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# Role of Sugar Residues for Recombinant Gastric H<sup>+</sup>,K<sup>+</sup>-ATPase<sup>a</sup>

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A major feature of the gastric H<sup>+</sup>,K<sup>+</sup>-ATPase  $\beta$  subunit is the presence of six or seven consensus sequences for *N*-linked glycosylation which are all cotranslationally glycosylated. In several reports of Na<sup>+</sup>,K<sup>+</sup>-ATPase, it was demonstrated that *N*-glycosylation is not essential for enzymatic activity.<sup>1-3</sup> The present study investigates whether *N*-glycosylation is essential for H<sup>+</sup>,K<sup>+</sup>-ATPase activity.

H<sup>+</sup>,K<sup>+</sup>-ATPase can be synthesized *in vitro* as an active enzyme using the baculovirus system.<sup>4,5</sup> In contrast to the mammalian enzyme, the  $\beta$  subunit is synthesized in both a nonglycosylated and a core-glycosylated form. Complex glycosylated  $\beta$  subunit is either present or absent in minor amounts.<sup>4</sup> 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<sup>+</sup>,K<sup>+</sup>-ATPase synthesized (FIG. 1). This decrease in H<sup>+</sup>,K<sup>+</sup>-ATPase activity is correlated with a simultaneous decrease in the amount of glycosylated  $\beta$  subunits. Tunicamycin treatment had no visible effect on the H<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha$  subunit. These results strongly suggest that *N*-glycosylation somehow is essential for H<sup>+</sup>,K<sup>+</sup>-ATPase activity.

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

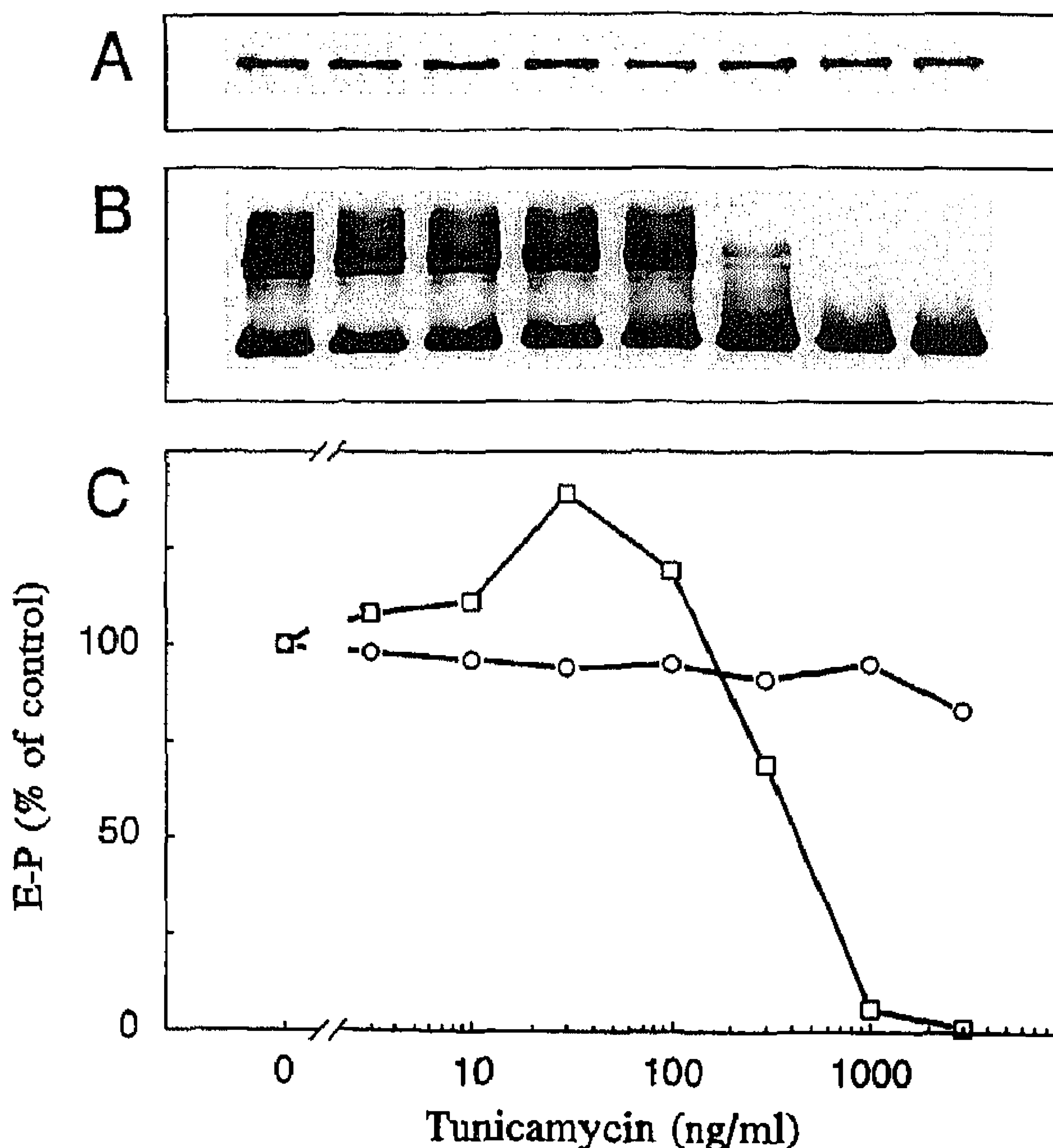
Functional H<sup>+</sup>,K<sup>+</sup>-ATPase subunits in the standard crude membrane preparation can be separated from nonfunctional H<sup>+</sup>,K<sup>+</sup>-ATPase subunits using a discontinuous sucrose density gradient. FIGURE 2 shows that the purified H<sup>+</sup>,K<sup>+</sup>-ATPase fraction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

