Biochem. J. (1990) 265, 161-167 (Printed in Great Britain)

Brefeldin A arrests the intracellular transport of viral envelope proteins in primary cultured rat hepatocytes and HepG2 cells

Kimimitsu ODA,* Toshiyuki FUJIWARA and Yukio IKEHARA
Department of Biochemistry, Fukuoka University School of Medicine, Jonan-ku, Fukuoka 814-01, Japan

We have studied the effect of brefeldin A (BFA) on the intracellular transport of the envelope proteins of vesicular stomatitis virus (VSV) and sindbis virus in primary cultured rat hepatocytes. BFA (2.5 μg/ml) inhibited not only the secretion of plasma proteins into the medium, but also the assembly of both G protein of VSV and E1 and E2 proteins (envelope proteins) of sindbis virus into respective virions. Concomitantly, both the acquisition of endo-β-*N*-acetylglucosaminidase H resistance by the G protein and the proteolytic conversion of PE2 to E2 were found to be inhibited in the BFA-treated cells, suggesting that the intracellular transport of the envelope proteins was arrested in the endoplasmic reticulum. Such inhibitory effects of the drug were variable depending upon the culture conditions of the hepatocytes. In the 1-day-cultured cells, even in the presence of the drug, newly synthesized envelope proteins were assembled into the virions after a 3 h chase period, at the same time as secretion of plasma proteins into the medium resumes. In contrast, in 4-day-cultured hepatocytes, BFA continuously blocked the entry of the envelope proteins into the virions and the release of plasma proteins into the medium for at least 5 h. BFA also completely inhibited the exocytotic pathway in HepG2 cells These results indicate that the duration time of the effect of BFA is different from one cell to another and may change depending upon the culture conditions of the cells.

INTRODUCTION

Brefeldin A (BFA) is a macrolide antibiotic produced by *Penicillium brefeldianum* which has an inhibitory effect on the multiplication of viruses (Tamura *et al.*, 1968). Previously, Takatsuki & Tamura (1985) have reported that BFA arrested G protein transport at an endo-β-*N*-acetylglucosaminidase H (Endo H)-sensitive stage in the intracellular pathway and consequently blocks cell surface expression of G-protein in vesicular stomatitis virus (VSV)-infected baby hamster kidney cells. These findings suggest not only that the antiviral effect of BFA may be caused by inhibition of virus assembly on the cell surface, but also that BFA represents a new perturbant of the exocytotic pathway.

In primary cultured rat hepatocytes, BFA inhibits the secretion of plasma proteins (Misumi *et al.*, 1986; Oda *et al.*, 1987). In agreement with the results of Takatsuki & Tamura (1985), BFA was found to cause the accumulation of α_1-proteinase inhibitor and haptoglobin containing Endo H-sensitive oligosaccharide chains within the BFA-treated cells (Misumi *et al.*, 1986). Furthermore, precursors of albumin and complement component C3, which are proteolytically converted into mature forms in the Golgi, were also found to accumulate in response to BFA (Misumi *et al.*, 1986; Oda *et al.*, 1987). These findings strongly suggest that BFA impedes the exit of secretory proteins from the endoplasmic reticulum (ER), although the mechanism by which BFA interrupts transport from the ER to the Golgi remains to be elucidated.

Interestingly, the effect of BFA on secretion is reversible depending on the drug dose used; plasma proteins are finally secreted from the BFA-treated hepatocytes at doses of 1–5 μg/ml (Misumi *et al.*, 1986; Oda *et al.*, 1987). This is in contrast with evidence on its effect on membrane protein transport (Takatsuki & Tamura, 1985). G protein of VSV did not appear on the cell surface of BFA-treated baby hamster kidney cells at a drug dose of 0.5 μg/ml even after prolonged incubation. The discrepancy may reflect the fact that the duration of the BFA potency differs from one cell type to another or that the drug effect on intracellular transport is different with secretory and membrane proteins.

In order to clarify this point, we compared the effects of BFA on the intracellular transport of viral envelope proteins with its effect on the transport of secretory proteins in rat hepatocytes and in a well-differentiated human hepatoma cell line (HepG2).

EXPERIMENTAL PROCEDURES

Materials

[^{35}S]Methionine (> 800 Ci/mmol) and EN^{3}HANCE were purchased from du Pont–New England Nuclear (Boston, MA, U.S.A.). Actinomycin D and aprotinin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Pansorbin (fixed *Staphylococcus aureus*) was from Behring Diagnostics (La Jolla, CA, U.S.A.). Antipain, chymostatin, elastatinal, leupeptin, pepstatin A and phosphoramidon were from Protein Research Foundation (Osaka, Japan). Eagle's minimum essential medium (MEM) was from Nissui Seiyaku (Tokyo, Japan). Collagenase was from Wako Pure Chemicals (Osaka,

Abbreviations used: BFA, brefeldin A; Endo H, endo-β-*N*-acetylglucosaminidase H; ER, endoplasmic reticulum; MEM, Eagle's minimum essential medium; PAGE, polyacrylamide-gel electrophoresis; pfu, plaque-forming units; VSV, vesicular stomatitis virus.

* To whom all correspondence should be addressed.

Japan). Endo H (*Streptomyces griseus*) was from Seikagaku Kogyo (Tokyo, Japan). Neuraminidase (*Arthrobacter ureafaciens*) was from Nakarai Chemicals (Kyoto, Japan). BFA was isolated in crystalline form from fungi, FL-24, as described previously (Tamura *et al.*, 1968). A stock solution of BFA (2.5 mg/ml) was prepared in methanol and stored at −20 °C until use. Antibodies against rat serum albumin (Ikehara *et al.*, 1977), transferrin (Miki *et al.*, 1983), and α_1-proteinase inhibitor (Ikehara *et al.*, 1981) were raised in rabbits as described previously.

