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Inhibition by brefeldin A of NADH oxidation activity of rat liver Golgi apparatus accelerated by GDP

D. James Morré[a,*], M. Paulik[a], J.L. Lawrence[a], D.M. Morré[b]

[a]*Department of Medicinal Chemistry and Pharmacognosy and* [b]*Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907, USA*

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Abstract

Reduced pyridine nucleotide has been reported to enhance cell-free transfer of membrane material from a radiolabeled Golgi apparatus donor fraction from rat liver to an acceptor fraction consisting of inside-out plasma vesicles immobilized on nitrocellulose [(1992) Biochim. Biophys. Acta 1107, 131]. As part of a continuing effort to identify NADH-requiring enzymes in the Golgi apparatus which may be important to membrane trafficking, highly purified fractions of Golgi apparatus from rat liver were tested for their ability to oxidize NADH and the inhibition of the oxidation of NADH by brefeldin A. The isolated Golgi apparatus fractions were found to oxidize NADH with a specific activity comparable to that of the plasma membrane of rat liver. The activity was inhibited by brefeldin A and this inhibition was augmented by GDP. At near optimal concentrations of 7 μM brefeldin A and 1 μM GDP, the activity was > 90% inhibited. Brefeldin A inhibition of NADH oxidation by the Golgi apparatus was time-dependent and GDP appeared to accelerate the inhibition by brefeldin A.

Key words:

1. Introduction

Brefeldin A (BFA) is a heterocyclic lactone (macrolide antibiotic) with potent inhibitory effects on trafficking to and through the Golgi apparatus [1]. Transport of G protein is inhibited in vesicular stomatitis virus-infected baby hamster kidney cells [2], and secretion of plasma proteins, such as albumin and α_1-protease inhibitor, are blocked in rat hepatocytes [3,4]. Despite earlier indications that brefeldin A resulted in more or less complete disassembly of the Golgi apparatus and its relocation to the endoplasmic reticulum [1–7], it now appears that, in most instances, a modified Golgi apparatus remains [8,9].

Brefeldin causes rapid dissociation of a 110 kDa protein from Golgi apparatus membranes [10]. This protein, β-COP, is a component of the non-clathrin-coated vesicles that accumulate as the Golgi apparatus-associated buds when intercisternal transport is blocked by GTP-γ-S [11,12]. Most recently, secretion of [^{35}S]SO_4-labeled secretogranin II from PC12 cells, a marker of secretory granule budding from the trans-Golgi network (TGN), was found to be blocked by brefeldin A [13]. These findings suggest that brefeldin A may exert similar effects on both pre- and post-Golgi apparatus compartments, blocking forward transport but not the return pathway [13]. This inhibition of transport by brefeldin A may be through interactions with low molecular weight GTP-binding proteins [14–16] that influence coatomer binding. An exchange enzyme that catalyzes guanine nucleotide exchange and promotes binding of ADP-ribosylation factor (ARF) and other accessory proteins by the Golgi apparatus, for example, has been described as the potential primary target for brefeldin A [17,18].

In our studies, we have reconstituted vesicular membrane trafficking between the transitional endoplasmic reticulum and the Golgi apparatus of rat liver (e.g. [19]). Progress has also been made in reconstituting vesicular membrane traffic between the Golgi apparatus and the plasma membrane [16]. Here a transient transfer of radiolabeled membrane constituents between isolated trans-Golgi apparatus elements and inside-out vesicles of the plasma membrane was found to be temperature-dependent but independent of ATP either in the presence or absence of cytosol. Instead of ATP, the transfer was stimulated by reduced pyridine dinucleotide (NADH) [20].

Consequently, NADH-requiring reactions potentially involved in membrane trafficking were sought. An NADH-ferricyanide oxidoreductase was localized previously to the Golgi apparatus of rat liver [21,22]. However, the natural endogenous acceptor of this activity was not determined. In this report we describe a Golgi apparatus NADH oxidase activity that is inhibited by brefeldin A. This inhibition appears to be accelerated by GDP.

2. Materials and methods

Golgi apparatus were purified from livers of male Holtzman rats as described [23]. The fractions were verified by electron microscopy and analysis of marker enzymes to be at least 90% Golgi apparatus-derived material.

*Corresponding author. Address: Department of Medicinal Chemistry, HANS Life Sciences Research Building, Purdue University, West Lafayette, IN 47907, USA. Fax: (1) (317) 494-4007.

