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# THE BIOENERGETICS OF GOLGI APPARATUS FUNCTION: EVIDENCE FOR AN ATP-DEPENDENT PROTON PUMP

Fang Zhang and Donald L. Schneider

Department of Biochemistry
Dartmouth Medical School
Hanover, NH 03756

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The energy requirement for the processing of newly-synthesized proteins by the Golgi was examined. Rat liver Golgi preparations enriched more than 100-fold have high ATPase activity that co-purified with the Golgi marker enzyme galactosyl transferase. The ATPase activity was 80% inhibited by dicyclohexylcarbodiimide and may represent a proton pump. Evidence is presented for a functional role of the ATPase in Golgi. First, measurement of [$^{14}$C]methylamine uptake demonstrated ATP-dependent acidification. Second, inhibition of the ATPase with dicyclohexylcarbodiimide resulted in a 3-fold accumulation of newly-synthesized protein in the Golgi.

The Golgi apparatus has an obligatory role in the cellular secretion of newly-synthesized proteins (1). Proteins enter the Golgi from the endoplasmic reticulum on their way for extrusion through the plasma membrane (1,2). Metabolic energy is required for the synthesis and secretion of proteins. Furthermore, treatment with the energy inhibitors monensin or carbonyl cyanide p-trifluoromethoxyphenylhydrazone blocks the transit of proteins through the Golgi (4-8). The mechanisms of this energy requirement are unknown but a possibility is a proton pump in the Golgi. In this communication, we report the inhibition of Golgi function *in vivo* by dicyclohexylcarbodiimide, an inhibitor of proton pump ATPases (9-14).

## MATERIALS AND METHODS

Golgi preparation. Golgi were isolated from rat liver as described (15,16). Livers were homogenized in 0.25 M sucrose, and large granules were removed by centrifuging the homogenate at 45,000 xg for 12 min. The Golgi and other microsomal elements were collected as pellets by centrifuging at 100,000 xg for 35 min and resuspended in 43% sucrose, w/w. Fifteen ml of the resuspension were layered below 6 ml of 0.25 M sucrose, 6 ml of 33% sucrose, w/w, and 6 ml of 36% sucrose, w/w, and above

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2 ml of 60% sucrose, w/w; after centrifugation in the Beckman SW 27 rotor at 100,000 xg for 2 h, the Golgi were recovered from the 0.25 M-33% sucrose interface by pumping 60% sucrose into the bottom of the gradient, using an inverted cone arrangement (17).

Assays. Galactosyl transferase activity was determined as trichloroacetic acid-precipitable radioactivity with UDP-[$^3$H]galactose (Amersham) and ovalbumin as substrates as described (16,18). ATPase activity was measured using 1.5 mM ATP and 3 mM $MgCl_2$ at pH 7 with 20 min of incubation at 37°C as described previously (14). Dicyclohexylcarbodiimide was added from a 25 mM stock solution in dimethylsulfoxide (fresh daily) to give a final concentration of 0.5 mM. Standard assays were performed for N-acetyl-β-D-glucosaminidase (19) and protein (20,21). Trichloroacetic acid-precipitable radioactivity was measured by counting liquid scintillations as described (21).

Continuous sucrose gradients. Golgi preparations were diluted with 1.5 volumes of 0.25 M sucrose, and 9 ml of the resulting suspension were applied to a 25-ml linear gradient, 14.3 to 45% sucrose, w/w, underlaid with 1 ml of 60% sucrose. After centrifugation in the Beckman SW 27 rotor at 100,000 xg for 2 h, fractions were collected from the top by pumping 60% sucrose into the bottom of the gradient. Densities were determined using a Fisher refractometer.

## RESULTS

ATPase Activity in the Golgi. In order to determine what the source of metabolic energy for Golgi function is, Golgi were isolated from rat livers. The isolation procedure consistently yielded Golgi preparations that were more than 100-fold enriched in galactosyl transferase activity (Table I). In fact, relative specific activity values, calculated as

Table I. Enzyme Activities of Golgi Preparations Isolated from Rat Liver

| Enzyme | Activity | | Enrichment |
|---|---|---|---|
| | Golgi | Homogenate | |
| | (nmol/min-mg) | | (fold) |
| Gal transferase | 0.237 ± 0.050 | 0.00199 ± 0.00032 | 119 |
| Total ATPase | 157 ± 5 | 105 ± 5 | 1.5 |
| DCCD-ATPase | 130 ± 30 | 82.5 ± 3 | 1.6 |
| NAβGase | 23.4 ± 3.7 | 28.7 ± 3.8 | 0.8 |

The Golgi, prepared from rat liver by discontinuous sucrose gradient centrifugation, were analyzed as described in Materials and Methods. The activity values given are the mean ± the standard deviation of at least three independent determinations. The yields were 15.5 ± 1.6% of the total galactosyl transferase. The abbreviations are Gal transferase, galactosyl transferase and NAβGase, N-acetyl-β-D-glucosaminidase and DCCD, dicyclohexylcarbodiimide.

described (22), indicate an enrichment of 140-fold (the discrepancy between 119 and 140 occurs because of activity losses). The high quality of the preparations was also evident from the very low content of the lysosomal marker enzyme N-acetyl-β-D-glucosaminidase.

The isolated Golgi have high ATPase activity (Table I), about 2% of the total activity in liver. This value corresponds to 20% of the non-plasma membrane, non-mitochondrial ATPase inasmuch as plasma membrane and mitochondria contain 90% of the liver ATPase activity (21). Significantly, the Golgi ATPase activity was 80% inhibited by dicyclohexylcarbodiimide (Table I).