Cells and viruses

Hepatocytes were isolated from adult male Wistar rats (200–250 g body wt). by the collagenase perfusion method of Seglen (1976). Isolated hepatocytes (7.5×10^5 cells), suspended in MEM supplemented with 5% newborn calf serum, 0.1 μM-insulin and 1 μM-dexamethasone, were transferred to collagen-coated Falcon dishes (35 mm) and cultured for 1 day or 4 days as described previously (Oda *et al.*, 1983). Fresh medium (MEM with the hormones) was changed after 24 h and then every 2 days. HepG2 cells were cultured in MEM supplemented with 10% fetal calf serum. VSV (Indiana Serotype) and sindbis virus (MP-684 strain), which were grown in human embryonic lung cells as described previously (Kuwano *et al.*, 1981), were kindly supplied by Dr K. Mifune (Oita Medical School, Japan). Stock viruses were suspended in 20 mM-Hepes buffer (pH 7.4) containing 150 mM-NaCl, 1 mM-EDTA and 0.1% bovine serum albumin (for VSV) or 20 mM-Hepes buffer (pH 7.4) containing 150 mM-NaCl and 0.1% bovine serum albumin (for sindbis virus) and stored at −80 °C until use.

Infection and labelling of cells

Hepatocytes which had been cultured for 1 or 4 days were infected with VSV at a multiplicity of infection of 10–50 plaque-forming units (pfu)/cell or with sindbis virus at 20–50 pfu/cell. HepG2 cells were infected with VSV at 70 pfu/cell. The cells were incubated for 1 h at 37 °C for adsorption. Then cells were washed once with MEM supplemented with 10% fetal calf serum and further incubated for 5 h in the above medium containing 2 μg of actinomycin D/ml before starting the labelling. The cells were preincubated for 1 h in methionine-free MEM, pulse-labelled with 25 μCi of [^{35}S]methionine for 5 or 10 min in fresh methionine-free MEM containing actinomycin D (2 μg/ml), and chased in complete MEM. Unless otherwise indicated, BFA was added 1 h before the start of labelling and was included throughout the experiments. At the indicated times, cells were harvested and lysed in 100 μl of 2 mM-Tris/HCl (pH 7.5) containing 1% SDS and 1% 2-mercaptoethanol (for enzyme digestion), or in 100 μl of phosphate-buffered saline (10 mM-phosphate/150 mM-NaCl, pH 7.4) containing 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% SDS (for immunoprecipitation). After vigorous sonication, the cell lysates were centrifuged at 15000 *g* for 10 min and the resulting supernatants were stored at −20 °C until use. A mixture of proteinase inhibitors (antipain, aprotinin, chymostatin, elastinal, leupeptin, pepstatin A and phosphoramidon) was added to cell lysates and media to a concentration of 10 μg of each/ml.

Virions were collected from the medium as described before (Oda *et al.*, 1988), except that sindbis virus was collected by centrifuging the medium at 200000 *g* for 2 h instead of 1 h.

Immunoprecipitation

Immunoprecipitation of albumin (Oda *et al.*, 1983), transferrin (Miki *et al.*, 1983) and α_1-proteinase inhibitor (Oda *et al.*, 1983) was performed with each purified carrier protein, and the precipitates were washed as described previously. For precipitation of VSV, the medium was incubated with mouse antibody against VSV at 4 °C overnight, and then with Pansorbin for 2 h. The Pansorbin immunocomplex was washed extensively as described previously (Takami *et al.*, 1988).

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) was performed on 10% gels by the method of Laemmli (1987). After electrophoresis, gels were fixed and processed for fluorography (Oda *et al.*, 1983).

Enzyme digestion

Samples dissolved in buffer containing 1% SDS were digested with either Endo H or neuraminidase as described previously (Oda *et al.*, 1986*b*, 1988). The samples were mixed with an equal volume of 2 × Laemmli's sample buffer (Laemmli, 1970), boiled for 3 min and analysed by SDS/PAGE.

RESULTS

BFA inhibits the assembly of G protein into virion

The effect of BFA on the assembly of VSV was examined by using 1-day-cultured rat hepatocytes. VSV-infected hepatocytes were pulse-labelled with [^{35}S]methionine and chased in the absence or the presence of BFA. At the indicated times, aliquots of the medium were taken and analysed by SDS/PAGE (Fig. 1). In this experiment, cells were infected with VSV at a low multiplicity of infection, allowing us to study the effects of BFA on VSV assembly and on secretion of plasma proteins simultaneously. Viral proteins and major plasma proteins in the medium were identified by immunoprecipitation with anti-VSV antibodies or monospecific antibodies against each plasma protein (Fig. 1*a*). G protein and albumin migrated to the same position on an SDS/polyacrylamide gel (lanes 2 and 3). Both newly synthesized viral components and plasma proteins appeared rapidly in the medium (Fig. 1*b*), and viral components were assembled into virions (Fig. 1*b*, lane 10). In contrast, the appearance of both G and plasma proteins in the medium was blocked for more than 2 h in the presence of 2.5 μg of BFA/ml (Fig. 1*c*), indicating that BFA inhibits the exocytotic pathway of proteins, irrespective of whether these are secretory or membrane-bound proteins. BFA did not apparently block the assembly of other components of VSV (Fig. 1*c*), which are synthesized on free polysomes and transported directly to the plasma membrane. The blockade by BFA, however, was temporary, and G protein was finally released into the medium 3 h after the start of the chase, concomitant with the discharge of plasma proteins (Fig. 1*c*, lanes 6–8). Enzyme digestion with either Endo H or neuraminidase demonstrated that the G proteins in virions released from the BFA-treated cells and the

