We have investigated the effect of nordihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenase, on the intracellular protein transport and the structure of the Golgi complex. Pulse-chase experiments and immunoelectron microscopy showed that NDGA strongly inhibits the transport of newly synthesized secretory proteins to the Golgi complex resulting in their accumulation in the endoplasmic reticulum (ER). Despite their retention in the ER, oligosaccharides of secretory and ER-resident proteins were processed to endoglycosidase H-resistant forms, raising the possibility that oligosaccharide-processing enzymes are redistributed from the Golgi to the ER. Morphological observations further revealed that α-mannosidase II (a cis-medial-Golgi marker), but not TGN38 (a trans-Golgi network marker), rapidly redistributes to the ER in the presence of NDGA, resulting in the disappearance of the characteristic Golgi structure. Upon removal of the drug, the Golgi complex was reassembled into the normal structure as judged by perinuclear staining of α-mannosidase II and by restoration of the secretory functions. These effects of NDGA are quite similar to those of brefeldin A. However, unlike brefeldin A, NDGA did not cause a dissociation of β-coatomer protein, a subunit of coatamer, from the Golgi membrane. On the contrary, NDGA exerted the stabilizing effect on β-coatomer protein/membrane interaction against the dissociation caused by brefeldin A and ATP depletion. Taken together, these results indicate that NDGA is a potent agent disrupting the structure and function of the Golgi complex with a mechanism different from those known for other drugs reported so far.

Newly synthesized secretory proteins are transported from the endoplasmic reticulum (ER) to the cell surface via the Golgi complex, which is comprised of structurally and functionally distinct subcompartments including the cis-Golgi network, the Golgi stack, and the trans-Golgi network (TGN) (1). In the secretory pathway, the Golgi complex plays a key role in the sorting and modification of proteins. Resident ER proteins are sorted at the cis-Golgi network and recycled back to the ER (2), and lysosomal proteins are sorted at the TGN (3). Modifications such as oligosaccharide processing (4), proteolytic processing (5), and sulfation (6) are carried out by enzymes with specific localizations in the Golgi subcompartments. Each transport step along the secretory pathway is mediated by small vesicles that bud from a donor compartment and fuse with a target compartment membrane. Biochemical and genetic studies have identified a number of proteins involved in the vesicular transport (1, 7–9). These include coat protein complexes (COPI and COPII) and ADP-riboseylation factor (ARF) or Sars1p for vesicle budding, and N-ethylmaleimide-sensitive fusion protein, soluble N-ethylmaleimide-sensitive fusion attachment proteins, soluble N-ethylmaleimide-sensitive fusion attachment protein receptors (SNAREs) for vesicle docking and fusion. It is postulated that the specificity of vesicle targeting is generated by complexes formed between membrane proteins on the transport vesicles (v-SNAREs) and those on the target compartments (t-SNAREs) (10).

The use of drugs affecting the secretory process at distinct sites in the cell may prove valuable for more detailed studies of specific steps in secretion and may lead to the understanding of the molecular basis of the mechanisms involved in intracellular transport. One of the most useful and characterized drugs is the fungal metabolite brefeldin A (BFA). BFA strongly blocks secretion by apparently inhibiting protein transport from the ER to the Golgi (11, 12) and causes redistribution of resident Golgi proteins into the ER (12–15). The primary action of the drug is now believed to be to inhibit Golgi membrane-catalyzed GDP/GTP exchange of ARF (16, 17) that is required for assembly of ARF and COPI onto the Golgi membrane (18), resulting in lack of formation of transport vesicles. The redistribution of resident Golgi proteins to the ER in the presence of BFA is microtubule-dependent (19) and suggests the existence of a retrograde transport pathway without involving COPI vesicles. This is in contrast to the recent evidence that resident ER proteins are retrieved from the cis-Golgi network by COPI-dependent transport vesicles (2), which also raises a question as to how COPI proteins are involved in both the anterograde and retrograde transport from the cis-Golgi network (9, 20). Thus, more data are required to elucidate the details of the vesicular transport mechanism.

