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Functional expression of gastric H,K-ATPase using the baculovirus expression system

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A novel approach to construct a single recombinant baculovirus expressing two protein subunits simultaneously by replacing polyhedrin as well as p10 coding sequences is described. The recombinant baculovirus expressed the α- as well as the β-subunit of the gastric H,K-ATPase. Sf9 cells infected with this virus exhibited a K^+- and SCH 28080-sensitive ATP-dependent phosphorylation capacity in purified Sf9 membranes similar to native H,K-ATPase. This activity was not present in control membranes containing only one of the two H,K-ATPase subunits. We therefore conclude that both subunits are essential for the phosphorylation capacity of H,K-ATPase.

H,K-ATPase; Baculovirus expression system; Sf9 cell; In vitro expression; SCH 28080

1. INTRODUCTION

H,K-ATPase is the enzyme responsible for gastric acid secretion catalysing the active exchange of intracellular H^+ for luminal K^+ ions and generating a proton gradient of more than 6 units across the apical membrane of gastric parietal cells. The enzyme belongs to the family of P-type ATPases [1,2], indicating the formation of a phosphorylated intermediate during its reaction cycle.

The enzyme consists of two subunits, a catalytic α-subunit of approximately 114 kDa and a heavily glycosylated β-subunit of 34 kDa with an apparent molecular mass of 60–80 kDa on SDS-PAGE gels due to its extensive glycosylation. Both subunits from several species have been cloned and analyzed [3–6].

The baculovirus expression system makes advantage of the high level expression of certain viral proteins not essential for viral replication in a cell line from the fall armyworm *Spodoptera frugiperda* (Sf9 cells) [7]. Two such proteins are polyhedrin and p10 protein. Both proteins can reach expression levels of more than 30% in Sf9 cells, dependent on the stage of infection. Replacing polyhedrin or p10 coding sequences by homologous recombination between wild-type viral DNA and a transfer vector DNA has led to the production of recombinant viruses expressing large amounts of recombinant proteins. Many complex membrane proteins as well as cytosolic proteins have successfully been expressed using this system [8–14]. In order to produce multiple subunit proteins, simultaneous expression of two or more proteins has been made possible by using either one transfer vector containing two or more promoters [15,16] or by co-infection of Sf9 cells using a mixture of two or more recombinant baculoviruses.

In this communication we report the in vitro synthesis of a functional H,K-ATPase using the baculovirus expression system and the production of a single recombinant baculovirus expressing both H,K-ATPase subunits by replacing the wild-type baculovirus polyhedrin coding sequences as well as the p10 coding sequences. To our knowledge this is the first report on the in vitro synthesis of a functional H,K-ATPase and the use of this method for simultaneous expression of two enzyme subunits in the baculovirus expression system.

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Abbreviations H,K-ATPase (E.C. 3.6.1.36), magnesium-dependent hydrogen ion transporting and potassium-stimulated adenosine triphosphatase, SCH 28080, 3-(cyanomethyl)-2-methyl-8(phenylmethoxy)imidazo[1,2a]pyridine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CAPS, cyclohexylamino-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; Tween 20, polyoxyethylene sorbitan monolaurate; PBST,, phosphate-buffered saline, 0.05% Tween 20; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

2. MATERIALS AND METHODS

2.1. *Cells and viruses*

Sf9 cells were maintained either as monolayers in tissue culture flasks (Costar, Cambridge, MS, USA) or as suspension cultures in spinner flasks (Bellco, Vineland, NJ, USA) in TNM-FH medium supplemented with 5 mg/ml bovine serum albumin, 5 U/ml penicillin, 5 μg/ml streptomycin and 10% fetal calf serum (Hyclone, Logan, UT, USA). Recombinant viruses were produced either by a modified cal-

cium phosphate transfection method [17] or by a combined infection/transfection protocol [18]. Recombinant viruses were purified by successive rounds of limited dilution dot blot hybridisation assays [18] and/or by plaque purification assays. For production of viral stocks, 100 ml spinner cultures with $2–3·10^6$ Sf9 cells/ml were infected with a multiplicity of infection of 0.1–1 and infection was allowed to proceed for 3–5 days Then the culture medium was centrifuged at $1,000 \times g$ for 5 min at ambient temperature and the supernatant was filter-sterilized and stored at 4°C. For production of recombinant protein(s), Sf9 cells were grown to $1.5–3·10^6$ cells/ml in 100 ml complete growth medium in spinner flasks, and infected with virus at $1·10^7$ cells/ml in complete growth medium with a multiplicity of infection of 10–15. After 1 h incubation at 27°C cells were transferred to spinner flasks in 100 ml complete growth medium and incubated at 27°C for the indicated periods of time.