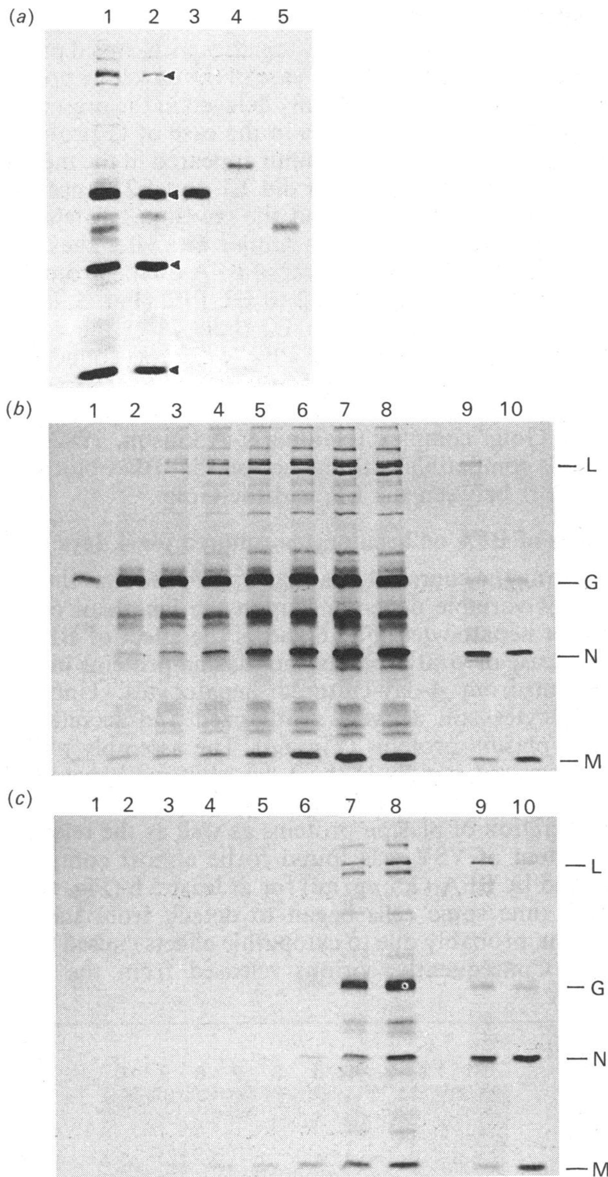

Fig. 1. Effects of BFA on release of plasma proteins and G protein from VSV-infected hepatocytes

Hepatocytes which had been cultured for 24 h were infected with VSV at a multiplicity of infection of 10 pfu/cell. In (*a*), after hepatocytes were labelled with 25 μCi of [^{35}S]methionine for 3 h, the medium was collected. An aliquot of the medium was subjected to SDS/PAGE (lane 1) or to immunoprecipitation of VSV (lane 2), albumin (lane 3, 68 kDa), transferrin (lane 4, 77 kDa) and α_1-proteinase inhibitor (lane 5, 56 kDa), followed by SDS/PAGE and fluorography. Arrow heads (lane 2) indicate viral components (from the top; L, G, N and M). In (*b*) and (*c*), the VSV-infected hepatocytes were pulse-labelled with 25 μCi of [^{35}S]methionine for 10 min and chased in the absence (*b*) or presence (*c*) of BFA (2.5 μg/ml). Aliquots of the medium were taken at the indicated times: lanes 1–8 were chased for 20, 40, 60, 90, 120, 180, 240 and 300 min respectively. At 300 min of chase, the remaining cells and medium were separated and subjected to preparation of cell lysates (lane 9) and VSV virions (lane 10) respectively. Each sample was analysed by SDS/PAGE, followed by fluorography. L, G, N and M denote large protein, glycoprotein, nucleocapsid protein and membrane protein respectively of VSV.

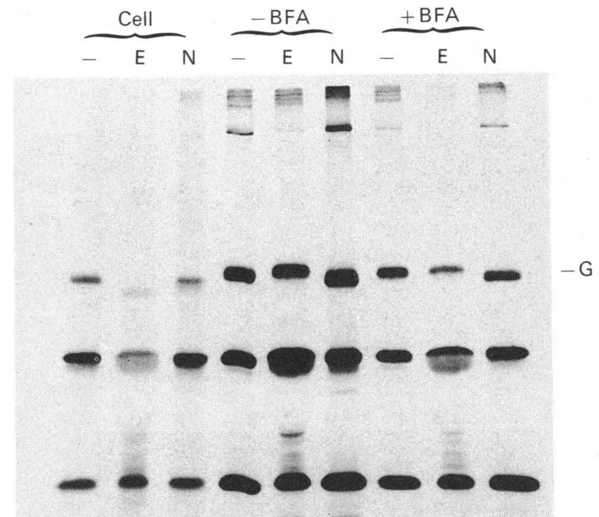

Fig. 2. Sensitivity of G protein of VSV virions to Endo H or neuraminidase

Hepatocytes, which had been cultured for 24 h, were infected with VSV (10 pfu/cell) as in Fig. 1, and labelled with 25 μCi of [^{35}S]methionine for 5 h in the absence or the presence of BFA. Virions were collected from the media and analysed by SDS/PAGE followed by fluorography before (−) and after enzyme digestion with either Endo H (E) or neuraminidase (N). VSV-infected cells were also pulse-labelled with [^{35}S]methionine for 5 min, lysed and subjected to the enzyme digestions.

control cells are indistinguishable from each other (Fig. 2), indicating that once the arrest by BFA is released, G protein undergoes the normal processing of *N*-linked oligosaccharide chains and is assembled into the virion.

