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Role of Sugar Residues for Recombinant Gastric H^+,K^+-ATPase[a]

CORNÉ H. W. KLAASSEN, HERMAN G. P. SWARTS, AND
JAN JOEP H. H. M. DE PONT[b]

Department of Biochemistry
Institute of Cellular Signalling
University of Nijmegen
P.O. Box 9101
6500 HB Nijmegen, The Netherlands

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.[1–3] 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.[4,5] 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.[4] 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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[b]To whom correspondence should be addressed. Tel: +31243614260; fax: +31243540525; e-mail: J.dePont@bioch.kun.nl

Confocal microscopy studies show that the α subunit of H^+,K^+-ATPase is found exclusively in intracellular membranous structures. No levels of α 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 β subunit is partly targeted to the plasma membrane and partly retained in intracellular membranous structures. In the presence of 5 μg/ml tunicamycin, the nonglycosylated β subunit can no longer be found on the plasma membrane (not shown). Apparently, proper processing of the H^+,K^+-ATPase β subunit onto the plasma membrane depends on the presence of *N*-linked oligosaccharides on this subunit. However, because the H^+,K^+-ATPase α subunit is found exclusively in intracellular membranous structures, processing of the H^+,K^+-ATPase β 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 β subunits are precipitated with the anti-α subunit antibody (not shown). This means that both forms of the β subunit must be engaged with the α