All other techniques regarding cell culture and baculovirus handlings were done according to Summers and Smith [17].

2.2. *Cloning of the α-subunit*

All DNA manipulations were done according to standard molecular biology techniques [19]. Two clones pRHK3.3 and pRHK1.8 [3] containing the carboxy-terminal and amino-terminal part of the rat α-subunit, respectively, were a gift from Dr. Gary E. Shull (University of Cincinnati, OH, USA). The clone pRHK1.8 was cut 5′ with *Cla*I, 3′ using *Bam*HI and subcloned into *Acc*I and *Bam*HI digested pUC18. A clone containing this fragment was cut with *Eco*RI and ligated to a 3.3 kbp *Eco*RI fragment of clone PRHK3.3, creating pUC18HKA1 In order to delete the internal *Bgl*II restriction site at position 2922 a 1.4 kbp *Bam*HI fragment (nt 1722 to 3092) of pUC18HK, was subcloned to M13mp19. The mutagenesis method from Kunkel [20] was followed using an in vitro mutagenesis kit from Bio-Rad (Veenendaal, The Netherlands). A synthetic oligonucleotide (5′ dCTTGAAGGTCTTGTA) (Pharmacia, Woerden, The Netherlands) was annealed to uracil containing ssDNA, extended using T7-DNA polymerase and closed covalently with T4-DNA ligase before transformation to *E. coli* CJ236. Successful mutagenesis was confirmed by restriction analysis of obtained clones The mutated 1.4 kbp *Bam*HI fragment was religated into PUC18, creating PUC18HK^m. For subcloning the entire α-subunit cDNA into a baculovirus transfer vector (including 5′ 54 nt and 3′ 158 nt untranslated sequences), a *Sfi*I–*Sca*I fragment from PUC18HK^m was blunt-ended using T4-DNA polymerase and ligated to *Bgl*II linkers (5′dGAAGACTCCT, New England Biolabs, Schwalbach, Germany), cut with *Bgl*II and after purification from agarose gels, ligated into *Bam*HI digested transfer vector DNA. Clones containing the α-subunit cDNA in the proper orientation relative to the polyhedrin promoter were identified by restriction analysis.

2.3. *Cloning of the β-subunit*

A cDNA clone (RS25–3) of the rat H,K-ATPase β-subunit [5] was a gift from Dr. Gary E. Shull (University of Cincinnati, OH, USA). A *Hin*dII–*Sph*I fragment from clone RS25–3 was subcloned into *Hin*dII and *Sph*I digested M13mp18. Site-directed mutagenesis using the in vitro mutagenesis kit from USB (Bad Homburg, Germany) based upon the mutagenesis method by Vandeyar et al. [21] was employed to create a *Bam*HI restriction site before the start codon using the synthetic oligonucleotide 5′-dCCCCCGTCCTAGGCTGTACCGTCG Restriction and sequence analysis were used to identify the correctly mutated clones. For subcloning into a baculovirus recombination vector the mutated clone was cut 3′ with *Sph*I, blunt ended using T4-DNA polymerase, ligated to *Bam*HI linkers and digested 5′ and 3′ with *Bam*HI. The purified fragment was ligated into *Bam*HI digested transfer vector DNA. Clones containing the β-subunit cDNA in the proper orientation relative to the polyhedrin or p10 promoter were identified by restriction analysis.

2.4. *Preparation of Sf9 membranes*

Cells were centrifuged at $1,000 \times g$ for 5 min at ambient temperature, resuspended at 1.10^7 cells/ml in icecold homogenisation buffer (HTSPI, 25 mM HEPES/Tris pH 7.0, 8.5% sucrose, 2 mM EDTA, 5 μg/ml leupeptin) and lysed by 3 pulses of 10 s with a probe sonicator (Branson Power Company, Denbury, USA) with subsequent cooling on ice. After centrifugation for 10 min at $4,000 \times g$ (4°C) the clarified cell lysate was centrifuged for 60 min at $100,000 \times g$ (4°C). Pelleted membranes, resuspended in 0.2 initial volume HTSE (25 mM HEPES/Tris pH 7.0, 8.5% sucrose, 2 mM EDTA), were further purified by centrifugation over a discontinuous gradient of 20% and 40% sucrose in HTSE for 60 min at $100,000 \times g$ (4°C). The pellet was resuspended in 0.1 initial volume HTSE and stored at −20°C. Unless stated otherwise, this membrane preparation was used in all activity measurements.