Abbreviations: BFA, brefeldin A; β-COP, β coatomer protein; GTP-γ-S, guanosine 5′-*O*-(3-thiophosphate).

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NADH oxidase was determined as the disappearance of NADH measured at 340 nm in a reaction mixture containing 25 mM Tris-MES buffer (pH 7.0), 1 mM KCN to inhibit any contaminating mitochondrial NADH oxidase activity, and 150 μM NADH at 37°C with constant stirring. Activity was measured with a Hitachi U3210 spectrophotometer with continuous recording over 5 min intervals. A millimolar extinction coefficient of 6.22 was used to determine the specific activity of the NADH oxidase.

Brefeldin A was added as an ethanol solution: an equivalent amount of ethanol was added to controls. Experiments were repeated in triplicate and averaged. Standard deviations were calculated from the means of the three experiments.

3. Results

Isolated rat liver Golgi apparatus exhibited steady-state NADH oxidase activities of 1.0 ± 0.2 nmol NADH oxidized/min/mg protein. Activity was proportional to protein concentration and exhibited linearity for 60 min or longer. Assays were at non-rate-limiting NADH concentrations and pH.

Increasing concentrations of brefeldin A within the normal 10 min NADH oxidase activity exhibited a maximum inhibition of about 30% at 7 μM BFA (Fig. 1). However, if 1 μM GDP was added to the assay mixture the inhibition was nearly doubled over a subsequent 5 min assay. An amount of ethanol equivalent to that added with the brefeldin A was without effect, and 1 μM GDP stimulated NADH oxidase activity slightly, either alone or added to the ethanol (Fig. 1).

The response of the NADH oxidase activity of rat liver

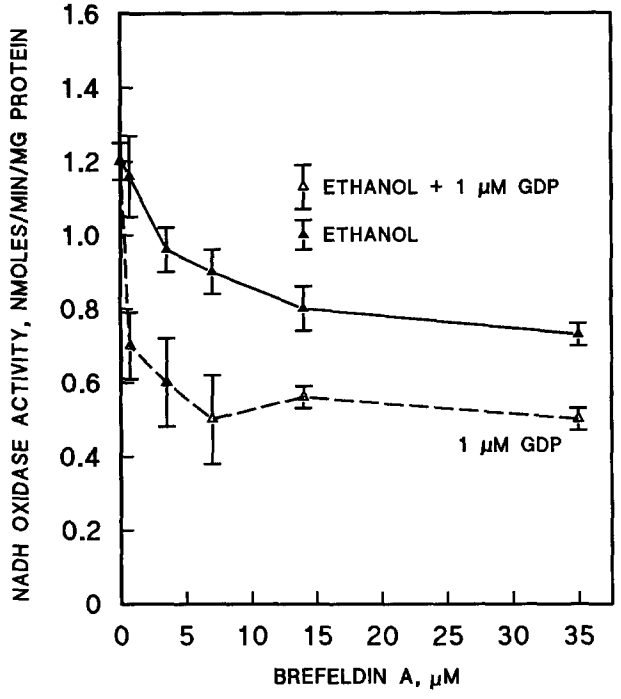

Fig. 1. Inhibition of the NADH oxidase activity of rat liver Golgi apparatus as a function of the concentration of brefeldin A and augmentation of the inhibition by 1 μM GDP. Ethanol alone (▲) and ethanol + 1 μM GDP (△) were largely without effect on the activity.

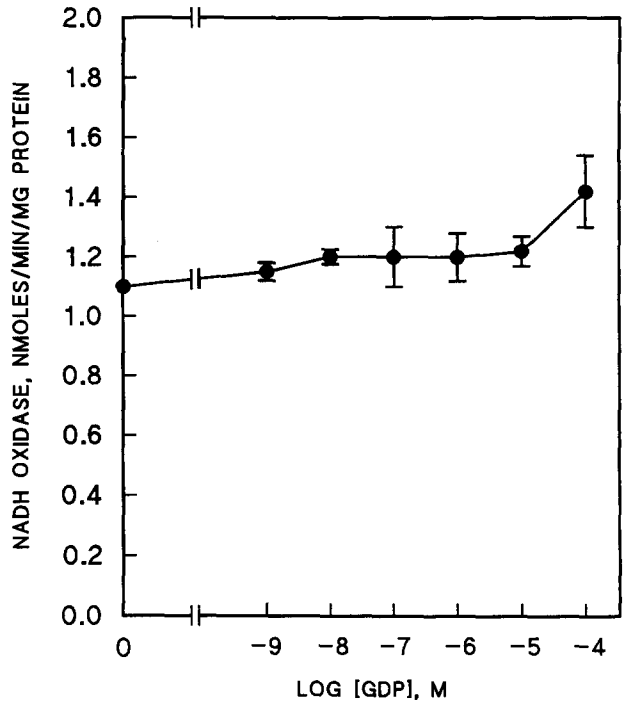

Fig. 2. Response of the NADH oxidase activity of rat liver Golgi apparatus to increasing concentrations of GDP. In the absence of brefeldin A, GDP was either inhibitory or without effect.

