate due to intermolecular interactions of its hydrophobic regions. This aggregate, as a consequence, will interact with hydrophobic ligands in a less efficient manner and displace under milder conditions. The lipophilic nature of gp 80 indicates that the protein has the potential to interact directly with the lipids of the virus envelope or of the cell membrane.

The hydrophobic properties of this protein coupled to the high content of hydrophilic carbohydrates, ie, 37% by weight, suggests an amphiphilic structure. Evidence for such structure comes from the behavior of the viral glycoprotein, gp 80, when subjected to charge shift electrophoresis. Amphiphilic proteins, in contrast to hydrophilic proteins, bind large amounts of non-ionic detergents such as Triton X-100 [28]. In the presence of mixtures of Triton X-100 and charged detergents, the amphiphilic proteins form detergent-protein complexes containing both neutral and charged detergent molecules. The net charge of the complexes is thus dependent on the charge of the detergent micelles bound to the hydrophobic part of the amphiphilic protein. The changes in net electric charge result in a shift in the electrophoretic mobility of the protein-detergent complex when compared to the mobility in Triton X-100 alone. Our data indicate that in the presence of a negatively charged detergent, the glycoprotein shows a shift toward the anode and with a positively charged

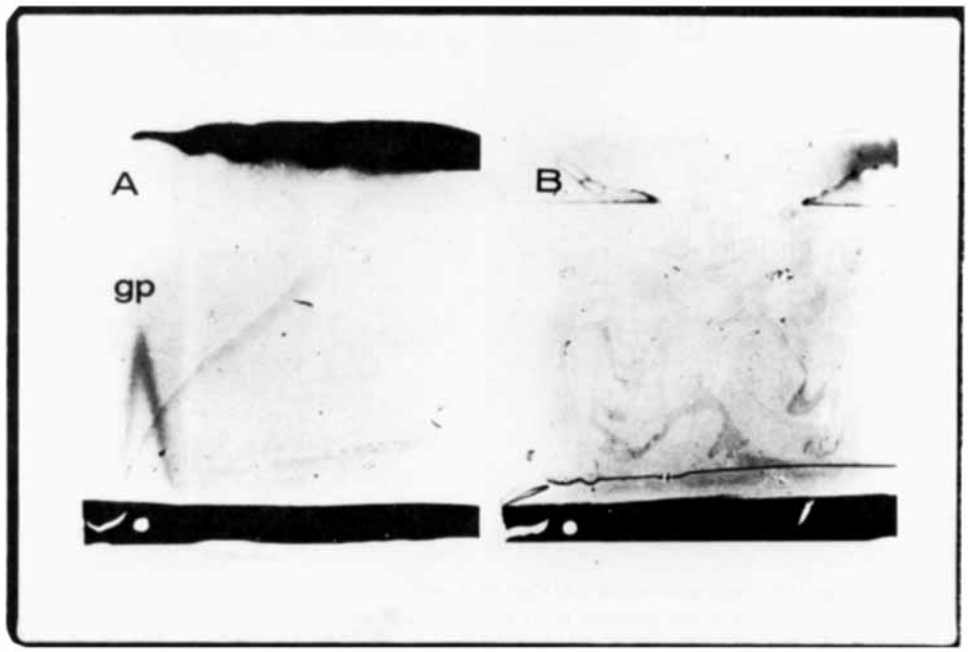

Fig. 4. Crossed hydrophobic interaction immunoelectrophoresis of 15 μg of purified AMV gp 80. First-dimension electrophoresis was performed in detergent-free gels containing octyl-agarose. Second dimension electrophoresis was performed in gels containing 5.4 μl/cm^2 of antiserum and with 1% (v/v) Triton X-100 (A) or without Triton X-100 (B). The position of the viral glycoprotein immuno-precipitate is indicated (gp). Plates were stained with Coomassie blue as described in the text.

detergent a shift to the opposite direction. These results are consistent with a model in which the detergents are bound in the form of micelle-like clusters around the hydrophobic domains of the protein. In effect, direct determination of ^{3}H-labeled Triton X-100 binding has shown that this viral glycoprotein binds a large amount of detergent, 0.4 mg per mg of protein [8], as expected for amphiphilic proteins. Additional evidence for the amphiphilic nature of gp 80 was provided by crossed hydrophobic interaction immunoelectrophoresis. Interactions of the glycoprotein with octyl agarose indicates the presence of significant hydrophobic domains in the protein structure.

The lipophilic area(s) or domain(s) of the viral glycoprotein may be involved in specific interaction between this glycoprotein (gp 80) and the smaller viral glycoprotein (gp 35) and the lipid bilayers. It is also possible to speculate that the lipophilic area(s) of gp 80 are responsible for the recognition of the virus receptor at the cell surface of the host and its physiological effects on chick fibroblasts.

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