Fig. 3 shows the effect of BFA on the maturation of G protein. In this experiment, hepatocytes were infected with VSV at a high multiplicity of infection to inhibit the

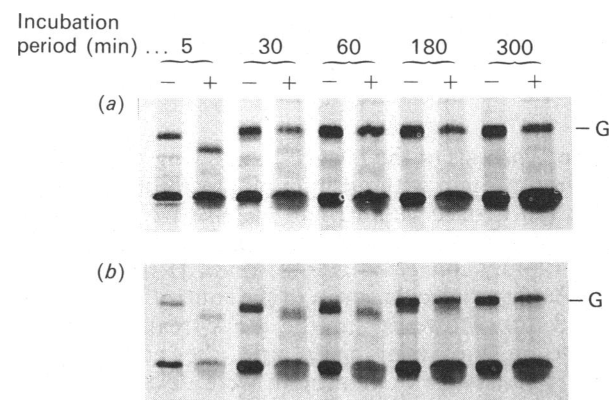

Fig. 3. Effect of BFA on acquisition by G protein of Endo H resistance

Hepatocytes which had been cultured for 24 h were infected with VSV at a multiplicity of 50 pfu/cell. The cells were pulse-labelled with 25 μCi of [^{35}S]methionine for 5 min and chased in the absence (*a*) or presence (*b*) of 2.5 μg of BFA/ml. Cell lysates were prepared at the indicated times and incubated at 37 °C for 16 h in the absence (−) or presence (+) of Endo H. Each sample was analysed by SDS/PAGE followed by fluorography.

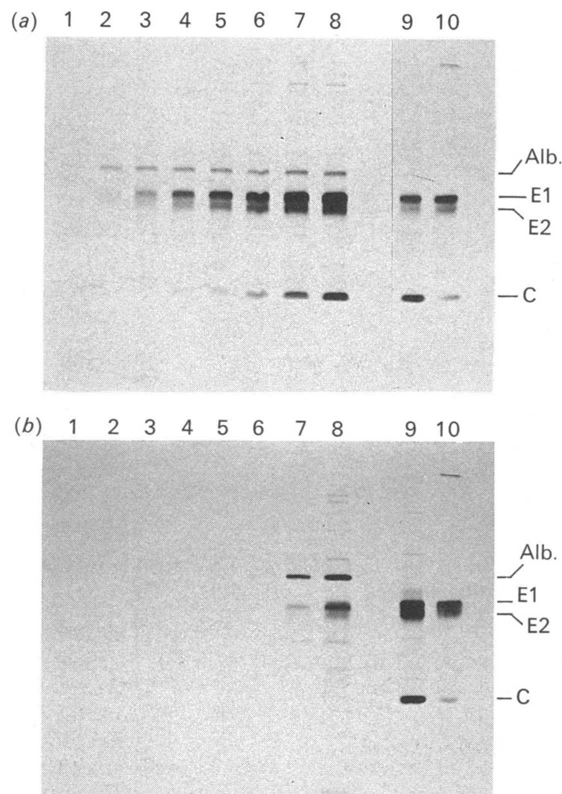

Fig. 4. Effect of BFA on the release of E1 and E2 proteins from sindbis-virus-infected hepatocytes

Hepatocytes which had been cultured for 24 h were infected with sindbis virus at a multiplicity of infection of 20 pfu/cell. The cells were pulse-labelled with 25 μCi of [^{35}S]methionine and chased in the absence (*a*) or presence (*b*) of BFA (2.5 μg/ml). At the indicated times, aliquots were removed from the medium: lanes 1–8 were chased for 20, 40, 60, 90, 120, 180, 240 and 300 min respectively. At 300 min of chase, the cells and medium were separated and used for preparing cell lysate (lane 9) and virions (lane 10). Each sample was analysed by SDS/PAGE followed by fluorography. E1, E2 and C (capsid protein) are components of sindbis virus. 'Alb.' denotes albumin.

protein synthesis of the host cells, allowing us to identify each viral protein without immunoprecipitation. G protein is synthesized as an Endo H-sensitive form with a relatively low molecular mass (Fig. 3*a*, lanes 1 and 2), and is rapidly converted into an Endo H-resistant form with a higher molecular mass in the control cells (Fig. 3*a*, lanes 3–10). However, in the BFA-treated cells, the increase in molecular mass and the acquisition of Endo H resistance of G protein were delayed (Fig. 3*b*, lanes 3–8). The results suggest that BFA inhibits the transport of G protein from the ER to the Golgi, where glycoproteins undergo oligosaccharide processing to become Endo H-resistant.

BFA inhibits the conversion of PE2 to E2 in sindbis virus

Fig. 4 shows the effect of BFA on the assembly of sindbis virus in 1-day-cultured hepatocytes after infection. In the control cells, newly synthesized envelope proteins (E1 and E2) of sindbis virus appeared rapidly in the medium (Fig. 4*a*, lanes 2–8), indicating the occurrence of their assembly into virions together with capsid protein C (lane 10). However, the release of the envelope proteins into the medium was markedly delayed in the presence of BFA (Fig. 4*b*, lanes 1–6), as in the case of G protein of VSV (Fig. 1). Note that albumin appeared in the medium at almost the same time as did E1 and E2 (lanes 5–8), suggesting that albumin and the envelope proteins migrate to the cell surface at a similar rate after they leave the ER. Fig. 5 shows the effect of BFA on the proteolytic conversion of precursor PE2 to E2. PE2 (Fig. 5, lane 1) was rapidly converted into E2 (lane 2) in the control cells, whereas conversion of PE2 to E2 was found to be delayed in the BFA-treated cells (lanes 6–8). Since the proteolytic conversion of PE2 to E2 is thought to occur in the Golgi complex (Hakimi & Atkinson, 1982), this result is compatible with the notion that BFA blocks the transport between the ER and the Golgi.