Golgi apparatus to GDP is illustrated further in Fig. 2. In the absence of brefeldin A, GDP at all concentrations tested was either without effect or slightly stimulatory. Not only was there no dose-dependent inhibition of NADH oxidase activity over a 5–10 min assay period by GTP at any concentration tested, but GDP was not inhibitory even with prolonged periods of incubation of up to 30 min.

At a near optimum concentration of 7 μM brefeldin A and varying concentrations of GDP, subsequent inhibition of the two substances added together was near maximal at about 1 μM GDP (Fig. 3). The combination of brefeldin A plus GDP was very effective in inhibiting NADH oxidase activity. Inhibitions of 90% or greater were routinely achieved.

Brefeldin A alone also exhibited substantial inhibition of NADH oxidase in a time-dependent manner (Fig. 4). Times of incubation of 40 min or longer were required in the absence of GDP before substantial inhibitions were observed. No loss of enzymatic activity was observed over the same period of time in the presence of equivalent amounts of ethanol alone.

4. Discussion

An established characteristic of the action of brefeldin A on cells is that the compound prevents the exit of newly synthesized proteins from the endoplasmic reticulum

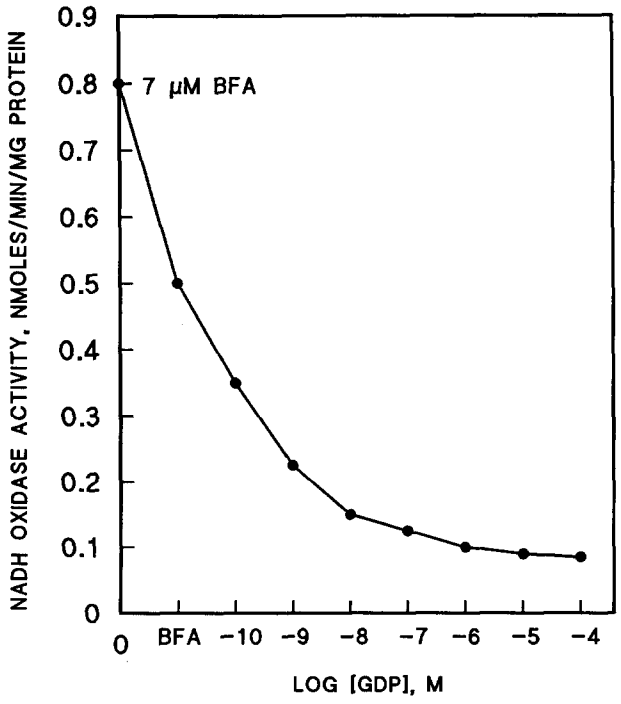

Fig. 3. Inhibition of the NADH oxidase of rat liver Golgi apparatus by 7 μM brefeldin A (BFA), and augmentation of the inhibition by increasing GDP concentrations. Activity was ca. 90% inhibited following the addition of 1 μM GDP.

(ER) [3,4]. The Golgi apparatus structure is rapidly disrupted and what has been interpreted as microtubule-dependent retrograde transport of Golgi apparatus enzymes back to the ER is induced [6,7,24,25]. Thus, brefeldin A blocks forward transport while retrograde transport appears to continue.

The basis for the block in forward transport has been attributed, at least in part, to an interruption of coatomer attachment to Golgi apparatus-associated vesicles [26]. Donaldson et al. [10] showed that brefeldin A caused the dissociation of a 110 kDa protein, β-COP, from Golgi apparatus membranes in less than 15 s. β-COP has been demonstrated to be present on non-clathrin-coated buds that accumulate when intercisternal Golgi apparatus transport is blocked by GTP-γ-S [11,12]. Formation of these buds is prevented in vitro by brefeldin A [27].