Nordihydroguaiaretic acid (NDGA), a polyhydroxyphenolic antioxidant, is known to exert the inhibitory effect on lipoygenase pathways of arachidonic acid metabolism (21, 22). It was recently demonstrated that NDGA also inhibits the secre-
Results of prolation from GH3 cells (23) and the intracellular transport of vesicular stomatitis virus G protein (24). The drug is likely to inhibit protein transport from the ER to the Golgi and also from the TGN to the cell surface. In the present study we examined the effect of NDGA on secretion and intracellular processing of secretory proteins, confirming that the drug indeed blocks the protein transport from the ER to the Golgi complex. In addition, we have found that NDGA rapidly disrupts the cisternal organization of the Golgi complex and causes the redistribution of resident Golgi proteins into the ER without dissociation of COPI from the membrane in contrast to the effects of BFA.

ExPERIMENTAL PROCEDURES

Materials—NDGA was obtained from Sigma. BFA was from Wako Chemicals (Osaka, Japan). Antibodies to β-COP (25) and dipeptidyl-peptidase IV (DPP IV) (26) were raised in rabbits as described. Anti-TGN38 antibody was generously provided by Dr. G. Banting (University of Bristol, U.K.) and monoclonal anti-α-mannosidase II (Man II) was from Berkeley Antibody Co. (Richmond, CA). Antibodies to albumin, third component of complement (C3), and α1-protease inhibitor (α1-PI) were purchased from Organon Teknika (Durham, NC). Fluorescein isothiocyanate-conjugated anti-rabbit IgG and tetramethylrhodamine isothiocyanate-conjugated anti-mouse IgG were from Dakopatts (Glostrup, Denmark). Peroxidase-conjugated goat anti-rabbit IgG was from Biosys (Compiegne, France). Biotinylated anti-mouse IgG and peroxidase-conjugated streptavidin were from Vector Laboratories (Burlingame, CA).

Cell Culture and Transfection—HepG2 and H35P15 cells were cultured in Eagle’s minimum essential medium containing 10% fetal calf serum. NRK and COS-1 cells were cultured in Dulbecco’s modified Eagle’s minimum essential medium with 10% fetal calf serum. Transfection experiments were carried out as described (27, 28), for which a plasmid encoding a mutant DPP IV (mDPP IV) with substitution of Gly633 was transfected into COS-1 cells with the Lipofectin Reagent. The transfected cells were cultured for 2 days before use.

Labeling of Cells and Immunoprecipitation—Cells in 60-mm plastic dishes were pulse-labeled for 10 or 20 min with [35S]methionine (50 μCi/dish) in methionine-free Eagle’s minimum essential medium and chased in the complete Eagle’s minimum essential medium in the presence or absence of NDGA (30 μm) or BFA (5 μg/ml) (11). Cycloheximide (50 μg/ml) was added to the chase medium. At the indicated times, the cells were separated from the medium, and cell lysates were prepared (29). [35S]Labeled proteins of cell lysates and medium were immunoprecipitated with the indicated antibodies, extensively washed, and subjected to enzyme digestion when indicated (29).

Enzyme Digestion—Samples were incubated with endo H (0.2 unit/ml) in 50 mM acetate buffer (pH 5.0) for 20 h. Polyacrylamide Gel Electrophoresis—Proalbumin (pl 6.0) and albumin (pl 5.8) were separated by electrofocusing on 5% polyacrylamide gels (pH range from 5 to 8) for albumin or by SDS-PAGE (7.5% gels) for C3, followed by fluorography. Lane 1, no chase; lane 2, chase 30 min; lane 3, 1 h; lane 4, 3 h; lane 5 and 6, 3 h. PA and SA (A-C) indicate proalbumin and serum-type albumin, respectively. P, α, and β (D-F) denote the proform and the α and β subunits, respectively, of C3.

Immunoelectron Microscopy—HepG2 cells (for albumin) and H35P15 cells (for Man II) were fixed for 2 h with the paraformaldehyde/lysine/periodate fixative (32) and permeabilized with 0.05% saponin (12). The HepG2 cells were incubated for 2 h with anti-albumin antibodies, followed by incubation for 1 h with peroxidase-conjugated goat anti-rabbit IgG. The H35P15 cells were incubated with a-Mann II antibodies and then with biotinylated anti-mouse IgG and peroxidase-conjugated streptavidin for 1 h each. After the peroxidase reaction, the cells were processed for electron microscopy as described previously (12).