2.5. *Analysis of recombinant proteins*

Protein determinations were done using the Bio-Rad protein assay with bovine serum albumin as a standard. Protein samples from infected cells were solubilised in SDS-PAGE sample buffer and separated on SDS gels containing 10% acrylamide Then separated proteins were either stained with Coomassie brilliant blue or, for immunoblot, transferred to Immobilon polyvinylidenedifluoride (PVDF) membranes (Millipore, Bedford, MA, USA) in 10 mM CAPS buffer pH 11.0 containing 10% methanol at 100 V for 1 h at 4°C. For detection of H,K-ATPase α-subunit or β-subunit expression, protein blots were blocked for 2 h in PBS, 1% gelatin and then incubated overnight in either anti-α-subunit or anti-β-subunit antibody in PBST, 1% gelatin. Detection of antibody binding was established using either swine anti-rabbit and rabbit peroxidase anti-peroxidase for polyclonal sera or rabbit anti-mouse and mouse peroxidase anti-peroxidase for monoclonal sera (all from Dako, Glostrup, Denmark) in PBST, 1% gelatin for 1 h, before staining with 4-chloro-1-naphtol (50 μg/ml) in PBS containing 0.015% H_2O_2 and 17% methanol

2.6 *Semi-quantitative assay of H,K-ATPase α-subunit and β-subunit in infected Sf9 cells*

For quantification of expression of H,K-ATPase subunits in infected Sf9 cells we used a two-step ELISA method as initially described by Schalken et al. [22]. Briefly, protein samples of infected cells were solubilized in 1% Tween 20 and diluted with PBS to reduce the detergent concentration to 0.05%. Then twofold serial dilutions of the sample were incubated with antiserum for 1 h at 20°C in PBST. Next, the remaining free antibody was titrated on purified H,K-ATPase from pig gastric mucosa using standard ELISA techniques and compared to samples containing known amounts of conventionally purified enzyme [23].

2.7. *Phosphorylation of Sf9 membranes*

For determining the phosphorylation capacity of Sf9 membranes, 10–50 μg of membrane proteins were incubated in 50 μl of assay buffer (25 mM HEPES/Tris pH 7.0, 1 mM $MgCl_2$) in the presence or absence of either 100 μM SCH 28080 (gift of Dr. B Wallmark, Astra Hässle, Mölndal, Sweden) or 10 mM KCl for 1 h at 20°C, before adding 10 μl of [γ-^{32}P]ATP (3 Ci/mmol; Amersham, Buckinghamshire, UK) in assay buffer at the indicated concentration(s). Reaction was allowed to proceed for 10 s and then stopped with 5 ml of icecold 5% trichloroacetic acid, 100 mM H_3PO_4. Free label was removed by filtration over 1.2 μm filters (Schleicher and Schull, Dassel, Germany) and filters were washed three times with stopping solution before counting with 4 ml scintillation solution (Opti-fluor; Packard, Groningen, The Netherlands).

3. RESULTS

3.1. *Construction of recombinant viruses expressing either one or both H,K-ATPase subunits*

Using the methods described above, both H,K-ATPase α-subunit and β-subunit cDNA's were subcloned separately into baculovirus transfer vectors pAcDZ1

and pAcAS3 and the respective recombinant viruses were purified (Table I, 1–6). We next used two successive recombination steps to produce a single recombinant baculovirus expressing both α- and β-subunit from the AcNPV polyhedrin and p10 promoter, respectively. First the LacZ coding sequences were deleted from pAcDZ1 by digesting this transfer vector with *Eco*RI and *Xba*I. DNA ends were made blunt end and were religated to form pAcDLZ1 (Table I, 7). After cloning of the H,K-ATPase α-subunit cDNA into the *Bam*HI cloning site of this new vector it was used for recombination into the AcNPV polyhedrin locus to produce recombinant virus DLZα (Table I, 8) expressing the H,K-ATPase α-subunit from the polyhedrin promoter. This virus was purified using a limited dilution dot blot hybridisation assay as well as a plaque assay screening for polyhedron negative plaques. Then the vector pAcASβ (Table I,6) was used for recombination into the p10 locus of DLZα virus generating the new virus DLZαASβ (Table I, 10) expressing the α-subunit from the polyhedrin promoter as well as the β-subunit from the p10 promoter and β-galactosidase from the HSP70 promoter. This virus was purified using conventional plaque assay screening for blue plaques after the addition of X-Gal to the agarose overlay.