In order to test for resolution of the ATPase and galactosyl transferase activities, Golgi preparations were centrifuged into continuous sucrose gradients. Figure 1 shows that they do not resolve but co-purify

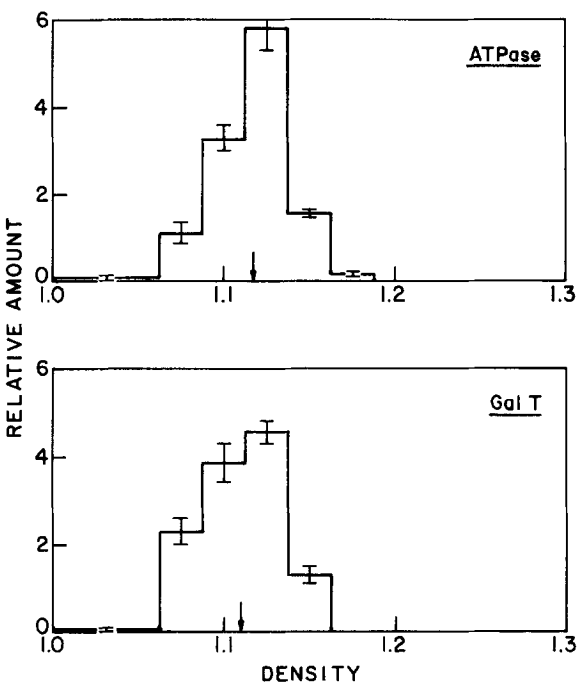


Fig. 1. Analysis of Golgi Preparations on Continuous Sucrose Density Gradients.

Golgi preparations were applied to linear sucrose gradients and fractions were analyzed as described in Materials and Methods. The density distributions represent the averages of three independent experiments; the error bars denote one unit of standard error. The arrows indicate median densities which were 1.110 g/ml for galactosyl transferase (Gal T) and 1.116 g/ml for ATPase.

and thus confirms that the ATPase is contained in the Golgi. Furthermore, the movement of newly-synthesized protein from cis- to trans-Golgi is blocked by energy inhibitors (4-8) and the Golgi is partially resolved on sucrose gradients into less dense trans-Golgi and more dense cis-Golgi with median densities of about 1.110 and 1.140 (2). Galactosyl transferase exhibited a median density of 1.110 g/ml, typical of trans-Golgi. On the other hand, ATPase exhibited a density of 1.116 which indicates a dual localization in cis- and trans-Golgi. A comparison of specific activities suggests that the energy-requiring cis-Golgi may be about 2.5-fold more enriched in ATPase than the trans.

ATPase Function in the Golgi. Since the preparations contained dicyclohexylcarbodiimide-sensitive ATPase, they might be expected to carry out ATP-dependent acidification. This possibility was tested by measuring the uptake of [$^{14}$C]methylamine by Golgi preparations in the absence and presence of ATP, using a rapid gel filtration technique previously developed in this lab for measuring acidification in lysosomes (14). Significant acidification of intra-Golgi pH was observed in the presence of MgATP: the ATP-dependent $\Delta$pH value was $0.98 \pm 0.22$. This corresponds to an internal pH of about 6.

The dependency of protein secretion through the Golgi on the purported proton pump ATPase was examined by treatment of rats with dicyclohexylcarbodiimide. These studies were facilitated by pre-labeling of newly-synthesized proteins with [$^{3}$H]leucine. As shown in Table II, dicyclohexylcarbodiimide appeared to inhibit secretion because a 3-fold accumulation of labeled protein occurred in the Golgi. These data demonstrate that the transit of newly-synthesized protein through the Golgi involves a dicyclohexylcarbodiimide-sensitive process, most probably ATP-dependent acidification.

## DISCUSSION

On the basis of these results, the bioenergetics of transit through the Golgi can be explained. Secretion requires ATP-dependent acidifi-

Table II. Effect of Dicyclohexylcarbodiimide on the Transit of Leucine-labeled Protein through the Golgi

| Experiment | Rat | Treatment | Radioactivity in Golgi | | |
|---|---|---|---|---|---|
| | | | (% of total in liver) | (cpm) | (cpm/mg) |
| 1 | 1 | Sham | 1.28 | 15,510 | 6,740 |
| | 2 | DCCD | 3.485 | 42,370 | 15,460 |
| 2 | 1 | Sham | 1.20 | 14,610 | 7,310 |
| | 2 | DCCD | 4.15 | 50,450 | 11,730 |

Rats were injected (i.p.) with 65 μCi of [$^3$H]leucine and 20 min later with dicyclohexylcarbodiimide, 1 ml per 100 g rat body weight of 6.5 mM dicyclohexylcarbodiimide in 10% dimethylsulfoxide-saline. After an additional 20 min, the rats were sacrificed by decapitation, the livers rapidly excised and chilled, and the Golgi were prepared. Trichloroacetic acid-precipitable protein and radioactivity were determined as described in Materials and Methods. The values presented are uncorrected for yields (corrected values indicate that about 8% of the labeled protein was in Golgi from sham treated rats and about 25% in Golgi from DCCD treated rats).

cation of the Golgi contents. The idea of obligatory acidification is consistent not only with a sensitivity to dicyclohexylcarbodiimide but also with sensitivity to monensin and to the uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (4-8). The relationship of acidification to the receptor-mediated sorting of newly-synthesized proteins that has been suggested to occur in Golgi (2) is intriguing. Furthermore, the similarities among the purported proton pump ATPase in Golgi presented here and those reported previously in lysosomes (14) and endocytic and/or coated vesicles (23-25) are remarkable. It now appears that all compartments within the vacuolar apparatus of the cell may require and be regulated by ATP-dependent acidification. The elucidation of the structural, functional and regulatory properties of these proton pumps will be of tremendous interest.

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