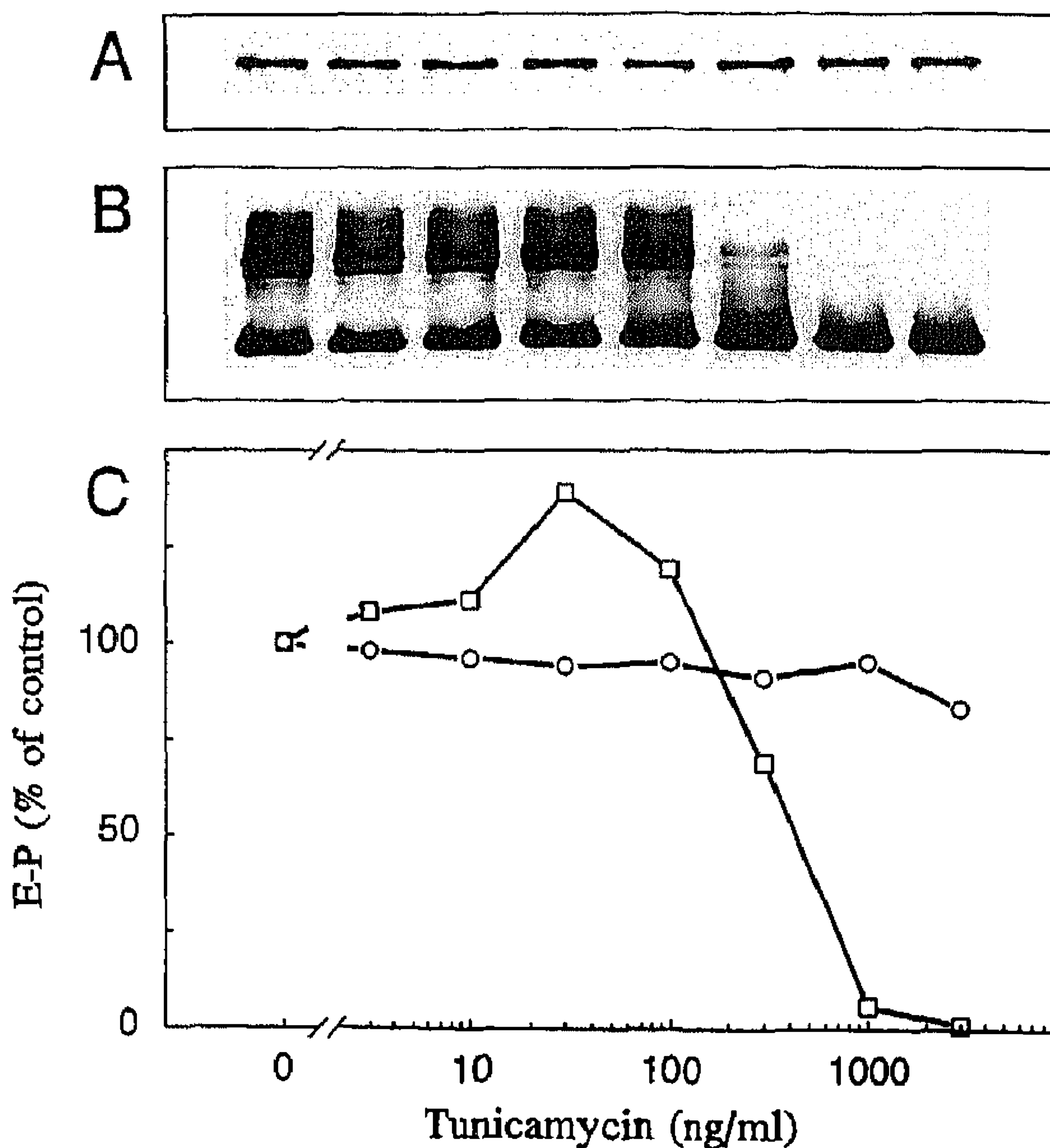

FIGURE 1. Effect of tunicamycin on glycosylation and activity of H^+,K^+-ATPase. The phosphorylation capacity of H^+,K^+-ATPase (*squares*) and endogenous (auto)phosphorylating enzymes (*circles*) in crude membrane fractions is plotted against the tunicamycin concentration in the culture medium. 100% values are 2.16 ± 0.28 pmol/mg (mean ± SEM) for H^+,K^+-ATPase and 0.75 ± 0.04 for endogenous (auto)phosphorylating enzymes from nine experiments. In the *upper panel*, western blot of the α subunit (**A**) and the β subunit (**B**) is shown. Each lane contains 2.0 μg 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.

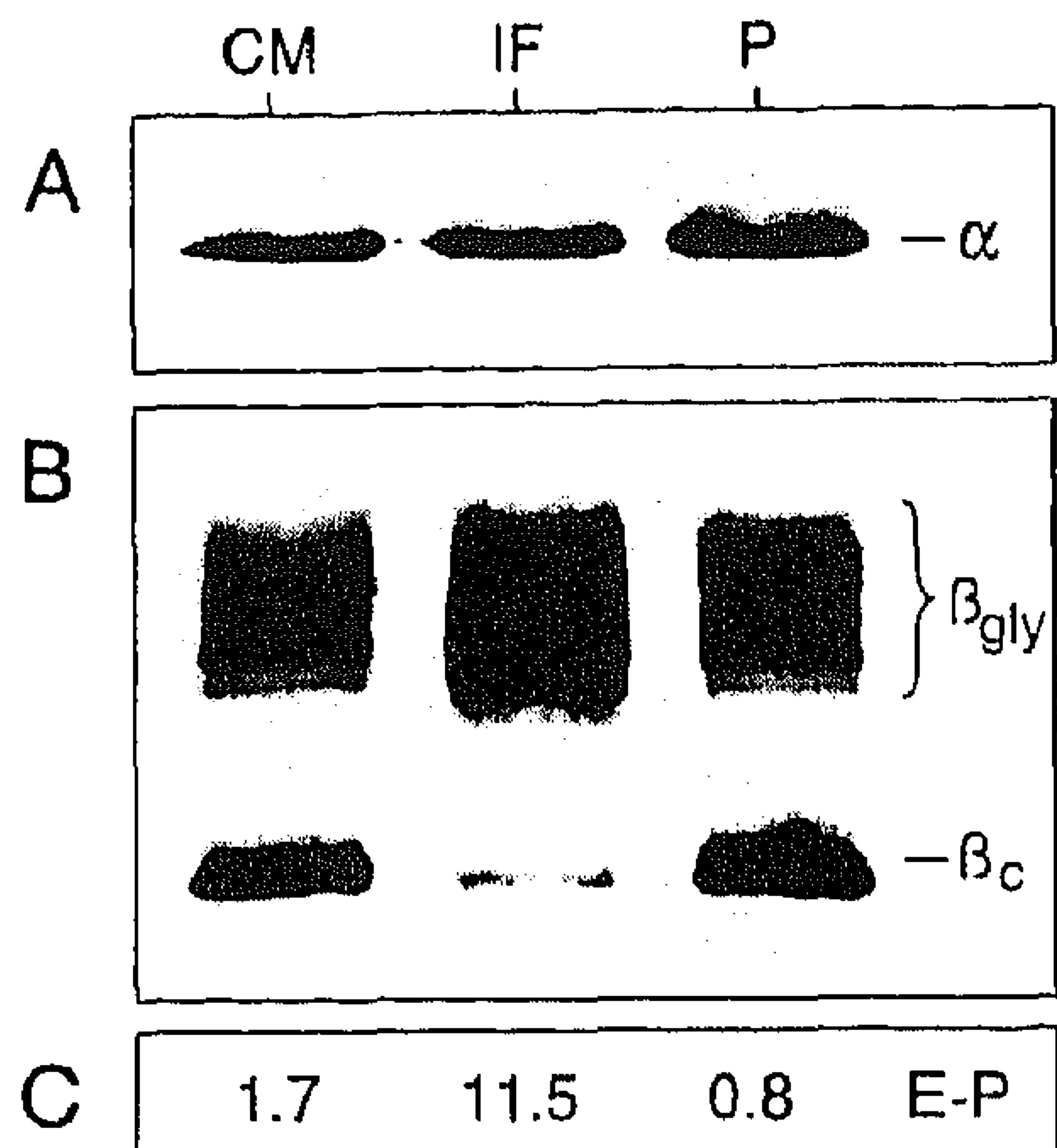

FIGURE 2. Glycosylated β subunits copurify with functional H^+,K^+-ATPase. The H^+,K^+-ATPase α subunit (**A**) or β subunit (**B**) in different *Sf*-9 membrane fractions was visualized with subunit-specific antibodies. β_c = β subunit core protein (34 kD); β_{gly} = glycosylated β subunits (40–50 kD). 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 interfase; 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.

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