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

\[ \text{FIGURE 1. Effect of tunicamycin on glycosylation and activity of H⁺,K⁺-ATPase. The phosphorylation capacity of H⁺,K⁺-ATPase (squares) and endogenous (auto)phosphorylating enzymes (circles) in crude membrane fractions is plotted against the tunicamycin concentration in the culture medium. 100% values are 2.16 ± 0.28 pmol/mg (mean ± SEM) for H⁺,K⁺-ATPase and 0.75 ± 0.04 for endogenous (auto)phosphorylating enzymes from nine experiments. In the upper panel, western blot of the α subunit (A) and the β subunit (B) is shown. Each lane contains 2.0 μg crude membrane protein. H⁺,K⁺-ATPase subunits were visualized using subunit-specific antibodies. Horizontal position of the lanes in A and B corresponds to the tunicamycin concentration below.} \]
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. 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 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.

REFERENCES