Effects of BFA on hepatocytes cultured for 4 days

During the course of this study, we found that the drug effect is variable depending upon the length of culture time of hepatocytes. Fig. 6 shows the effect of BFA on the release of viral proteins and plasma proteins into the medium from 4-day-cultured hepatocytes. Untreated hepatocytes still actively synthesized and secreted the major plasma proteins (Fig. 6*a*). The assembly of VSV also occurred rapidly in the 4-day-cultured hepatocytes, as in the 1-day-cultured hepatocytes (Fig. 1*b*). In contrast, the secretion of plasma proteins as well as the release of G protein of VSV was found to be almost completely blocked by BFA (2.5 μg/ml) for at least 5 h (Fig. 6*b*), at which time some cells began to detach from the substratum, probably due to cytopathic effects caused by the virus. Consequently, virions released from the BFA-

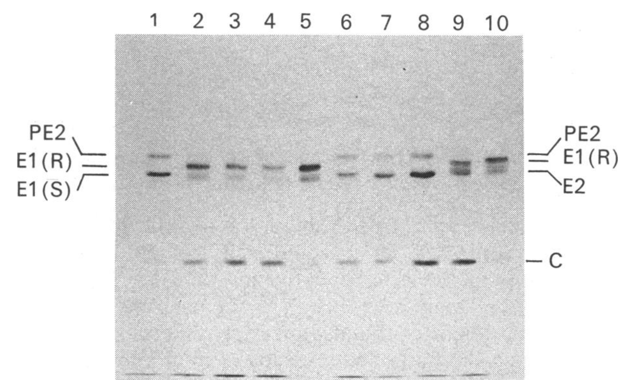

Fig. 5. Effect of BFA on proteolytic conversion of PE2 to E2

Hepatocytes which had been cultured for 24 h were infected with sindbis virus at a multiplicity of infection of 50 pfu/cell. The cells were pulse-labelled with 25 μCi of [^{35}S]methionine for 10 min and chased in the absence (lanes 1–5) or presence (lanes 6–10) of BFA (2.5 μg/ml). At the indicated times, the cell lysates (lanes 1–4 and 6–9) and media (lanes 5 and 10) were prepared and analysed by SDS/PAGE followed by fluorography. Lanes 1 and 6, no chase; lanes 2 and 7, 60 min chase; lanes 3 and 8, 180 min chase; lanes 4, 5, 9 and 10, 300 min chase. PE2, E2, E1 and C are viral components. E1(S) and E1(R) denote Endo H-sensitive and Endo H-resistant forms of E1 protein respectively.

