The Spleen Focus-forming Virus Envelope Glycoprotein Is Defective in Oligomerization*

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The gp52 envelope glycoprotein of Friend spleen focus-forming virus (SFFV) is a recombinant molecule derived from Friend murine leukemia virus (MuLV) by various deletions, insertions, and substitutions. The SFFV gp52 glycoprotein, unlike MuLV envelope glycoproteins, is defective in transport to the cell surface. Only 3–5% of gp52 eventually reaches the cell surface as a processed form (gp65). Although gp52 lacks cytoplasmic tail residues found in MuLV glycoproteins, we have previously shown that this deletion is not responsible for its defective transport. In order to investigate the basis for the defective transport of gp52, we have examined the folding and assembly of gp52 molecules into oligomeric molecules. CV-1 cells infected with vaccinia virus recombinants expressing SFFV gp52 were pulse labeled and the cell extracts were fractionated by velocity centrifugation through sucrose gradients. Immediately after a 10-min pulse, gp52 was detected as a monomer in the upper part of the sucrose gradient (fractions 12 and 14) and it remained as such after a 2-h chase period. However, the processed form, gp65, was found in a lower part of the gradient (fraction 8) after a 2-h chase. The position of gp65 was found to correspond to the position of trimeric influenza hemagglutinin which was analyzed on a parallel sucrose gradient, suggesting that gp65 also exists as a trimer in this fraction. These results indicate that changes in the external domain of gp52 result in improper folding of the glycoprotein molecule, and suggest that this lack of oligomerization is responsible for the defective transport of the molecules. Only those molecules that do form oligomeric structures are transported to the Golgi complex and undergo further oligosaccharide processing, and transport to the cell surface.

Friend spleen focus-forming virus (SFFV)1 is a defective murine leukemia virus (MuLV) that causes an acute erythroleukemia in newborn and adult mice (Friend, 1957; Rauscher, 1962; Mirand et al., 1968; Steeves et al., 1971). SFFV has been identified as a recombinant virus containing substitutions in the envelope gene as well as deletions in the env, gag, and pol regions of the genome (Clark and Mak, 1983). gp52 is the primary translation product of the SFFV envelope gene, and its expression is necessary for the disease process. The analysis of SFFV mutants has previously shown that mutations within the envelope gene eliminated the transporting activity of the genome, while mutations outside the envelope gene had no effect on the leukemogenicity of the virus (Linemeyer et al., 1982; Ruta et al., 1983; Machida et al., 1984). Nucleotide sequence analyses (Amanuma et al., 1983; Wolff et al., 1983; Clark and Mak, 1983) indicate that the amino-terminal portion of gp52 is closely related to the amino-terminal region of the envelope gene of mink cell focus-forming viruses, whereas the carboxyl-terminal region of the SFFV env gene is closely related to the 3'-half of MuLV p15E.

The envelope proteins of replication-competent MuLVs are cleaved into an external subunit, designated gp70, and a transmembrane protein designated p15E. The SFFV envelope gene contains a large deletion of 585 bases that removes the carboxyl-terminal portion of gp70 sequences and the amino-terminal portion of the p15E sequences, resulting in an uncleaved protein. In addition, the sequences coding for the membrane-spanning region of gp52, as compared to similar domains in MuLV p15E, reveal the insertion of a six-base tandem repeat at position 1408 which adds 2 hydrophobic leucine residues. There is also a single base insertion at position 1426 that causes a frameshift mutation and results in premature termination of the molecule 34 codons prior to the termination codon in MuLV p15E (Amanuma et al., 1983; Wolff et al., 1983; Clark and Mak, 1983). These changes result in a hydrophobic carboxyl terminus which appears buried in the membrane with no portion of the molecule exposed on the cytoplasmic side of the membrane (Srinivas and Compans, 1988b). Unlike the MuLV envelope proteins, gp52 is defective in transport to the cell surface; only 3–5% is expressed on the surface of cells in a processed form designated gp65 (Ruta and Kabat, 1980). However, once gp56 does get to the surface it is apparently secreted from cells, at least in part (Pinter and Honnen, 1985). The proteins specified by other known retroviral oncogenes appear to possess a substantial diversity as to their subcellular location and biological properties (Weber and McClure, 1987). The glycoprotein of SFFV has not been shown to possess protein kinase activities, as is the case with several other retroviral transforming proteins. It is still unknown how the SFFV env gene product functions in the induction of oncogenesis.

