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PDMP blocks the BFA-induced ADP-ribosylation of BARS-50 in isolated Golgi membranes

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Abstract We reported that an inhibitor of sphingolipid biosynthesis, D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), blocks brefeldin A (BFA)-induced retrograde membrane transport from the Golgi complex to the endoplasmic reticulum (ER) (Kok et al., 1998, J. Cell Biol. 142, 25–38). We now show that PDMP partially blocks the BFA-induced ADP-ribosylation of the cytosolic protein BARS-50. Moreover, PDMP does not interfere with the BFA-induced inhibition of the binding of ADP-ribosylation factor (ARF) and the coatomer component β-coat protein to Golgi membranes. These results are consistent with a role of ADP-ribosylation in the action of BFA and with the involvement of BARS-50 in the regulation of membrane trafficking.

Key words: Golgi complex; ADP-ribosylation; D,L-Threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; Brefeldin A; Membrane transport

1. Introduction

The Golgi complex (GC) regulates post-translational and sorting events of the biosecretory pathway. However, the mechanism(s) of transport from the endoplasmic reticulum (ER) to the GC, between the Golgi cisternae and from Golgi to plasma membrane or intracellular destinations are controversial [2–4]. Despite the continual discovery of molecules involved in membrane transport, the molecular mechanisms remain rather obscure. The use of the fungal macrocyclic lactone brefeldin A (BFA) revealed a retrograde membrane flow pathway from the GC to the ER [5–8], which under normal conditions is in balance with the anterograde membrane flow that ensures the transfer of lipids and proteins from the ER to the GC. BFA causes a rapid and explosive disruption of the GC, involving the formation of long tubules that redistribute most of the Golgi-resident membrane components into the ER [9]. The Golgi disorganization produced by BFA is initiated by the release of coat proteins (COPs) from Golgi membranes [9,10] and the small GTP binding protein ADP-ribosylation factor (ARF) [11,12]. This sensitivity to BFA is a test of the association of these components with Golgi membranes in vivo. Although the molecular mechanisms of the BFA effects are in part due to the inhibition of GDP-GTP exchange of ARF [11,12], it is also known that this drug induces specific mono-ADP-ribosylation of two cytosolic proteins of 38 and 50 kDa [13]. The former is the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the later, named BARS-50, binds GTP and is regulated by heterotrimeric G proteins [14]. Recently, it has been shown that (i) inhibitors of the BFA-dependent ADP-ribosylation prevent the Golgi disassembly induced by BFA [15], (ii) NAD^+ (a substrate of the ADP-ribosylation reaction) is required for the BFA-induced disassembly of the GC, (iii) pre-ADP-ribosylated cytosol mimics the effect of NAD^+ and (iv) the further addition of native BARS-50 abolishes the ability of pre-ADP-ribosylated cytosol to sustain the effect of BFA (in the absence of NAD^+) [16]. Thus, there is correlative evidence for a role of ADP-ribosylation in BFA-induced Golgi disassembly, while BARS-50 appears to be an essential component involved in the membrane flow in this organelle [16–18].

It is possible to antagonize the effect of BFA without preventing the β-COP detachment from the GC [1,19]. This observation suggests that this early event is necessary but not sufficient to induce the complete disassembly of the GC by BFA. Of particular interest is the inhibitor of sphingolipid biosynthesis D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) [20]. Apart from its effect on glucosylceramide biosynthesis, PDMP inhibits anterograde membrane transport through the GC and from the GC to plasma membrane, probably by increasing the intracellular ceramide levels [21,22]. More recently, PDMP has been shown to block the BFA-induced retrograde membrane flow from Golgi to ER through local changes in calcium homeostasis [1].

We now provide evidence that the inhibitory effect of PDMP on the BFA-induced retrograde membrane flow involves inhibition of BFA-dependent ADP-ribosylation of the cytosolic protein BARS-50.

2. Material and methods

2.1. Antibodies and other reagents

The anti-ARF monoclonal antibody (mAb 1D9) was a gift from R.A. Kahn (National Cancer Institute, NIH, Bethesda, MD, USA), the anti-β-COP monoclonal antibody, NAD^+ and BFA were obtained from Sigma (St. Louis, MO, USA). PDMP and lyso-PDMP were purchased from Matreya (Pleasant Gap, PA, USA).

2.2. ARF and β-COP binding assay

Measurement of ARF and β-COP binding to Golgi membranes was performed as described [23].

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2.3. ADP-ribosylation assay

The ADP-ribosylation assay using Golgi membranes and rat brain cytosol was performed as previously described [16]. The maximal ADP-ribosylation of BARS-50 (>90%) was obtained using 200 μM NAD^+ and 100 μM BFA for 60 min at 37°C. The radioactivity bound to BARS-50 and GAPDH was measured by electronic autoradiography using an Instant Imager (Packard).

3. Results and discussion

3.1. PDMP inhibits the BFA-induced ADP-ribosylation of BARS-50

Fig. 1 shows the dose-response effect of PDMP on the BFA-induced ADP-ribosylation of the cytosolic protein BARS-50. The specific ADP-ribosylation induced by BFA was partially inhibited by PDMP. The inhibitory effect of PDMP was specific for PDMP, as lyso-PDMP did not block the BFA-induced ADP-ribosylation of BARS-50. This was consistent with the absence of an effect of lyso-PDMP on BFA-induced GC to ER membrane flow in intact cells [1]. The PDMP inhibition of BFA-induced ADP-ribosylation was lower than that of other inhibitors such as dicumarol [15]. However, both agents produce similar morphological effects by inhibiting the fusion of the GC with the ER in NRK cells [1,16]. Importantly, the blocking effect of the BFA-induced relocalization of Golgi lipids and proteins to the ER is absolute when PDMP was administered 15 min before BFA. However, when cells are co-incubated with PDMP and BFA, the GC stained with NBD-C_6-Cer presents long tubules [1]. Here, we have observed that PDMP inhibited BFA-induced ADP-ribosylation of BARS-50 more strongly when it was added 15 min before BFA than when membranes were co-incubated with PDMP and BFA (Fig. 2). Again, lyso-PDMP had no significant effects.

