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Interleaflet clear space is reduced in the membrane of COP I and COP II-coated buds/vesicles

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ABSTRACT **Intracellular transfers between membrane-bound compartments occur through vesicles that bud from a donor compartment to fuse subsequently with an acceptor membrane. We report that the membrane that delimits COP I or COP II-coated buds/vesicles from the endoplasmic reticulum and the Golgi complex has a thinner interleaflet clear space as compared with the surrounding, noncoated parental membrane. This change is compatible with a compositional change of the membrane bilayer during the budding process.**

The fundamental structure of biological membranes is a phospholipid bilayer containing a variable proportion of amphipathic, transmembrane, or integral proteins (1, 2). On conventional thin sections for electron microscopy after osmium tetroxide fixation and lead staining, all membranes display the characteristic "trilaminar" appearance, i.e., a clear line sandwiched between two dense layers. Current models of membrane organization imply that the dense layers (or leaflets) correspond to the hydrophilic portion of phospholipid head groups and peripheral and integral proteins, whereas the clear middle space is the site where the fatty acyl chains are packed (1, 2). The thin section appearance of membranes is deceptively uniform in view of their known biochemical heterogeneity, except for the fact that the overall thickness of the trilaminar structure varies from about 6–10 nm (3). For example, the membranes delimiting the endoplasmic reticulum and cis-Golgi cisternae are thin (about 6–7 nm), whereas those delimiting the trans-Golgi cisternae and the plasma membrane are thick (about 8–9 nm) (3). Here, we report a decrease in the interleaflet clear space at sites of budding of COP-coated transfer vesicles, as compared with the noncoated parental membrane.

MATERIALS AND METHODS

COP I or COP II membrane preparations were fixed with glutaraldehyde, post-fixed in osmium tetroxide, stained with tannic acid and embedded in Epon as described (4). Pictures of thin sections of these preparations were printed on 26 × 23 cm Kodak diapositive films; coated and noncoated membrane profiles suitably oriented in cross section were scanned with a double beam recording microdensitometer Joyce–Loebl MK III C. The slit-shaped beam was moved across the membrane bilayer perpendicularly to the dense leaflets. The density pattern was drawn to an enlarged scale, by the pen fitted to the microdensitometer, on millimetric paper. The interleaflet distance in coated and noncoated membrane profiles was measured directly on the millimetric paper. COP I and COP II membrane preparations fixed in glutaraldehyde alone and directly sectioned with an ultracryomicrotome (5) for the immunolabeling of the coat components (6–10) were also qualitatively assessed.

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RESULTS

The morphology, molecular composition, and assembly mode of COP I or COP II-coated buds, which result in coated transfer vesicles, have been extensively described (4, 6–10). At a suitable magnification showing the trilaminar structure of the membrane on thin sections of Epon-embedded preparations, we observed that the membrane delimiting coated buds/vesicles had a reduced interleaflet clear space, as compared with the neighboring noncoated membrane. The decrease was observed on both COP I-coated buds/vesicles from Golgi cisternae (Fig. 1 *A* and *B*), or COP II-coated buds/vesicles from yeast endoplasmic reticulum fractions (Fig. 1 *C* and *D*). The thinning was detectable not only in fixed, dehydrated, and embedded membranes (Fig. 1), but also in membranes that had been fixed in glutaraldehyde alone and directly cryosectioned without dehydration or embedding (Fig. 2). We quantified the size of the clear space on Epon-embedded samples by densitometry. Each densitometric trace across a perpendicularly cut bilayer comprised two high-density peaks, corresponding to the dense outer and inner leaflets, separated by a deep valley of low density representing the interleaflet clear space (Fig. 3). We measured the width of the interpeak valley at fixed distances (+2, +4, +6 cm) from the bottom of the densitometric trace (Fig. 3). In traces with shallow valleys, i.e., delimited by short peaks, the inner slope of the valley was extrapolated to enable the +4 or +6 measures (Fig. 3). In pilot trials, the mean value obtained by pooling the measures at +2, +4, and +6 cm from the bottom of the densitometric trace was nearly identical to the single value taken at +4 cm. We thus completed our measures of the interpeak valley on coated and noncoated regions on the basis of the +4 cm value alone (Table 1). The reduction of the interleaflet clear space was found statistically significant for both COP I and COP II-coated buds/vesicles. The means had a rather large dispersion reflecting the fact that not all coated profiles in either COP I or COP II preparations showed the same degree of thinning. Converted into nanometers, the centimetric data indicated a clear interleaflet space ranging from 2.8 nm in coated bilayers to 3.3 nm in noncoated membranes. These values fully fit the classical trilaminar membrane model (11). The thinning of the membrane interleaflet space on COP-coated buds/vesicles was also observed on Epon sections of intact cells (not shown).