RESULTS

Inhibition of Secretion and Accumulation of Secretory Proteins in the ER—HepG2 cells synthesize various plasma proteins including albumin, the C3, and α1-PI. In addition, albumin and C3 are initially synthesized as proforms, which are converted into mature forms at the TGN (6, 11). In control cells, newly synthesized albumin was rapidly converted to the mature form and secreted into the medium (Fig. 1A). The presence...
of a small amount of proalbumin in the medium may be due to
the relatively low activity of the converting enzyme in HepG2
cells as compared with that of hepatocytes (33). In the presence
of 30 μM NDGA, however, the processing and secretion of
albumin were strongly blocked (Fig. 1B). Upon removal of
the drug, the labeled albumin was normally processed and secreted
into the medium (Fig. 1C), indicating the reversibility of the
drug effect. Since protein synthesis was found to be signifi-
cantly inhibited at higher concentrations of the drug, we used
NDGA at 30 μM throughout the following experiments. Esse-
tially the same results were obtained for the processing and
secretion of pro-C3. In the control cells, pro-C3 synthesized as
a single polypeptide of 180 kDa and was processed into the α
(115 kDa) and β (65 kDa) subunits, which were secreted into
the medium, although a considerable amount of the proform
was also secreted (Fig. 1D). The processing and secretion of
pro-C3 were completely blocked by NDGA (Fig. 1E), and this
inhibitory effect was reversible (Fig. 1F). These results suggest
that NDGA blocks secretion by inhibiting the intracellular
transport of proteins before the site where the proforms of
albumin and C3 are processed into the mature forms.

Immunofluorescence microscopy showed that albumin was
markedly concentrated in the juxtanuclear region in the con-
trol cells (Fig. 2A), whereas the protein was distributed in
reticular network structures extending throughout the cyto-
plasm in the NDGA-treated cells (Fig. 2B). The alteration in
localization of albumin was examined in more detail by immu-
noelectronic microscopy. In the control cells, albumin was most
heavily stained in the Golgi complex, although also detectable
in the ER and nuclear envelope (Fig. 2C). In the cells treated
with the drug for 2 h (Fig. 2D), however, we could not identify
the characteristic Golgi stack structure where albumin had
been concentrated. The immunoreaction product was detected
exclusively in the ER and nuclear envelope. Although some
mitochondria appeared to be slightly swollen, structures of
other organelles were not significantly changed by treatment
with the drug. These results suggest that NDGA primarily
blocks the protein transport from the ER, resulting in the
accumulation of secretory proteins in the ER.

**Effect of NDGA on Oligosaccharide Processing**—If NDGA
blocks the transport of secretory glycoproteins out of the ER, it is
expected that their oligosaccharides will remain as high
mannose type sensitive to endo H digestion. To test this, we
examined the biosynthesis and processing of the secretory glyco-
protein α₁-PI in HepG2 cells. In control cells, α₁-PI was
initially synthesized as a 51-kDa form and subsequently con-
verted to a 56-kDa form, which was secreted into the medium
(Fig. 4A, lanes 1–6). The 51-kDa form was sensitive to endo H
digestion, whereas the 56-kDa form was resistant to endo H
(Fig. 4A, lanes 7–11). In addition, the latter form decreased the
molecular size to 51 kDa when treated with neuraminidase
(Fig. 4A, lane 12). In contrast, in NDGA-treated cells, the newly
synthesized 51-kDa form was neither converted into the ma-
ture 56-kDa form nor secreted into the medium (Fig. 4B, lanes
1–6). The 51-kDa form in the treated cells, however, showed
Effects of NDGA on the Golgi Complex

**FIG. 4.** Effect of NDGA on oligosaccharide processing of α1-PI. HepG2 cells were pulse-labeled and chased in the absence (A) or presence (B) of 30 μM NDGA as in Fig. 1. Immunoprecipitates of α1-PI were prepared from cell lysates (lanes 1–5, 7–11, and 10 and media (lanes 6 and 12). The samples before (lanes 1–6) and after endo H (lanes 7–11) or neuraminidase (lanes 12 and 13) digestion were analyzed by SDS-PAGE (10% gels), followed by fluorography. Lanes 1 and 7, no chase; lanes 2 and 8, chase 30 min; lanes 3 and 9, 1 h; lanes 4 and 10, 2 h; lanes 5, 6, 11, 12, and 13, 3 h. Molecular masses (kDa) are indicated at the left. In C and D, cells were incubated at 37 °C for 3 h in the absence (C) or presence (D) of NDGA. The cells were fixed, permeabilized, and stained with goat anti-α1-PI in combination with tetramethylrhodamine isothiocyanate-conjugated anti-goat IgG. Bar = 5 μm.