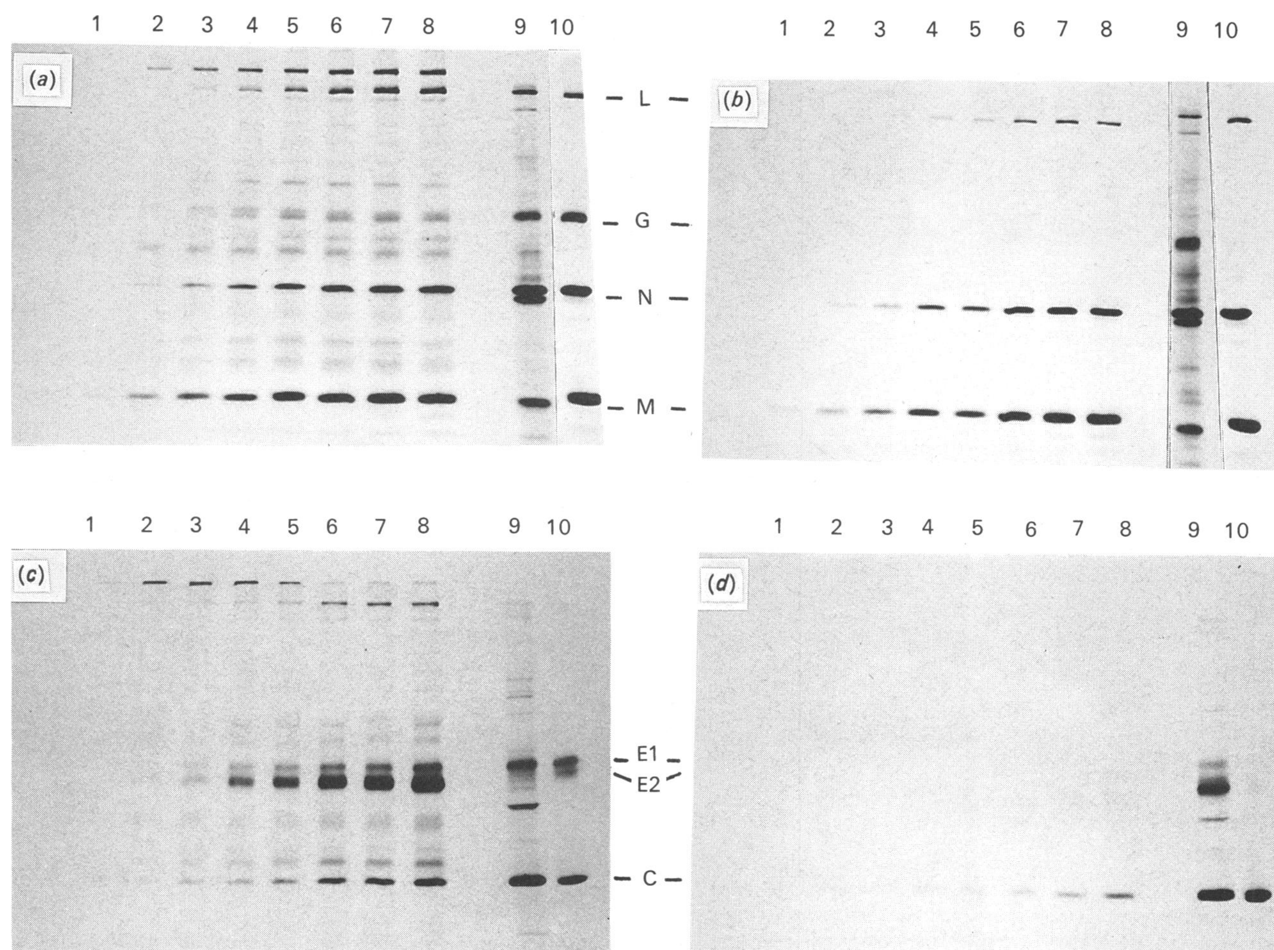

Fig. 6. Effect of BFA on release of G protein and E1 and E2 proteins from 4-day-cultured hepatocytes

Hepatocytes which had been cultured for 4 days were infected either with VSV (*a* and *b*) at 10 pfu/cell or with sindbis virus (*c* and *d*) at 20 pfu/cell. The cells were pulse-labelled with 25 μCi of [^{35}S]methionine for 10 min and chased in the absence (*a* and *c*) or presence (*b* and *d*) of BFA (2.5 μg/ml). At the indicated times, aliquots were removed from the media; lanes 1–8, chased for 20, 40, 60, 90, 120, 180, 240 and 300 min respectively. At 300 min of chase, the cells and medium were separated and used for preparing cell lysates (lane 9) and virions (lane 10). Each sample was analysed by SDS/PAGE followed by fluorography. Letters are as defined in Figs. 1 and 4.

treated cells lacked newly synthesized G protein (Fig. 6*b*, lane 10). These results are quite different from those shown in Fig. 1, where delayed release of both plasma proteins and G protein of VSV occurred. This suggests that BFA exerts its potency in the 4-day-cultured cells for much longer than in the 1-day-cultured cells. Such an effect of BFA was also observed on the assembly of sindbis virus in 4-day-cultured hepatocytes (Fig. 6).

Effect of BFA on the exocytotic pathway in HepG2 cells

Since 4-day-cultured hepatocytes were affected for a longer time by BFA treatment compared with 1-day-cultured cells, it was of interest to know the effect of BFA on an established hepatocyte-derived cell line. The HepG2 cell is a well differentiated human hepatoma cell line and still has the potential to synthesize at least 20 plasma proteins (Knowles *et al.*, 1980). The intracellular transport of plasma proteins and viral components has been extensively studied in HepG2 cells, (Strous & Lodish, 1980; Strous *et al.*, 1983). The appearance of both plasma proteins (Fig. 7) and G protein of VSV (Fig. 8) into the medium was found to be completely blocked by BFA (2.5 μg/ml) in HepG2 cells, as observed in the 4-day-cultured rat hepatocytes.

DISCUSSION

We have investigated the effects of BFA on the intracellular transport and accompanying processing of both envelope proteins and plasma proteins in virus-infected hepatocytes. It is generally assumed that these membrane glycoproteins follow the same intracellular route as do secretory proteins (Johnson & Schlesinger, 1980; Green *et al.*, 1981; Strous *et al.*, 1983; Farquhar, 1985). After synthesis on the rough ER, envelope proteins migrate to the Golgi complex and then finally reach the plasma membrane, where the assembly of virion occurs. Incubation of the virus-infected cells in the presence of BFA yielded no detectable G protein (VSV) or E1 and E2 (sindbis virus) in the medium (Figs. 1 and 4), demonstrating that BFA strongly blocks the surface

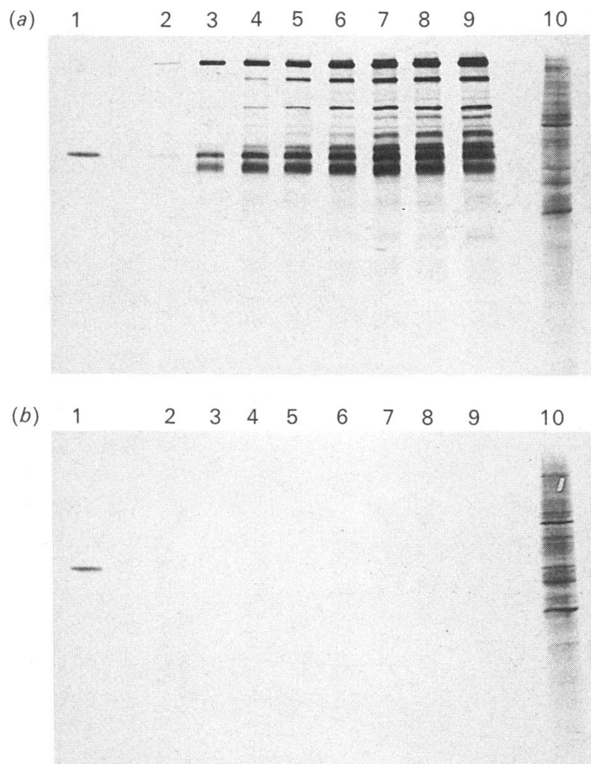

Fig. 7. Effect of BFA on the secretion of plasma proteins from Hep G2 cells

The HepG2 cells were pulse-labelled with 25 μCi of [^{35}S]methionine for 10 min and chased in the absence (*a*) or presence (*b*) of BFA (2.5 μg/ml). Aliquots of the medium were taken from the medium at the indicated times. Lanes 2–9 were chased for 20, 40, 60, 90, 120, 180, 240 and 300 min respectively. At 300 min of chase, the remaining cells were separated from the medium and subjected to preparation of cell lysate (lane 10). Each sample was analysed by SDS/PAGE followed by fluorography. Lane 1 indicates the position of [^{35}S]methionine-labelled rat serum albumin.