It was previously thought that the leukemogenicity of SFFV may be related to the defective transport of gp52 (Dressler et al., 1979), and that this defective transport was possibly the result of the deletion of the cytoplasmic tail residues from gp52 (Srinivas and Compans, 1988a). However, we have recently shown that a chimeric envelope gene containing the 5' ectodomain sequences from F-MuLV and the transmembrane domain of SFFV (designated MuLVpSFFV') was not defective in transport to the surface of CV-1 cells, even though the
chimeric molecule lacks cytoplasmic tail residues (Kilpatrick et al., 1987). Another chimeric envelope gene (designated SFFV<sub>e</sub>/MuLV<sub>Tc</sub>) with the transmembrane domain and cytoplasmic tail residues of F-MuLV and the ectodomain of SFFV gp52 was also defective in transport (Srinivas et al., 1987; Kilpatrick et al., 1988), although the addition of the cytoplasmic tail residues to gp52 did eliminate the leukemogenicity of this chimeric construct (Srinivas et al., 1987). These studies have shown that the lack of the cytoplasmic tail residues in gp52 does not determine the transport defect of the molecule, and that the transport defect per se is not responsible for leukemogenesis.

The reason why a small amount of the envelope protein slowly reaches the cell surface as gp65, and why the majority of gp52 accumulates within the rough endoplasmic reticulum, has not been determined. We thought it likely that the large 585-base deletion in the external domain could change the folding of the protein molecule. Recent studies have shown that the successful achievement of the correct quaternary structure is a general prerequisite for transport of membrane-bound or secretory glycoproteins. Incompletely assembled subunits of immunoglobulins (Mains and Sibley, 1983), or monomeric forms of retinal binding protein (Ronne et al., 1983), and the major histocompatibility complex class I antigens (Severinsson and Peterson, 1984), were not transport-competent. Efficient transport of influenza virus hemagglutinin (HA) requires correct folding and assembly of monomeric subunits into trimeric molecules (Copeland et al., 1986; Gething et al., 1986). The G glycoprotein of vesicular stomatitis virus was also shown to undergo a similar process (Kreis and Lodish, 1986; Doms et al., 1987). In this study, we have investigated the folding of the SFFV gp52 molecules by using sucrose density gradient centrifugation. We demonstrate that gp56 has undergone oligomerization while gp52 is defective in oligomerization and remains as a monomer in the rough endoplasmic reticulum.

**MATERIALS AND METHODS AND RESULTS**

*Oligomerization of SFFV Glycoproteins*—We investigated whether the defective transport of gp52 could be correlated with a block in oligomerization. To obtain high levels of glycoprotein expression, we used vaccinia virus recombinants expressing SFFV gp52 in these studies (Kilpatrick et al., 1988). CV-1 cells were infected with the vaccinia virus recombinant, pulse-labeled using <sup>35</sup>S)methionine at 6 h post-infection, and either harvested immediately or after a chase period of 2 h. To identify the formation of possible gp52 oligomers, cell extracts were fractionated by velocity centrifugation through sucrose gradients containing octylglucoside (Gething et al., 1986), and the SFFV glycoprotein molecules were immunoprecipitated from gradient fractions using polyclonal antisera. Fig. 1A shows that all of the gp52 molecules collected immediately after the radioactive pulse remain in the upper part of the gradient (fractions 12-14). After a 2-h chase, the bulk of the radioactivity remains associated with gp52 and remains in the upper part of the gradient (fractions 12-16). However, a portion of the label is found as gp65, which is the processed form of gp52, and is found in a lower fraction (Fig. 1B, fraction 8). To identify the relative size of these faster sedimenting gp65 molecules, Madin-Darby canine kidney cells were infected with influenza virus (WSN), radiolabeled, and analyzed on parallel sucrose gradients to determine the position of the known trimers of the HA molecules. The conversion of HA monomers to trimers can be seen in a pulse-chase, with the peak of HA trimers sedimenting in fraction number 8 (Fig. 1D). The results for HA are similar to those of Gething et al. (1986) and Copeland et al. (1986) who reported that HA trimers sedimented in fractions 6-8 when analyzed under identical gradient conditions. Thus, the sedimentation profile indicates that gp65 is found as an oligomer. It is likely that the oligomers are comprised of trimers, based on the comparison with the sedimentation profile of influenza HA, although other possibilities cannot be excluded.