 Different responses to endo H depending on the chase times. Upon digestion with endo H, the protein was initially converted to the completely sensitive 41-kDa form, then to partially sensitive forms, and finally to a single resistant 51-kDa form (Fig. 4B). The final 51-kDa form obtained at 3-h chase was insensitive to neuraminidase treatment (Fig. 4B). The samples before (lanes 1–5, 7–11, and 10 and media (lanes 6 and 12). The samples before (lanes 1–6) and after endo H (lanes 7–11) or neuraminidase (lanes 12 and 13) digestion were analyzed by SDS-PAGE (10% gels), followed by fluorography. Lanes 1 and 7, no chase; lanes 2 and 8, chase 30 min; lanes 3 and 9, 1 h; lanes 4 and 10, 2 h; lanes 5, 6, 11, 12, and 13, 3 h. Molecular masses (kDa) are indicated at the left. In C and D, cells were incubated at 37 °C for 3 h in the absence (C) or presence (D) of NDGA. The cells were fixed, permeabilized, and stained with goat anti-α1-PI in combination with tetramethylrhodamine isothiocyanate-conjugated anti-goat IgG. Bar = 5 μm.

The finding that the oligosaccharides of α1-PI retained in the ER are converted to the complex type might suggest that the processing enzymes localized in the Golgi complex are redistributed into the ER in the presence of NDGA. This possibility was confirmed by another set of experiments. DPP IV is an ectoenzyme with N-linked oligosaccharide chains (28). We found that a mutant with substitution of Gly633 → Arg (mDPP IV) is retained in the ER and degraded there without being transported to the Golgi complex (27, 28). When mDPP IV was expressed in COS-1 cells by transfection, the protein was retained in the ER (Fig. 5D) and remained completely sensitive to endo H even after 4-h chase in control cells (Fig. 5A). In the presence of NDGA, however, the protein acquired the resistance to endo H after the chase (Fig. 5D) as observed in BFA-treated cells (Fig. 5B). There was no significant difference in distribution of mDPP IV between the control and treated cells, demonstrating its retention in the ER (Fig. 5, D and E). These results suggest that NDGA causes the redistribution of the Golgi-resident processing enzymes to the ER as effectively as BFA.

**Redistribution of Resident Golgi Proteins to the ER—Man II has been used as a cis medial–Golgi marker (4, 14), whereas TGN38 is a membrane protein localized in the TGN (34). Changes in localizations of Man II and TGN38 were examined in NRK cells as a function of time after exposure to NDGA. Man II localized in perinuclear regions in control cells (Fig. 6A) still remained in the same regions with slightly fragmented and dispersed structures in cells treated with the drug for 5 min (Fig. 6C). After 30 min of incubation, Man II was distributed on punctate or reticular structures over the cytoplasm, and no significant perinuclear staining was detected (Fig. 6E). A more intense staining pattern for Man II on the reticular structures was observed in cells exposed for 2 h (Fig. 6D). This effect of NDGA was found to be reversible. The reticular distribution of Man II in cells treated with the drug for 1 h (Fig. 7A) completely reversed to a perinuclear localization after 2 h of incubation without the drug (Fig. 7D) through intermediate stepwise changes (Fig. 7B and C) reciprocal to those observed in the presence of the drug (Fig. 6, C and E). In contrast to Man II, the localization of TGN38 was not so significantly changed by the drug (Fig. 6, B, D, F, and H), indicating that NDGA has little effect, if any, on the TGN structure.

The change in localization of Man II was examined by immunoelectron microscopy, for which rat hepatoma H35P15 cells were used since Man II was more intensely stained in these cells than in the NRK cells. Immunofluorescence micros-
and treated with NDGA for 10 min, Golgi complex in control cells (Fig. 9, panel A). Bar = 10 μm. Fixed, permeabilized, and incubated with antibodies to Man II (Fig. 8, data not shown) as observed for Man II (Fig. 8B). Localization to the ER and nuclear envelope in the treated cells (Fig. 8C). The cells were incubated with BFA (Fig. 8D). In contrast, when cells were first treated with BFA for 2 min followed by incubation together with NDGA for 5 min, β-COP was completely dissociated from the Golgi (Fig. 10C), whereas Man II was still associated with the Golgi (Fig. 10D). Depletion of cytosolic ATP is also known to cause the dissociation of the coatomer from the Golgi membrane (35). When cells were treated for 10 min with 2-deoxyglucose and sodium azide, β-COP was completely dissociated into the cytoplasm while the Golgi structure with Man II remained unaffected (Fig. 10, G and H). Once COPI was dissociated from the membrane by prior ATP depletion treatment, however, the addition of NDGA exerted no effect (Fig. 10, I and J). Thus, it is likely that NDGA does not primarily dissociate the coatomer from the Golgi membrane but rather exerts the stabilizing effect, even against the dissociation caused by BFA and by ATP depletion.