Using similar approaches, a number of different viruses expressing H,K-ATPase subunits and control viruses (i.e. after recombination with a transfer vector with no subunit cloned) listed in Table I were produced.

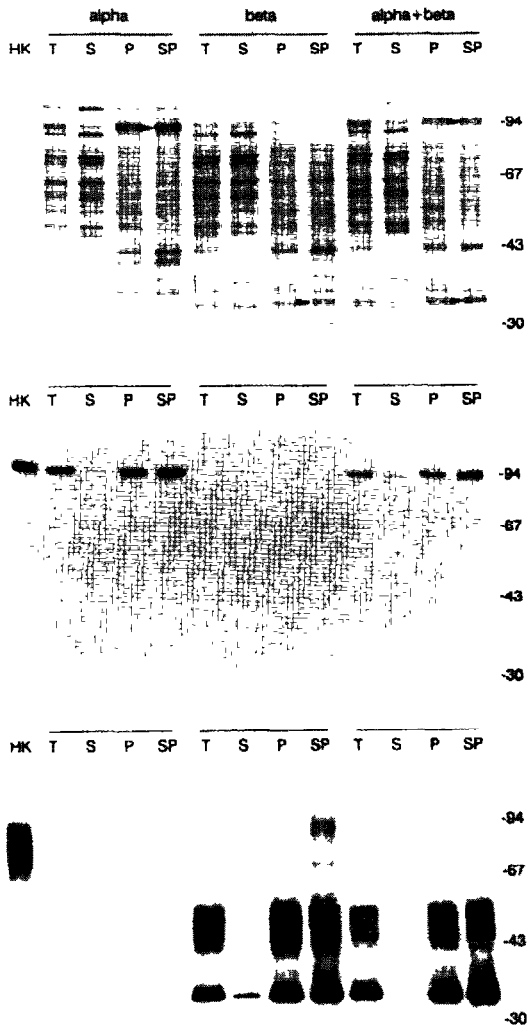

Fig. 1. Analysis of recombinant proteins expressed in Sf9 cells. The top figure shows a Coomassie stained SDS-polyacrylamide gel of protein samples from cells infected with either DLZαAS3 virus expressing the H,K-ATPase α-subunit (alpha), DZβ virus expressing the H,K-ATPase β-subunit (beta) or DLZαASβ virus expressing both H,K-ATPase subunits (alpha + beta). Protein samples were taken 48 h after infection. Except for HK (500 ng HK-ATPase from pig gastric mucosa) each lane contains 2.5 μg of protein. T, total protein sample from infected cells; S and P represent supernatant and pelleted membranes, respectively, after 1 h centrifugation of clarified cell lysate at 100,000 × g; SP, pellet obtained by centrifugation of pelleted membranes (P) over a discontinuous gradient of 20% and 40% sucrose (see section 2). The arrows indicate the position of protein bands representing H,K-ATPase α-subunit (at 95 kDa) and unglycosylated β-subunit (at 34 kDa) in the purified membrane fraction (SP). The middle figure shows Western blot of proteins in top figure identified by a monoclonal antibody against the H,K-ATPase α-subunit (95–111) [32,33]. The bottom figure shows a Western blot using a monoclonal antibody directed against the H,K-ATPase β-subunit (2G11) [34]. At the right, the positions of molecular mass standards (in kDa) are indicated.