*Oligomerization of Chimeric gp55 Molecules*—Previous studies have provided evidence for the importance of the transmembrane domains of glycoproteins in the oligomerization process. Interactions between the transmembrane glycoprotein, gp37, of the Rous sarcoma virus envelope glycoprotein complex (gp85/gp37) appeared to be the most important feature of the envelope glycoprotein needed for oligomerization (Einfeld and Hunter, 1988). We therefore also analyzed the oligomerization of a chimeric gp52 molecule which has the normal gp52 transmembrane domain replaced with the transmembrane domain and cytoplasmic tail of MuLV gp70/p15E. The construction of this chimeric molecule has been described previously (Srinivas et al., 1987; Kilpatrick et al., 1988). The chimeric molecule is 3000 daltons larger due to the addition of the cytoplasmic tail from MuLV gp70/p15E, and is designated gp55. Using a vaccinia virus-gp55 recombinant, we investigated whether the mutations in the transmem-
brane domain of gp52 have any effect on the oligomerization of the molecule. Fig. 2 shows that the chimeric gp55 molecule remains as a monomer in the upper portions of the gradient (fractions 12, 14, and 16). The processed form of the chimeric molecule (designated gp68) sediments in fraction 8, the same as that found with the wild-type gp65 molecules. Thus, the substitution of the SFFV transmembrane domain with the transmembrane and cytoplasmic regions of MuLV resulted in the majority of the chimeric gp55 still remaining as monomers. However, under identical labeling conditions, the chimeric gp68 oligomer was more easily visible on the autoradiograms than the wild-type gp65 oligomer (data not shown). When the gels were scanned using a soft-laser densitometer, the ratio of chimeric gp68 to gp55 was found to be 3-fold higher than the ratio of wild type gp65 to gp52. The increased oligomerization of the chimeric molecules could be due to a change in their folding because of either the addition of cytoplasmic tail residues or changes within the transmembrane domain.

DISCUSSION

We have used sucrose density gradients to analyze the folding of newly synthesized SFFV glycoproteins. Molecules of gp52 were shown to predominantly remain as monomeric structures, even after a 2-h chase period. In contrast, gp65, the processed form of gp52, sedimented predominantly as oligomers after a similar chase period. The sedimentation coefficient for these oligomers was found to be about 8 S, when compared to the internal standard influenza hemagglutinin, and suggests a trimeric organization. However, since the shape of the proteins is not known, it is also possible that they are dimers or tetramers. In this context, it is interesting to note that, based on the electrophoretic mobility of cross-linked proteins, Pinter and Fleissner (1979) concluded that the envelope proteins of MuLV exist as oligomers consisting of 4 or 6 individual protein units. The oligomerization of chimeric gp68 was more readily detected than the wild-type gp65 protein under the same conditions. Wild-type gp65 oligomers were only detected after increasing the labeling time and the amount of radioactive isotope that was used. This difference was correlated with a 5-fold increase in the ability of the chimeric molecules to form disulfide-linked molecules, possibly due to the presence of an additional cysteine residue in the transmembrane domain of the chimeric molecules. These disulfide bridges may increase the intermolecular stability of the oligomeric molecules, and increase their chances of transport from the endoplasmic reticulum. Most of the fully processed molecules, of both the wild-type as well as the chimeric type, were found to exist as disulfide-linked dimers.