**DISCUSSION**

In this study we have demonstrated that NDGA strongly blocks the secretion and causes the redistribution of resident
Golgi proteins into the ER. It is important to use about 30 μm NDGA because higher concentrations such as 100 μm Golgi proteins into the ER. It is important to use about 30 μm ATP level to 40% of normal has little effect, if any, on the recovery of cellular ATP levels as was previously shown by electron microscopy that the intracellular distribution of Golgi is only slightly blocked at 30% of the normal ATP level, although strongly blocked below 10% of the normal level (44).

In the presence of NDGA, the newly synthesized glycoproteins, α3-PI and the mutant DPP IV, despite accumulating in the ER, acquired endo H resistance, indicating that their oligosaccharides are modified by processing enzymes localized in the cis/medial Golgi (4). This is in contrast to a previous finding that a glycoprotein accumulated in the ER by NDGA was sensitive to endo H (24). The discrepancy may be explained by the difference of incubation times with the drug; for 30 min (24) and up to 3 h (this study). On the other hand, no sialylation of the oligosaccharides nor proteolytic processing of the proforms, which are carried out by the TGN-localized enzymes (5, 45), took place. These results indicate that the early (the cis/medial- and possibly trans-) Golgi enzymes are redistributed into the ER, whereas the TGN enzymes are not. Supporting these biochemical data, immunocytochemical analysis demonstrated that the early Golgi marker Man II was rapidly redistributed into the ER, whereas the TGN marker TGN38 was not. The observations confirm the proposal that the TGN is a compartment distinct from the cis/medial/trans-Golgi cisternae (46). These effects of NDGA are quite similar to those of BFA; proteins resident in the early Golgi subcompartments (cis-, medial-, and trans-cisternae) are rapidly redistributed into the ER in the presence of BFA (47), resulting in disappearance of the characteristic Golgi stack structure (12–14). The fate of the TGN after treatment with NDGA, however, is different from that in the BFA-treated cells. Long tubular processes emanating from the TGN in the BFA-treated cells (48, 49) could not be observed in the NDGA-treated cells. Those tubular processes caused by BFA are supposed to reflect the mixing of the TGN with early endosomes. Therefore, it is unlikely that NDGA causes the fusion of TGN with endosomes. It was also confirmed by electron microscopy that the intracellular distribution of horseradish peroxidase, a marker for fluid phase endocytosis, was not significantly changed after treatment with NDGA (data not shown).

To elucidate the mechanism by which NDGA inhibits the protein transport from the ER to the Golgi, one would focus on the formation of transport vesicles. Two sets of non-clathrin coats, COPI and COPII, drive the formation of vesicles that mediate the transport between the ER and the Golgi (9, 50). The blockade of transport by BFA could be explained by no assembly of ARF and COPI onto the membranes, which is due to the inhibition of Golgi membrane-catalyzed exchange of GDP/GTP bound to ARF (16, 17). Contrary to our expectation, and unlike BFA, NDGA did not cause a dissociation of β-COP from the Golgi membrane. Instead, β-COP was stably associated with the membrane during incubation with NDGA. In addition, NDGA was found to stabilize the binding of β-COP to the membrane, preventing its dissociation from the membrane caused by BFA or ATP depletion. It is thought that the active vesicular transport is carried out by dynamic cycling of ARF and COPI between the Golgi membrane and the cytosol (10). NDGA probably interrupts this dynamic cycling by locking the COPI components onto the membrane thereby preventing the functional formation and/or fusion of transport vesicles with the target membrane in the secretory pathway.