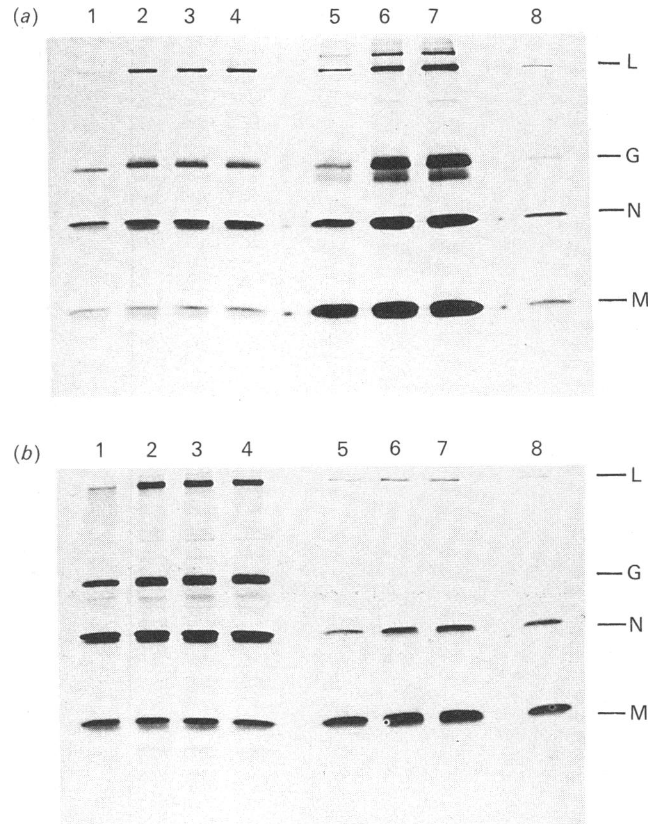

Fig. 8. Effect of BFA on the formation of VSV virions from HepG2 cells

HepG2 cells were infected with VSV at 70 pfu/cell and labelled with 25 μCi of [^{35}S]methionine for 10 min and chased in the absence (*a*) or presence (*b*) of BFA (2.5 μg/ml). At the indicated times, the cell lysates (lanes 1–4) and media (lanes 5–7) were prepared. At 180 min of chase, the medium was subjected to preparation of VSV virions (lanes 8). Each sample was analysed by SDS/PAGE followed by fluorography. Lane 1, no chase; lanes 2 and 5, 60 min chase; lanes 3 and 6, 120 min chase; lanes 4 and 7, 180 min chase. The gels were exposed for 6 h (*a*) or 12 h (*b*).

expression of the envelope proteins and their assembly into virions. Concomitant with the blockade of cell-surface expression, the acquisition of Endo H resistance of G protein and the proteolytic conversion of PE2 to E2 were inhibited (Figs. 3 and 5). Since both the acquisition of Endo H resistance of *N*-linked oligosaccharides (Kornfeld & Kornfeld, 1985) and the proteolytic conversion of PE2 are thought to occur in the Golgi complex (Hakimi & Atkinson, 1982), these results suggest that BFA blocks transport from the ER to the Golgi. These findings are in accordance with our previous studies on plasma proteins. BFA caused the accumulation of Endo H-sensitive forms of both α_1-proteinase inhibitor and haptoglobin within the cells (Misumi *et al.*, 1986). Furthermore, proalbumin and complement pro-C3, both of which are proteolytically converted to their mature forms in the Golgi (Ikehara *et al.*, 1976; Judah & Quinn, 1976; Oda *et al.*, 1986*a*), accumulated within the BFA-treated cells (Misumi *et al.*, 1986; Oda *et al.*, 1987).

We have demonstrated recently by an immunocytochemical technique that BFA causes marked accumulation of albumin in the ER, nuclear envelope and small vesicles (Fujiwara *et al.*, 1988), strongly indicating that the release of albumin from the ER is blocked by BFA. Similar results have been reported recently with regard to the intracellular transport of the T-cell antigen receptor (Lippincott-Schwartz *et al.*, 1989). The T-cell receptor subunits were found to accumulate in the ER and nuclear membrane in the BFA-treated T-cell hybridoma cells. Taken together, these results strongly indicate that BFA blocks the exocytotic pathway at the exit from the ER. Of interest is the finding that the Golgi stacks disappeared in the BFA-treated cells (Fujiwara *et al.*, 1988; Lippincott-Schwartz *et al.*, 1989). Since the disassembly of the Golgi stack occurs rapidly after the addition of BFA, it is conceivable that the primary target site of BFA is the Golgi stacks, although the mechanism whereby BFA causes the disassembly of the Golgi stacks and retards the intracellular transport at the ER and nuclear membrane remains unknown.