The accumulation of gp52 in the rough endoplasmic reticulum is possibly due to a selective retention of this protein or due to an improper folding of the molecule. Selective retention of unfolded proteins in the lumen of the ER was first reported with hetero-oligomeric proteins in which one of the subunits was synthesized without its partner. Kvist et al. (1982) reported that the γ chain of the histocompatibility antigen HLA-DR accumulated in the ER unless it was able to complex with HLA-DR α or β chains. Mains and Sibley (1983) also reported that the heavy chain of IgM accumulates in the ER unless it is able to complex with its light chain partner. Several reports have indicated that it is necessary for influenza HA to correctly fold into an oligomeric structure in order to be efficiently transported out of the ER (Gething et al., 1986; Copeland et al., 1986; Doms et al., 1987; Boulay et al., 1988), although in another study (Yewdell et al., 1988) it was reported that trimerization occurs only after HA has been transported from the ER. In the present study, the Golgi and surface forms of the SFFV proteins (gp65 and gp68) were found as oligomers. In contrast, the ER forms of the proteins (gp52 and gp55) were found as monomers in the sucrose density gradients. Interestingly, under nonreducing conditions, all the processed forms, and a small portion of the unprocessed forms of the protein were found to exist as disulfide-linked oligomers. Our present results therefore suggest that oligomerization precedes exit from ER, and may be a prerequisite for the SFFV glycoprotein to be transported to the Golgi complex. However, a direct causal relationship between oligomerization and transport competence cannot be established from these results, nor has this been established in other reports. Examples of mutant viral proteins which do oligomerize, but are defective in transport, have been documented (Doms et al., 1988).

Several possible mechanisms may be considered for the retention of gp52 in the endoplasmic reticulum. It was shown that some unfolded or unassembled proteins associate with a 77-kDa protein which was identified as an immunoglobulin heavy chain binding protein (Haas and Wabl, 1983; Bole et al., 1986). Mutants of influenza HA which are defective in transport from the ER were found to be associated with this ER resident protein (Gething et al., 1986). The wild-type HA also appears to associate with binding protein as an intermediate step along its normal pathway of ER export. It was thought, therefore, that binding protein may facilitate oligomerization and/or folding in the lumen of the ER. Our results show that binding protein does not associate with gp52, indicating that such association is not involved as the mechanism for ER retention of the SFFV glycoproteins. Also, the transport-competent MuLV proteins did not show any transient association with binding protein, as has been reported for influenza HA. The G protein of a temperature-sensitive mutant of vesicular stomatitis virus also was not observed to associate with binding protein (Doms et al., 1987).

It was alternatively possible that gp52 is nonspecifically retained in the ER due to the formation of protein aggregates. Pfeffer and Rothman (1987) suggested that such aggregates may form with unfolded or partially folded proteins, and that proteins such as binding protein and protein disulfide isomerase may facilitate dissolution of such aggregates, since hydrophobic interactions and incorrect (inter- and intramolecular) disulfide bridges may be involved in aggregate formation. The ER lumen is an oxidizing environment designed to favor disulfide bond formation, and any nonspecific contacts among unfolded protein chains could lead to protein aggregation. Indeed, our results show that the gp52 molecules that are retained in the ER consist of monomeric and oligomeric components that exhibit heterogeneity when electrophoresed in the absence but not the presence of a reducing agent (Figs. 5 and 6). Our results confirm previous findings by Gliniak and Kabat, who suggested that these components may consist of monomers and dimers with different intrachain and interchain disulfide bonds. Another indication of this microheterogeneity is the spreading of gp52 molecules throughout the upper one-half of the sucrose gradient (Fig. 1B), which would seem to indicate a heterogeneous population of molecules present after the 2-h chase period. However, previous studies by Ruta et al. (1982) indicate that the selection of gp52 molecules for transport occurs within minutes of their synthesis and that retained gp52 does not subsequently become competent for export, indicating that protein disulfide isomerase is also probably not involved in slowly correcting the incorrectly disulfide-linked gp52 molecules. Recently, Maclachan and Rose (1988) have shown that aberrant intermo-
lecular disulfide bonding, leading to the accumulation of large protein complexes in the ER, was detected with mutants of vesicular stomatitis virus G protein. We did not detect such protein aggregates of gp52 in either sucrose gradients or within the stacker region of an SDS-PAGE analyzed under nonreducing conditions (data not shown). It was also previously found, using microsomal vesicles prepared from SFFV persistently infected normal rat kidney cells, that gp52 did not form aggregates (Srinivas and Compans, 1983b).