It is possible that β-COP and the coatomer complex also function in more distal parts of the Golgi complex. For example, Miller et al. [13] showed that secretion of [^{35}S]SO_4-labeled secretogranin II from PC12 cells was blocked by brefeldin A whereas endocytic traffic was not. The sulfated secretogranins have been widely employed to study secretory granule budding from the trans-Golgi apparatus network [28].

One explanation for how brefeldin A might prevent the assembly of coatomer onto the membrane has come from studies that demonstrate inhibition of the GTP-dependent interaction of ADP-ribosylation factor (ARF) with the Golgi apparatus membranes [17,18,29,30]. Additionally, recruitment of the Golgi-specific AP-1 adaptor complex onto Golgi membrane involved in clathrin coat assembly required ARF [31], and this recruitment was also sensitive to brefeldin A. ARF binding to isolated Golgi apparatus was hormone receptor- and protein kinase C-mediated [32], and inactive analogs of brefeldin A did not inhibit nucleotide exchange and accessor protein binding by the Golgi apparatus [18,33]. The target for brefeldin A would then be the exchange enzyme that catalyzed guanine nucleotide exchange on the ARF-1 protein [17,18]. ARF-1(T31N) mutant protein, a protein with a putative preferential affinity for GDP compared to the wild-type protein, was shown to inhibit export from the ER and trigger a brefeldin A-like phenotype in HeLa cells [35].

Interaction with an exchange enzyme that catalyzes guanine nucleotide exchange may be important as well as help explain our findings concerning the brefeldin A plus GDP inhibition of the NADH oxidase activity. The NADH oxidase of rat liver plasma membranes does respond to guanine nucleotides, GTP-γ-S, mastoparan and aluminum fluoride [36]. The activity is growth factor- and hormone-responsive with plasma membranes of rat liver [37], but is constitutively activated and no longer growth factor- and hormone-responsive in plasma membranes from rat hepatomas [38]. Alteration of a guanine nucleotide exchange activity similar to that involved with

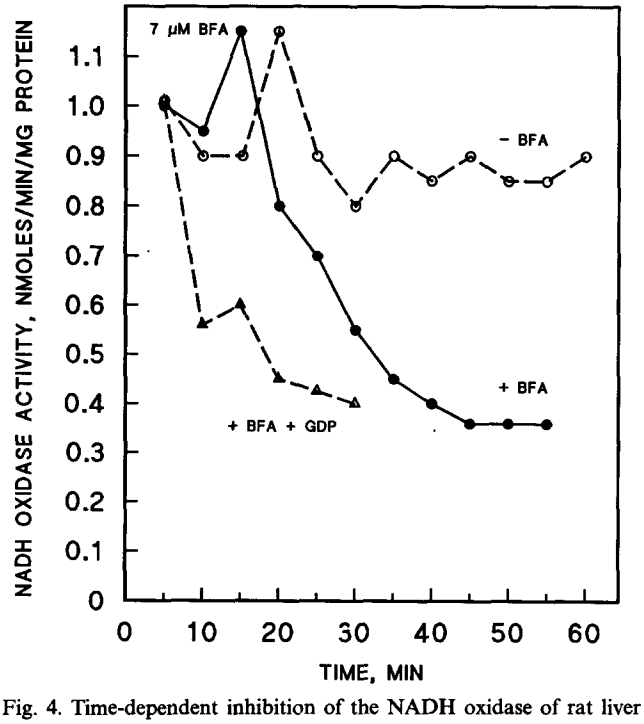

Fig. 4. Time-dependent inhibition of the NADH oxidase of rat liver Golgi apparatus by 7 μM brefeldin A (BFA): GDP may only serve to accelerate the rate of BFA inhibition of the oxidase. Even in the absence of added GDP, BFA inhibited the NADH oxidase in a time-dependent manner to a maximum at about 45 min. In the absence of BFA (equivalent amount of ethanol alone), the activity was not inhibited.

ARF and accessory protein binding to Golgi apparatus membranes [17,18] could help to explain how GDP and brefeldin A interact to inhibit Golgi apparatus NADH oxidase activity.

Added interest in the inhibition of the NADH oxidase activity by brefeldin A is provided by the previously reported enhancement of cell-free transfer from Golgi apparatus to plasma membrane augmented by NADH [20]. As such, the activity may provide a convenient tool for the future elucidation of both the action of brefeldin A in blocking Golgi apparatus membrane traffic, and for the possible resolution of the role of reduced pyridine nucleotide in Golgi apparatus to plasma membrane vesicle transfer.

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