DISCUSSION

COP II vesicles mediate anterograde endoplasmic reticulum to Golgi transport (9, 10); COP I vesicles are likely to be responsible for both anterograde and retrograde transport steps in the endoplasmic reticulum–Golgi region and within the Golgi complex (10, 12–16). We ruled out that the thinner interleaflet space seen on the membrane of COP-coated buds and vesicles was an artifact of looking at spherical structures (i.e., buds and vesicles) similar in diameter to the thickness of the section. Indeed, the uncoated membrane at the terminal rim of a Golgi cisterna has a radius of curvature comparable to that of a coated bud/vesicle. Yet, the interleaflet space of

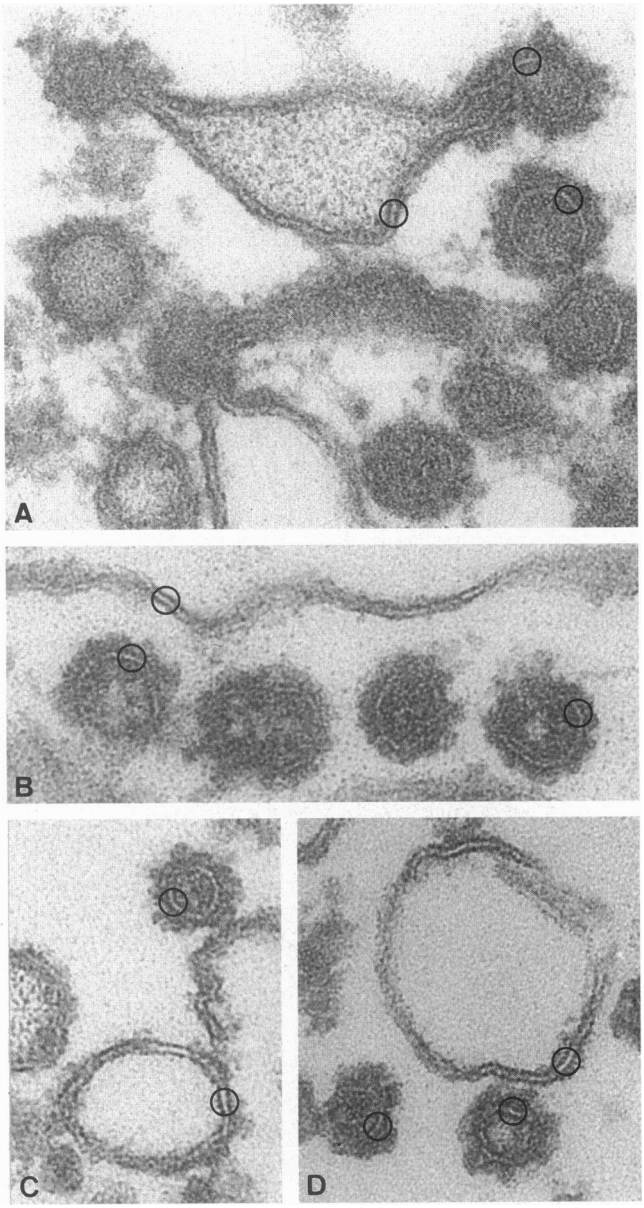

FIG. 1. (*A* and *B*) Thin sections of isolated Golgi cisternae with COP I-coated buds and associated coated vesicles. The trilaminar membrane structure is distinctly visible on segments of the noncoated membranes and of the coated bud/vesicle profiles. When these are close to one another, enabling a visual comparison, the clear interleaflet space appears distinctly reduced in coated regions as compared with noncoated regions. Golgi fractions primed to bud COP I vesicles in the presence of GTPγS (4). (*C* and *D*) Thin sections of semi-purified yeast endoplasmic reticulum fractions primed to bud COP II vesicles (9). As for COP I-coated membranes, the interleaflet clear space on COP II-coated vesicles appears thinner than on the noncoated parental membrane. Circles were drawn on coated and noncoated membrane regions to show examples of bilayers that were submitted to densitometric tracing to quantify the interleaflet clear space (see Fig. 3). (*A*, ×186,000; *B*, ×162,000; *C*, ×180,000; *D*, ×174,000.)

the curved membrane does not appear thinner than the interleaflet space in the flat part of the cisterna.