We had previously suggested (Srinivas and Compans, 1983a) that the deletion events involved in the generation of gp52 may have resulted in the loss of sequences that act as recognition signals for protein sorting. However, it was subsequently observed that the processed form of gp52 is expressed exclusively on basolateral surfaces of polarized epithelial cells, like the glycoproteins of replication-competent MuLVs (Kilpatrick et al., 1988), demonstrating that the SFFV glycoproteins are recognized by the cellular sorting mechanisms responsible for polarized glycoprotein transport in these cells. The present results, as well as the findings of Gliniak and Kabat, indicate that the oligomeric forms of SFFV glycoproteins selectively undergo oligosaccharide processing and are transport-compatible, suggesting that the defect in oligomerization of gp52 is primarily responsible for the transport defect.

A possible explanation of the block in transport of gp52 monomers is that oligomerization per se may be a positive requirement for movement of these membrane glycoproteins to the cell surface. Each stage of the transport process, beginning with exit from the rough endoplasmic reticulum, probably involves the transport of the transport competent molecules into vesicular carriers involved in movement of proteins between membrane compartments. Selective incorporation of oligomeric proteins into such transport vesicles may occur following clustering of the molecules, either by lateral interactions among the oligomers themselves, or by interactions mediated by host proteins. In either case, oligomers can form identical protein-protein bonds, leading to clustering, whereas monomers cannot. The lateral interactions leading to clustering of membrane proteins may be the stimulus that results in the formation of the transport vesicles, which contain the oligomeric proteins.

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Oligomerization of SFFV Glycoproteins

Supplemental material to:

The SPLEEN FOCUS-FORMING VIRUS ENVELOPE GLYCOPROTEIN IS DEFECTIVE IN Oligomerization.

MATERIALS AND METHODS

Cells and Virus. Conditions for propagation of MDCK cells (Roth et al., 1979), MDBK cells (Chopra, 1981), and CV cells (Kipriyanov et al., 1987) were described previously. Virus stock preparations were performed and titrated using CV cells as described previously (Chopra, 1981). Influenza virus, A/WSN strain, was grown in MDCK cells and titered on MDCK cells as described previously (Roth and Chopra, 1981).

Construction of Recombinant Vaccinia Virus. The recombinant virus containing the wild type envelope glycoproteins of SFFV was designated as VV-gp52, while the chimeric envelope gene recombinant of SFFV is designated as VV-gp52, and have been described previously (Kipriyanov et al., 1988). VV recombinants containing wild type or chimeric SFFV gp52/p15E envelope genes have also been described previously (Kipriyanov et al., 1987) and are designated as VV-wild type gp52/p15E and VV-chimeric gp52/p15E.