3.2. *Expression of H,K-ATPase subunits*

Fig. 1 shows that infection of Sf9 cells with purified recombinant virus leads to expression of both H,K-ATPase α- and β-subunits. Expression of the α-subunit leads to formation of an extra protein band clearly visible in Coomassie stained SDS-PAGE gels, with an apparent molecular mass of 95 kDa, which is exactly the same size as that of native H,K-ATPase purified from pig gastric mucosa [23]. This protein band is furthermore identified by several different antisera to the α-

Table I

Overview of recombinant baculoviruses produced.

no.	Viral code	Polyhedrin locus	p10 locus
1	DZ1	β-gal	p10
2	DZα	α-subunit, β-gal	p10
3	DZβ	β-subunit, β-gal	p10
4	AS3	polyhedrin	β-gal
5	ASα	polyhedrin	α-subunit, β-gal
6	ASβ	polyhedrin	β-subunit, β-gal
7	DLZ1	-	β-gal
8	DLZα	α-subunit	p10
9	DLZαAS3	α-subunit	β-gal
10	DLZαASβ	α-subunit	β-subunit, β-gal
11	DLZβ	β-subunit	p10
12	DLZβAS3*	β-subunit	β-gal
13	DLZβASα*	β-subunit	α-subunit, β-gal

Behind each viral code, H,K-ATPase subunit(s) expressed in the polyhedrin locus or p10 locus are indicated; β-gal, β-galactosidase.
*indicates viruses in the process of being purified

subunit (monoclonal as well as polyclonal antibodies, not shown).

Expression of the β-subunit also leads to the production of an extra protein band visible in Coomassie stained gels and could be visualised by different antisera to the H,K-ATPase β-subunit. The β-subunit appears both as a single protein band of 34 kDa and partly as a smear of approximately 40–50 kDa. This smear can be explained by different glycosylation patterns of the protein produced in the insect cells since the rat β-subunit contains 7 potential N-glycosylation sites and Sf9 cells are known to be capable of formation of N-linked sugars on peptide chains expressed by infection with recombinant baculoviruses [7]. The protein band at 34 kDa could be identified as the naked core protein of the β-subunit. Indeed when infected cells were incubated in the presence of 5 μg/ml tunicamycin, an inhibitor of the N-glycosylation process, the smear identified by the anti-β-subunit antisera disappeared, leading to an increase of the immunoreactive protein band at 34 kDa (Fig. 2). Although the in vitro produced β-subunit in the absence of tunicamycin is glycosylated, glycosylation patterns were not as extensive as in the native enzyme which appeared on SDS-PAGE as a protein with an apparent molecular mass of 60–80 kDa, while the glycosylated β-subunit as produced by Sf9 cells had a molecular mass between 40 and 50 kDa.

The majority of H,K-ATPase subunits produced in insect cells were located in Sf9 membranes as demonstrated by immunofluorescence analysis (not shown). This is furthermore supported by the finding that purified Sf9 membranes show an enrichment of each subunit by a factor 2.8 compared to total protein samples as calculated by ELISA (not shown) and demonstrated by immunoblot (fig 1, lanes SP vs. T). Nearly all of the immunoreactive protein in the clarified cell lysate (lane T) can be recovered by centrifugation at $100{,}000 \times g$ (lane P vs. S), indicating either membrane association or membrane localisation.

3.3. *Phosphorylation capacities of Sf9 membranes*

Native H,K-ATPase can be phosphorylated by the addition of ATP to purified enzyme. This autophosphorylation is a very fast reaction reaching saturation in less than 1 s and can be inhibited by preincubation of H,K-ATPase with SCH 28080 [24,25]. Since the K^+-dependent dephosphorylation reaction is the rate limiting step in the reaction cycle of H,K-ATPase [26], phosphorylation levels are maximal in the complete absence of K^+ ions. Addition of K^+ to the reaction mixture leads to a lowering of the phosphorylation level. Furthermore, K^+ reduces the affinity of H,K-ATPase for ATP. Therefore, the inhibitory effect of K^+ on phosphorylation levels is more pronounced at low ATP concentrations.

In order to investigate whether the in vitro produced subunits were functionally active, we measured phosphorylation levels of purified Sf9 membranes from cells infected with different recombinant baculoviruses. Phosphorylation reactions were measured at 0.1 μM ATP in the presence of 1 mM $MgCl_2$. Only membranes containing both H,K-ATPase subunits exhibit a K^+-and SCH 28080-sensitive phosphorylation capacity (20–27% of total; Table II), indicating that only cells expressing both subunits contain an functional H,K-ATPase. In cells expressing either α- or β-subunit alone, some K^+-sensitive (but SCH 28080-insensitive) phosphorylation was found. Absolute phosphorylation levels ranged between 3.90 and 12.9 pmol P/mg membrane protein. When an ATP concentration of 2.0 μM instead of 0.1 μM was used the absolute SCH 28080-sensitive phosphorylation capacity increased approximately twofold but phosphorylation levels not inhibitable by SCH 28080, increased even more (up to tenfold), resulting in relatively less inhibition of total ATP-sensitive phosphorylation by SCH 28080. The nature of the nonspecific phosphorylation capacity is under investigation.