Another potential artifact that would reduce the interleaflet clear space (but not the overall membrane thickness) is that the coat proteins per se, or some transmembrane anchors, would reinforce the staining and apparent extension of the dense leaflets, and decrease by the same amount the apparent width of the interleaflet space. This is a difficult alternative to solve with the present data, because the dense content of the

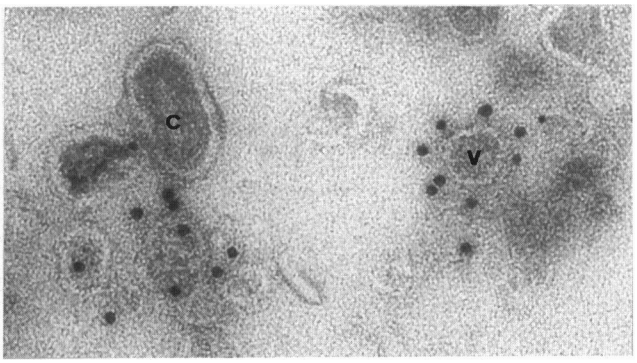

FIG. 2. Thin cryosection made without dehydration or embedding and immunolabeled for a component of the COP I coat (Sec 21p). Although the typical trilaminar pattern of membranes is less sharp on cryosections than on Epon-embedded sections (see Fig. 1), the clear middle space is distinctively thinner on the membrane delimiting a Sec 21p-labeled coated vesicle (v), than on the membrane limiting a nonlabeled cisternal element (c). Protein A-gold labeling (4–9); size of gold particles = 10 nm. (×160,000.)

bud/vesicle, as well as the dense coat make it most often impossible to tell the true limits of the bilayer itself. There are good reasons, however, to believe that the measured thinning of the interleaflet space reflects a local compositional change of the membrane. Recent biochemical evidence indicates that the process of vesicular budding from intracellular compartments is associated with the activation of enzymes involved in phospholipid metabolism (17, 18). For example, ADP-

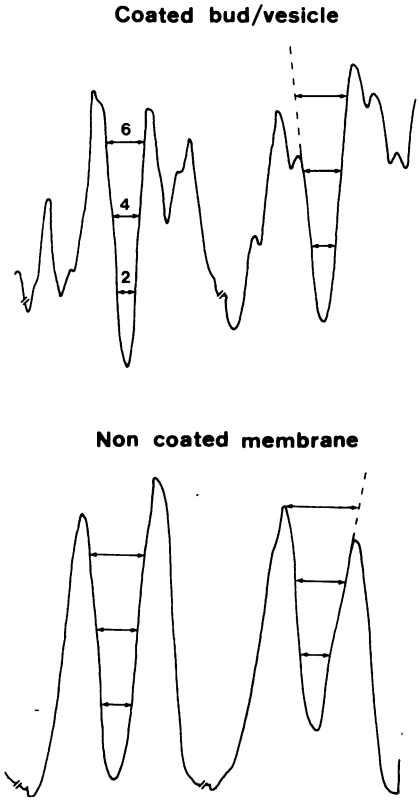

FIG. 3. Examples of densitometric traces obtained by moving the beam across the membrane of two different coated buds/vesicles (*Top*) and two different noncoated membranes (*Bottom*). The horizontal arrows indicate the level at which the measurements of width were made on each trace (+2, +4, and +6 cm from the bottom of the valley). On the right hand traces, the dotted line indicates the extrapolation of the slope of the valley to enable the measurement of width at +6 cm. The quantitation of all measures at +4 cm is shown in Table 1.

Table 1. Width of the membrane interleaflet clear space on coated buds/vesicles and uncoated cisternal membrane measured by densitometry

	n	Width, cm	Width, nm	*P*
COP I				
Coated	54	1.25 ± 0.05	2.8 ± 0.11	<0.05
Noncoated	54	1.48 ± 0.06	3.3 ± 0.13	<0.05
COP II				
Coated	63	1.33 ± 0.04	3.0 ± 0.09	<0.05
Noncoated	54	1.47 ± 0.04	3.3 ± 0.09	<0.05

Width in centimeters/nanometers at +4 from the bottom of the densitometric trace (see Fig. 3).

ribosylation factor (ARF), which is required for the budding of COP I-coated vesicles (15, 16), activates phospholipase D (19), an enzyme that cleaves the polar head groups of phospholipids, thereby producing phosphatidic acid. This could result in the formation of membrane microdomains with a distinctive lipid composition. The segregation of integral proteins with short transmembrane domains (20) might also account for the reduction of the interleaflet clear space. The present work thus calls the attention of the investigators active in this field to a potentially interesting and important process that remains to be substantiated by future work.

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