Labeling and Immunoprecipitation of SFFV and MUV Glycoproteins. CV-1 cells were infected with vaccinia recombinants and at 8 hours post-infection were either labeled with 1-hour with 35S-methionine (100 μCi/ml) or labeled for 2 hours with 1 mM-glycosamine (200 μCi/ml). Cells extracts were prepared by adding lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 100 mM NaCl, 50 mM Tris-Cl, pH 7.4, 5 mM EDTA). Nuclease were left at 37°C for 1 hour at 37°C. Each 30 of Protein A-agarose suspension (6%) was added to each sample and mixed for 1 hour at 4°C. The samples were washed 3 times and the final pellet was resuspended in either 30 ml of 50 mM reducing buffer (1% SDS, 1% glycerol, 0.5% 2-mercaptoethanol, 10% DMSO, 0.125 M Tris-Cl, pH 6.8, 0.001% Bromophenol Blue) or 3 ml of non-reducing buffer (Reducing Buffer minus 2-mercaptoethanol) and boiled for 3 minutes. The samples were analyzed on SDS-PAGE and fluorography as described previously (Kipriyanov et al., 1988).

RESULTS

Oligomerization of SFFV and MUV enveloped viruses. A quantitative analysis of the oligomerization process of the VV-gp52 and gp52/p15E was performed using the same sedimentation conditions for SFFV gp52/p15E as described above. The experiment was performed at different times post-infection in the presence or absence of reducing agents. MUV-52/p15E was analyzed under the same sedimentation conditions for SFFV gp52/p15E glycoproteins with the exception of 60°C incubation at 37°C was used as the radioactive label. Incubated cells were pulse labeled for either 5 minutes (gp52/gp55 and VV-gp52/gp55) or 10 minutes (gp52/gp55-gp55) and harvested immediately after the pulse, or after 2 hour incubation in media containing an excess of nonradioactive methionine or leucine. MDCK cells (35 mm tissue culture plates) were also infected with the MDCK strain of influenza virus and allowed to infect for 2 hours. Samples were pulse labeled with 10 μCi/ml of [35S]-methionine (200 μCi/ml). WSN-infected cells were pulse labeled for 5 minutes and harvested extremely immediately after the pulse or after 2 hours. Samples were lysed with ice-cold Tris-buffered saline and then adding 0.5 ml of 50 mM Tris-Cl, pH 7.4, 150 mM NaCl and 4 μg/ml of protease inhibitors. The pellets of the nuclei by centrifugation, the lysates were applied to sucrose gradients (11.5% to 5% sucrose in 50 mM Tris-Cl, pH 7.4, 150 mM NaCl and 4 μg/ml of protease inhibitors) and the samples were centrifuged at 1°C, 25,000 rpm for 16 hours at 1°C before fractionation from the bottom of the tube into 20 aliquots (Sethi et al., 1989). titer and gdm fractions were immunoprecipitated as described above and analyzed by SDS-PAGE and fluorography.
To further examine oligomeric molecules under non-reducing conditions, we used N-\text{glucosamine} to enhance the labeling of processed forms of the glycoproteins (Fig. 6). Only small amounts of gp52 and gp56 could be seen in the nonreducing lanes, indicating that the majority of processed molecules are disulfide-linked. Dimers of the unprocessed gp52 molecules (61) and a broad band of higher mol. wt. (71 and 122) are shown in Figure 6, lane A. Dimers of gp56 (52) are not clearly detectable in lane a, although they can be seen on the original x-ray film, and are evident in Figure 5, lane A. In contrast, the trimers and higher forms of the chimeric molecules were more readily detected. Figure 6, lane D shows the presence of dimers (104kDa and 116kDa) and higher forms (120kDa and 130 kDa) for the chimeric gp56 and gp56 molecules. The higher percentage (5 to 6 fold) of chimeric molecules that form disulfide-linked molecules could account for the increased levels of oligomerized molecules of chimeric gp66 in the sucrose gradients due to their increased intermolecular stability. To determine the relationship of these disulfide-linked dimers to the oligomers detected in sucrose density gradients, the oligomeric gp52 fractions from a sucrose gradient was analyzed under nonreducing conditions. Only monomeric forms were detected under these conditions, indicating that hydrophobic interactions may be involved in stabilizing the oligomers detected by sucrose gradients. We believe that the disulfide-linked dimers may represent a folding intermediate, that facilitates formation of the mature oligomers.