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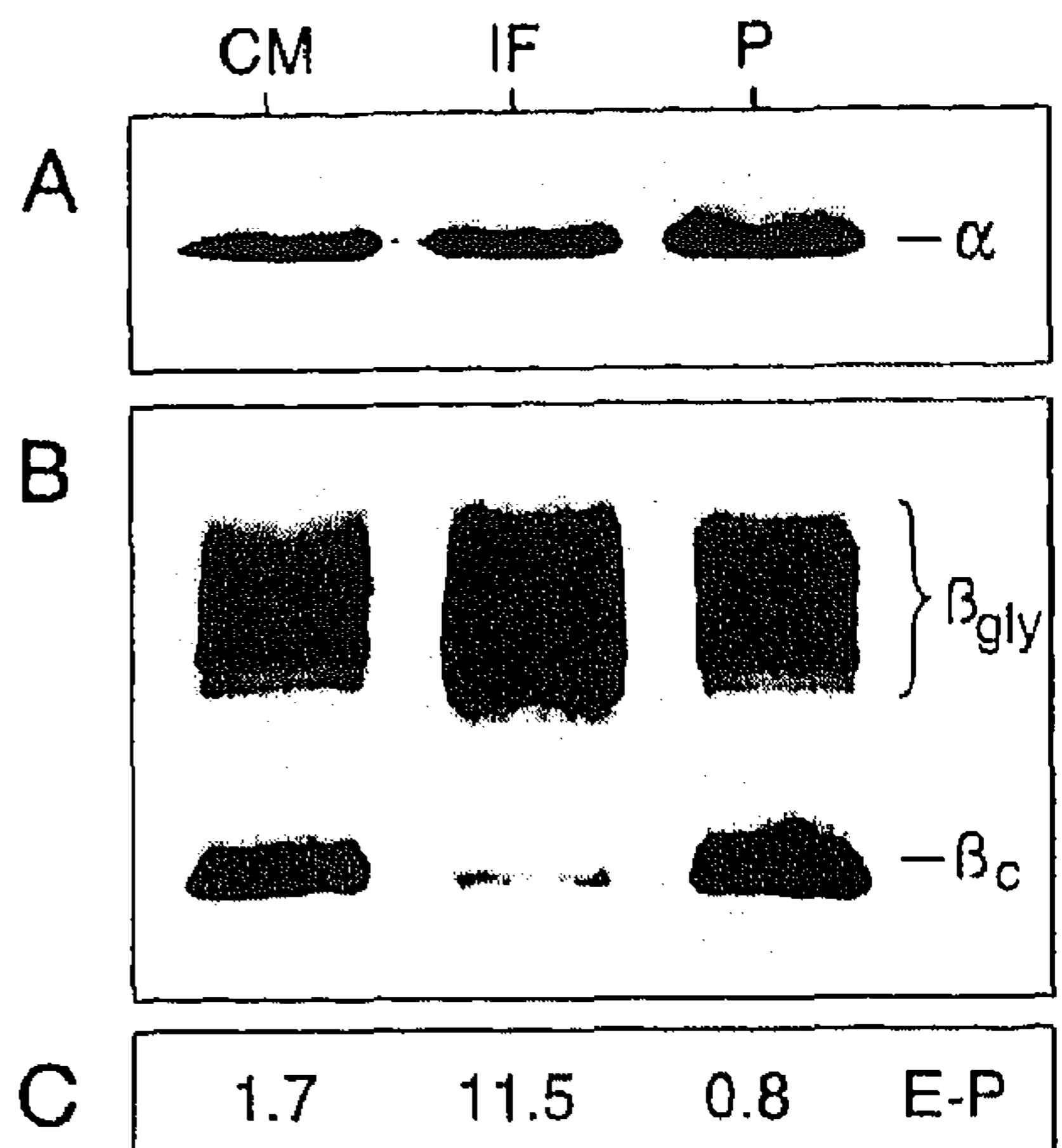
Confocal microscopy studies show that the  $\alpha$  subunit of  $H^+,K^+$ -ATPase is found exclusively in intracellular membranous structures. No levels of  $\alpha$  subunit are detectable in the plasma membrane. This means that the catalytically active  $H^+,K^+$ -ATPase fraction also originates from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

