A major feature of the gastric H⁺,K⁺-ATPase β subunit is the presence of six or seven consensus sequences for N-linked glycosylation which are all cotranslationally glycosylated. In several reports of Na⁺,K⁺-ATPase, it was demonstrated that N-glycosylation is not essential for enzymatic activity. The present study investigates whether N-glycosylation is essential for H⁺,K⁺-ATPase activity.

H⁺,K⁺-ATPase can be synthesized in vitro as an active enzyme using the baculovirus system. In contrast to the mammalian enzyme, the β subunit is synthesized in both a nonglycosylated and a core-glycosylated form. Complex glycosylated β subunit is either present or absent in minor amounts. The presence of increasing concentrations of tunicamycin, an inhibitor of N-glycosylation, in the culture medium of SF-9 cells resulted in a highly reproducible dose-dependent decrease in the amount of functional H⁺,K⁺-ATPase synthesized (Fig. 1). This decrease in H⁺,K⁺-ATPase activity is correlated with a simultaneous decrease in the amount of glycosylated β subunits. Tunicamycin treatment had no visible effect on the H⁺,K⁺-ATPase α subunit. These results strongly suggest that N-glycosylation somehow is essential for H⁺,K⁺-ATPase activity.

By using deoxymannojirimycin, a specific inhibitor of α-mannosidase I, trimming of the high-mannose oligosaccharide precursor can be blocked, preventing formation of complex glycosylated forms. Analysis of glycosylated forms of the β subunit indicated that the compound was active. However, no effect on the activity of the recombinant expressed H⁺,K⁺-ATPase was measured. Thus, only the presence and not the exact structure of the oligosaccharide moieties is essential for H⁺,K⁺-ATPase activity.

Functional H⁺,K⁺-ATPase subunits in the standard crude membrane preparation can be separated from nonfunctional H⁺,K⁺-ATPase subunits using a discontinuous sucrose density gradient. Figure 2 shows that the purified H⁺,K⁺-ATPase fraction contained more glycosylated and almost no nonglycosylated β subunits. The nonglycosylated β subunits were more abundant in the pellet fraction, in which only little H⁺,K⁺-ATPase activity was found. This supports our conclusion that glycosylation is essential for H⁺,K⁺-ATPase activity.

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Confocal microscopy studies show that the \( \alpha \) subunit of \( \text{H}^+,\text{K}^+-\text{ATPase} \) is found exclusively in intracellular membranous structures. No levels of \( \alpha \) subunit are detectable in the plasma membrane. This means that the catalytically active \( \text{H}^+,\text{K}^+-\text{ATPase} \) fraction also originates from an intracellular source. The \( \text{H}^+,\text{K}^+-\text{ATPase} \) \( \beta \) subunit is partly targeted to the plasma membrane and partly retained in intracellular membranous structures. In the presence of 5 \( \mu \text{g/ml} \) tunicamycin, the nonglycosylated \( \beta \) subunit can no longer be found on the plasma membrane (not shown). Apparently, proper processing of the \( \text{H}^+,\text{K}^+-\text{ATPase} \) \( \beta \) subunit onto the plasma membrane depends on the presence of \( N \)-linked oligosaccharides on this subunit. However, because the \( \text{H}^+,\text{K}^+-\text{ATPase} \) \( \alpha \) subunit is found exclusively in intracellular membranous structures, processing of the \( \text{H}^+,\text{K}^+-\text{ATPase} \) \( \beta \) subunit to the plasma membrane is apparently not essential for synthesis of a functional \( \text{H}^+,\text{K}^+-\text{ATPase} \) in insect cells.

In immunoprecipitates from untreated cultures, both glycosylated and nonglycosylated \( \text{H}^+,\text{K}^+-\text{ATPase} \) \( \beta \) subunits are precipitated with the anti-\( \alpha \) subunit antibody (not shown). This means that both forms of the \( \beta \) subunit must be engaged with the \( \alpha \)

**FIGURE 1.** Effect of tunicamycin on glycosylation and activity of \( \text{H}^+,\text{K}^+-\text{ATPase} \). The phosphorylation capacity of \( \text{H}^+,\text{K}^+-\text{ATPase} \) (squares) and endogenous (auto)phosphorylating enzymes (circles) in crude membrane fractions is plotted against the tunicamycin concentration in the culture medium. 100% values are 2.16 ± 0.28 pmol/mg (mean ± SEM) for \( \text{H}^+,\text{K}^+-\text{ATPase} \) and 0.75 ± 0.04 for endogenous (auto)phosphorylating enzymes from nine experiments. In the upper panel, western blot of the \( \alpha \) subunit (A) and the \( \beta \) subunit (B) is shown. Each lane contains 2.0 \( \mu \text{g} \) crude membrane protein. \( \text{H}^+,\text{K}^+-\text{ATPase} \) subunits were visualized using subunit-specific antibodies. Horizontal position of the lanes in A and B corresponds to the tunicamycin concentration below.
FIGURE 2. Glycosylated β subunits copurify with functional H⁺,K⁺-ATPase. The H⁺,K⁺-ATPase α subunit (A) or β subunit (B) in different Sf-9 membrane fractions was visualized with subunit-specific antibodies. βc = β subunit core protein (34 kD); βgly = glycosylated β subunits (40–50 kD). The activity of the resulting fraction is given as the steady-state phosphorylation capacity in pmol mg⁻¹ protein and is given below in C. CM = crude membranes; IF = 25% (w/v) – 38% (w/v) sucrose interface; P = 38% (w/v) sucrose pellet. Each lane contains 1.0 μg protein.

subunit in a detergent-resistant complex and hence are tightly associated. This conclusion is supported by the finding that in tunicamycin-treated cultures, where no glycosylated β subunits are produced, the amount of immunoprecipitated nonglycosylated β subunit is increased relative to untreated cultures. Thus, N-glycosylation plays no role in the interaction between α and β subunits.

In conclusion, N-glycosylation is essential for H⁺,K⁺-ATPase activity and for targeting the β subunit to the plasma membrane. Core glycosylation seems to be sufficient for enzyme activity. Moreover, the presence of sugar residues is not essential for α-β interaction.

REFERENCES