Figure 6. 1) Identification of SFFV glycoprotein dimers and trimers. Wild type (lanes A and B) and chimeric (lanes C and D) glycoproteins were labeled with N-glucosamine. Lanes in indicate nonreducing conditions, and r show reducing conditions. Disulfide linked molecules were labeled as follows: 1) unprocessed gp52 and gp56 dimers, 2) processed gp52 and gp56 dimers, 3) unprocessed gp52 and gp56 trimers, and 4) processed gp52 and gp56 trimers. The small circles indicate the following size of protein markers: 104kDa, 116kDa, 120kDa, 130kDa, 140kDa, 150kDa, and 160kDa. 2) Analysis of wild type MULV gp70 and chimeric MULV gp70. Wild type MULV gp70(153) was expressed in VV recombinant and is shown under nonreducing (lane E) and reducing (lane F) conditions. Chimeric MULV gp70(315) which has the transmembrane domain from SFFV and therefore lacks the cytoplasmic tail region, was also expressed using a VV recombinant and is shown under nonreducing (lane H) and reducing (lane G) conditions. No disulfide linked molecules were detected for either glycoprotein.

It is also evident in Fig. 6 that, when analyzed under nonreducing conditions, both gp52 and gp56 migrate slightly faster than the bands under reducing conditions. It was previously suggested that this increased mobility is due to the presence of at least one intramolecular disulfide bridge. Only monomeric forms of gp70 were detected under nonreducing conditions; no disulfide linked dimers were detected, indicating that the acquisition of disulfide bonds is not a significant factor in the formation of dimers (gp70 (Figure 6, panel B)). These results also show that disulfide-linked gp70(315) complexes are not detected under these conditions, which is consistent with our conclusion that these two proteins are associated by weak, noncovalent interaction.

**gp52 is not associated with BIP.** Previous studies have suggested the possible involvement of a resident endoplasmic reticulum protein, the immunoglobulin heavy chain binding protein (BIP), in the selective retention of improperly folded protein immunoglobulin molecules (Has and Wabl, 1983; Bole et al., 1986). Kohler and co-workers (1986) have also reported that influenza HA mutant proteins that were defective in transport were associated with the BIP protein. We observed a prominent band of a host cell derived protein, of a size consistent with BIP, in sucrose gradient fractions containing gp52 analyzed in Figure 1. We therefore used monoclonal antibodies to BIP (Bole et al., 1986) to investigate the possible association of BIP with gp52 (Fig. 7). Lysates from cells infected with vesicular virus recombinants expressing gp52 following a 30 min radioactive pulse (lanes 1 and 2); a pulse followed by a 2 hour chase (lanes 3 and 4), and from fraction 13 of the sucrose gradient used in Figure 1 (lanes 5 and 6) were divided in half and immune precipitated either with antisera to gp52 (lanes 1, 3, and 5) or with antisera to BIP (lanes 2, 4, and 6). As shown in Fig. 5, BIP was not found to be associated with gp52 in any of the samples, as indicated by the lack of co-precipitation. Therefore, association with BIP is not apparently involved in the retention of gp52 in the endoplasmic reticulum.

Figure 7. Lack of association of BIP with gp52. Autoradiograph of samples immune precipitated with antisera against either gp52 (odd numbered lanes) or against BIP (even numbered lanes). Lysates from cells infected with vesicular virus recombinants expressing gp52 (Figure 5, panel C) were divided in half and immune precipitated (lane 1, 2, 3, 4, 5, and 6), following a 30 min pulse (lanes 1 and 2) or after a pulse followed by a 2 hour chase period (lanes 3 and 4). Fraction 13 of the sucrose gradient (even fractions shown in Figure 1) was also similarly analyzed for the association of BIP with gp52 (lanes 5 and 6).