In contrast to the situation with baby hamster kidney cells (Takatsuki & Tamura, 1985) and murine T-cell

hybridoma cells (Lippincott-Schwartz *et al.*, 1989), the blockade by BFA is temporary and fully processed forms of plasma proteins are finally secreted from the BFA-treated hepatocytes (Misumi *et al.*, 1986; Oda *et al.*, 1987; Fujiwara *et al.*, 1988). This is also the case for membrane glycoproteins (Figs. 1 and 4). The envelope proteins of virions budded from either the control cells or the BFA-treated cells were indistinguishable from each other with regard to their sensitivities to Endo H or neuraminidase (Fig. 2), indicating that once the arrest by BFA is released, the envelope proteins move to the Golgi and undergo oligosaccharide processing and proteolytic processing normally before reaching the cell surface. The release of the arrest, which accompanies reassembly of the Golgi stacks in the cytoplasm, probably reflects the metabolic conversion of BFA to an inert form (Fujiwara *et al.*, 1988).

However, in the 4-day-cultured hepatocytes, BFA persistently blocked the intracellular transport of both envelope proteins and plasma proteins for up to 5 h (Fig. 6), and the Golgi stacks disappeared from BFA-treated cells during the entire experiment (results not shown). Thus hepatocytes appear to lose the capability to metabolize BFA on being cultured for a long time. This may be the same situation as observed in HepG2 cells, in which the appearance of both plasma proteins and G protein in the medium is blocked even after prolonged incubation (Figs. 7 and 8). Thus in the 1-day-cultured hepatocytes, disassembly and reassembly of the Golgi stacks occurs in response to BFA treatment, giving us a clue to the mechanisms of not only the dynamic process of organization of the Golgi stacks, but also the metabolism of BFA.

We thank N. Takami and M. Shibata for assistance with the culture of HepG2 cells. We are grateful to Dr. K. Mifune (Oita Medical School), Dr. A. Takatsuki (University of Tokyo) and Dr. H. Tanahashi (Kyushu University) for generously providing viruses and antibodies against viruses, brefeldin A and the HepG2 cell line respectively. We thank Dr. E. Yamada and Y. Koriyama (Fukuoka University) for preliminary electron microscopic observations of BFA-treated hepatocytes. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Farquhar, M. G. (1985) Annu. Rev. Cell Biol. **1**, 447–488

Fujiwara, T., Oda, K., Yokota, S., Takatsuki, A. & Ikehara, Y. (1988) J. Biol. Chem. **263**, 18545–18552

Green, J., Griffiths, G., Quinn, P., Louvard, D. & Warren, G. (1981) J. Mol. Biol. **152**, 663–698

Hakimi, J. & Atkinson, P. H. (1982) Biochemistry **21**, 2140–2145

Ikehara, Y., Oda, K. & Kato, K. (1976) Biochem. Biophys. Res. Commun. **72**, 319–326

Ikehara, Y., Oda, K. & Kato, K. (1977) J. Biochem. (Tokyo) 81, 1293–1297

Ikehara, Y., Miyasato, M., Ogata, S. & Oda, K. (1981) Eur. J. Biochem. **115**, 253–260

Johnson, D. C. & Schlesinger, M. J. (1980) Virology **103**, 407–424

Judah, J. D. & Quinn, P. S. (1976) Trends Biochem. Sci. **1**, 107–109

Knowles, B. B., Howe, C. D. & Aden, D. P. (1980) Science **209**, 497–499

Kornfeld, R. & Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631–644

Kuwano, M., Tabuki, T., Akiyama, S., Mifune, K., Takatsuki, A., Tamura, G. & Ikehara, Y. (1981) Somatic Cell Genet. **7**, 507–521

Laemmli, U. K. (1987) Nature (London) **227**, 680–685

Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S. & Klausner, R. D. (1989) Cell **56**, 801–813

Miki, K., Misumi, Y., Ogata, S. & Ikehara, Y. (1983) in Structure and function of Iron Storage and Transport Proteins (Urushizaki, I., Aisen, P., Listowsky, I. & Drysdale, J. W., eds.), pp. 267–270, Elsevier, Amsterdam

Misumi, Y., Misumi, Y., Miki, K., Takatsuki, A., Tamura, G. & Ikehara, Y. (1986) J. Biol. Chem. **261**, 11398–11403

Oda, K., Misumi, Y. & Ikehara, Y. (1983) Eur. J. Biochem. **135**, 209–216

Oda, K., Miki, K., Hirose, S., Takami, N., Misumi, Y. & Ikehara, Y. (1986*a*) J. Biochem. (Tokyo) **100**, 1669–1675

Oda, K., Koriyama, Y., Yamada, E. & Ikehara, Y. (1986*b*) Biochem. J. **240**, 739–745

Oda, K., Hirose, S., Takami, N., Misumi, Y., Takatsuki, A. & Ikehara, Y, (1987) FEBS Lett. **214**, 135–138

Oda, K., Ogata, S., Koriyama, Y., Yamada, E., Mifune, K. & Ikehara, Y. (1988) J. Biol. Chem. **263**, 12576–12583

Seglen, P. O. (1976) Methods Cell Biol. **13**, 29–83

Strous, G. J. A. M. & Lodish, H. F. (1980) Cell **22**, 709–717

Strous, G. J. A. M., Willemsen, R., van Kerkhof, P., Slot, J. W., Geuze, H. J. & Lodish, H. F. (1983) J. Cell Biol. **97**, 1815–1822

Takami, N., Ogata, S., Oda, K., Misumi, Y. & Ikehara, Y. (1988) J. Biol. Chem. **263**, 3016–3021

Takatsuki, A. & Tamura, G. (1985) Agric. Biol. Chem. **49**, 899–902

Tamura, G., Ando, K., Takatsuki, A. & Ariama, K. (1968) J. Antibiot. **21**, 160–161

Received 26 June 1989/22 August 1989; accepted 24 August 1989