Recent studies revealed that COPI coatamer also plays an essential role in retrograde transport of resident ER proteins with the COOH-terminal KXXX motif from the Golgi to the ER (2), suggesting that COPI is primarily involved in retrograde transport rather than in anterograde transport (2, 20, 51). The apparent blockade of anterograde transport by BFA (11–15) or by mutation of COPI subunits (52) may be explained by no retrieval of cargo receptors to the ER. The coated transport vesicles...
vesicles must be uncoated before fusing with the target membrane (53). The blocking of the uncoating step for example with GTP\textsubscript{S} causes an accumulation of the coated vesicles that do not fuse with the target membrane, resulting in no transport of cargo (1, 53, 54). In the presence of NDGA, COPI or at least \(\beta\)-COP was not dissociated from the Golgi membranes. The Golgi membranes, however, was initially converted into aggregate-like or fragmented structures and finally fused with the ER, suggesting that the Golgi membranes without detachment of COPI are able to fuse with the ER membrane in the presence of NDGA. In the presence of BFA that prevents the association of COPI with the membranes, a direct membrane fusion also occurs between the ER and the Golgi in a microtubule-dependent manner (19). These observations suggest that there would be another pathway for redistribution of the resident Golgi proteins into the ER, independent from the COPI-dependent pathway for retrieval of resident ER proteins (2). The pathway would become evident only when vesicular transport is blocked, regardless of the blocking sites at the formation or fusion of transport vesicles.

Rab6, a ubiquitous Rab associated with the Golgi membranes, was found to function in intra-Golgi transport (55). Most recently it has been reported that overexpression of a GTP-bound mutant of Rab6 (Rab6 Q72L) inhibits the anterograde transport (55) and induces the redistribution of Golgi proteins into the ER (56). It was also pointed out that the typical necklaces and tubular structures emanating from the Golgi of BFA-treated cells (19) were never observed in cells overexpressing Rab6 Q72L even at early times of overexpression (56). Similarly our immunocytochemical stainings failed to identify such structures in the NDGA-treated cells. Although the NDGA treatment and overexpression of Rab6 Q72L cause almost the same phenotypic effects as does the addition of BFA, it is unlikely that these effects arise via analogous mechanisms. Despite recent remarkable advances, details of the mechanisms for anterograde and retrograde transport in the secretory pathway still remain to be elucidated. NDGA could provide a useful tool for investigating vesicular transport and its role in the formation and maintenance of the Golgi complex.

During revision of this manuscript, Yamaguchi et al. (57) also reported that NDGA caused disassembly of the Golgi complex and suggested the possible involvement of heterotrimeric GTP-binding proteins in the organization of the Golgi complex. 

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Fig. 9. Effect of NDGA on the distribution of \(\beta\)-COP. In panel A, NRK cells were treated at 37 °C with 30 \(\mu\)M NDGA for 0 (a and b), 10 (c and d), or 30 (e and f) min. The cells were fixed, permeabilized, and double-stained for \(\beta\)-COP (a, c, e, and I) and Man II (b, d, f, and j) Bar = 10 \(\mu\)m.

In panel B, cells were incubated with 30 \(\mu\)M NDGA (a and b) or 5 \(\mu\)g/ml BFA (c and d) at 37 °C for 30 min. The cells were immediately fixed (a and c) or fixed after being perforated and washed (b and d), and stained for \(\beta\)-COP by immunofluorescence. Bar = 10 \(\mu\)m.

In panel C, membrane (lanes 1, 3, 5, and 7) and cytosol (lanes 2, 4, 6, and 8) fractions were prepared from NRK cells that had been incubated with 30 \(\mu\)M NDGA for 0 (lanes 1 and 2), 30 (lanes 3 and 4), or 60 (lanes 5 and 6) min or with 5 \(\mu\)g/ml BFA for 30 min (lanes 7 and 8). The samples (25 \(\mu\)g of protein/lane) were analyzed by SDS-PAGE (8% gel) and immunoblotting with anti-\(\beta\)-COP IgG.

Fig. 10. Effect of NDGA on the dissociation of \(\beta\)-COP from the Golgi caused by BFA or by ATP depletion. In A and B, NRK cells were treated at 37 °C first with NDGA for 5 min and then together with BFA (2 \(\mu\)g/ml) for 2 min. In C and D, cells were incubated with BFA for 2 min and then together with NDGA for 5 min. In E and F, cells were incubated with 50 \(\mu\)M 2-deoxyglucose and 0.05% sodium azide for 10 min. In G and H, cells were treated first with NDGA for 5 min and then together with 2-deoxyglucose and sodium azide for 10 min. In I and J, cells were treated with 2-deoxyglucose and sodium azide for 10 min and then together with NDGA for 5 min. The cells were fixed, permeabilized, and double-stained for \(\beta\)-COP (A, C, E, G, and I) and Man II (B, D, F, H, and J). Bar = 10 \(\mu\)m.