In immunoprecipitates from untreated cultures, both glycosylated and nonglycosylated  $H^+,K^+$ -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  $H^+,K^+$ -ATPase. The phosphorylation capacity of  $H^+,K^+$ -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  $2.16 \pm 0.28$  pmol/mg (mean  $\pm$  SEM) for  $H^+,K^+$ -ATPase and  $0.75 \pm 0.04$  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.  $H^+,K^+$ -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  $\beta$  subunits copurify with functional H<sup>+</sup>,K<sup>+</sup>-ATPase. The H<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha$  subunit (A) or  $\beta$  subunit (B) in different *Sf*-9 membrane fractions was visualized with subunit-specific antibodies.  $\beta_c$  =  $\beta$  subunit core protein (34 kD);  $\beta_{gly}$  = glycosylated  $\beta$  subunits (40–50 kD). The activity of the resulting fraction is given as the steady-state phosphorylation capacity in pmol.mg<sup>-1</sup> protein and is given below in C. CM = crude membranes; IF = 25% (w/v) – 38% (w/v) sucrose interfase; P = 38% (w/v) sucrose pellet. Each lane contains 1.0  $\mu$ g 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  $\beta$  subunits are produced, the amount of immunoprecipitated nonglycosylated  $\beta$  subunit is increased relative to untreated cultures. Thus, *N*-glycosylation plays no role in the interaction between  $\alpha$  and  $\beta$  subunits.

In conclusion, *N*-glycosylation is essential for H<sup>+</sup>,K<sup>+</sup>-ATPase activity and for targeting the  $\beta$  subunit to the plasma membrane. Core glycosylation seems to be sufficient for enzyme activity. Moreover, the presence of sugar residues is not essential for  $\alpha$ - $\beta$  interaction.

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