3.2. PDMP does not alter the effect of BFA on ARF and β-COP binding to isolated Golgi membranes

One of the earliest effects of BFA is the redistribution of ARF and the coatomer component β-COP from the GC into the cytosol [10]. This is followed by the formation of tubules, emerging from the GC, that instantly fuse with the ER. Consequently, the resident Golgi membrane components redistribute into the ER [5,6]. Importantly, PDMP does not impair the

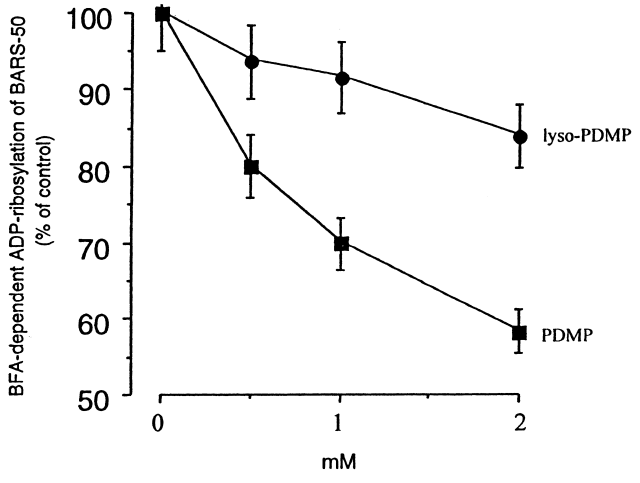

Fig. 1. PDMP but not lyso-PDMP inhibited the BFA-induced ADP-ribosylation of BARS-50 in a dose-dependent manner.

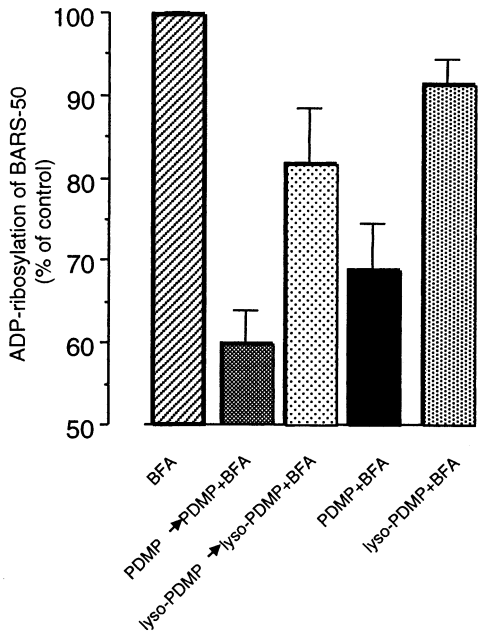

Fig. 2. The PDMP-induced inhibition of the ADP-ribosylation of BARS-50 was more marked when Golgi membranes are first pre-incubated with PDMP than when co-incubated with BFA+PDMP.

β-COP redistribution induced by BFA but completely preserved the GC morphology and prevented the redistribution of Golgi enzymes (such as mannosidase II) or lipids (NBD-C_6-Cer) into the ER [1]. Since the ARF and coatomer binding to Golgi membranes is a crucial step in the formation of transport carriers [8], we here studied whether the ARF and β-COP binding to GC in the presence of BFA was altered by PDMP. PDMP did not alter the effect of BFA on ARF (Fig. 3) or β-COP (data not shown) binding to isolated Golgi membranes. This result indicates that the target of PDMP is an

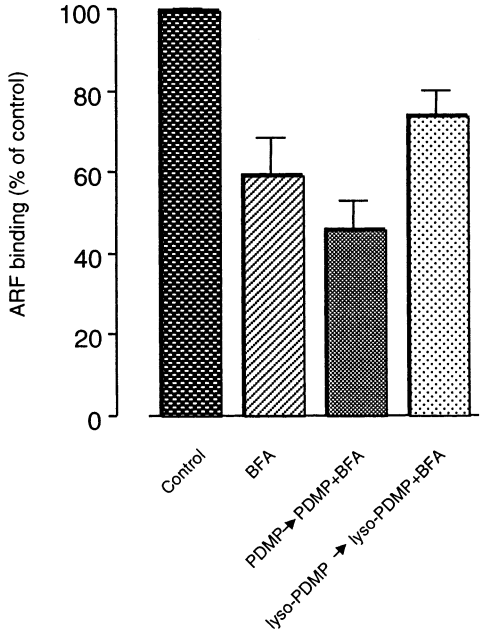

Fig. 3. PDMP did not affect the effect of BFA on ARF binding to Golgi membranes in vitro.

event that occurs after the binding of these two molecular components to Golgi membranes.

Previous experiments suggest that BFA-induced ADP-ribosylation is important in the regulation of the structure and function of the GC [16,17]. BARS-50 is the main target of BFA-induced ADP-ribosylation and it might downregulate the tubulation process [18], for example, regulating the membrane fission process of Golgi-derived tubular intermediates. Although the inhibitory effect of PDMP on the BFA-induced ADP-ribosylation of BARS-50 is not complete, it is reasonable to conclude that this post-translational modification plays a role in the inhibitory effect of PDMP on BFA-induced retrograde membrane flow. This effect of PDMP appears to be independent of its effect on calcium homeostasis [1], since the ADP-ribosylation reaction is not regulated by calcium.

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