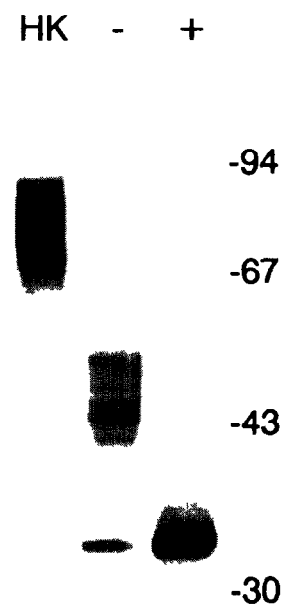

Fig. 2. Western blot analysis of total protein samples from Sf9 cells infected with DZβ virus expressing the H,K-ATPase β-subunit. Protein samples (approximately 2.5 μg) were taken 48 h after infection. Cells were incubated in the presence (+) or absence (−) of 5 μg/ml tunicamycin. HK represents 500 ng of H,K-ATPase from pig gastric mucosa. The blot was incubated with monoclonal antibody 2G11 [34] to detect expression of the β-subunit. At the right, the positions of molecular mass standards (in kDa) are indicated.

4. DISCUSSION

The baculovirus expression system has become one of the most popular expression systems for eukaryotic proteins due to the formation of high levels of active

Table II

Phosphorylation capacities of purified Sf9 membranes

	+ SCH 28080	+ KCl
alpha	103.2 (1 9)	90.9 (5.1)
beta	99.5 (2.2)	88.2 (7.4)
alpha + beta	80.7 (11.1)	72.9 (5.9)

Cells were infected with DLZαAS3 virus expressing the H,K-ATPase α-subunit (alpha), DZβ virus expressing the β-subunit (beta) or DLZαASβ virus expressing both H,K-ATPase subunits (alpha + beta) at 48 h post infection. Phosphorylation capacities were measured according to Materials and Methods in the presence of 0.1 μM ATP and 1.0 mM $MgCl_2$, with and without either 100 μM SCH 28080 or 10 mM KCl. Phosphorylation values are expressed as percentage of values in the absence of inhibitor. 100% phosphorylation values are: alpha 7.3 (4.9) pmol/mg, beta 7.0 (2.9), alpha+beta 5.9 (2.1) pmol/mg. Values between parenthesis indicate the standard error of the mean of three independent infections

protein. Expression of multisubunit proteins with the baculovirus expression system has been reported using a mixture of recombinant viruses coding for different subunits. This approach, however, needs precise titration of each virus to reach a homogeneous distribution of both proteins between individual Sf9 cells. A problem using this approach might be that a cell once infected by one virus could become less susceptible for infection by a second virus. Infected cells therefore are likely to express one subunit in favour over the other subunit. An alternative approach for simultaneous expression of two proteins has become possible using recombination vectors with multiple promoters.

We used a simple but efficient method to create a single recombinant baculovirus expressing both H,K-ATPase subunits using a double recombination event. We used two different transfer vectors for recombination at different loci in the wild-type baculovirus genome using both polyhedrin and p10 promoters to drive expression of H,K-ATPase α-subunit and β-subunits. The use of this method for simultaneous expression of two protein subunits has been postulated before [15] but to our knowledge the use of it has never actually been reported.

Since no apparent function for the β-subunit of gastric H,K-ATPase is known, it was postulated that a presynthesized β-subunit could be necessary for correct assembly with the α-subunit for transport and insertion into the cell membrane. Expression of either the α- or β-subunit alone, however, leads to accumulation of recombinant protein subunits in Sf9 membranes, suggesting that one subunit is not essential for correct transport and insertion of the other subunit into Sf9 membranes. This seems to be a unique feature of Sf9 cells, since this has also been described for Na,K-ATPase [27] while studies with other expression systems indicate that both with H,K-ATPase and with Na,K-ATPase both subunits are necessary for transport and insertion into cell membranes [28,29].

In order to determine wether or not the in vitro produced protein was functional, we measured phosphorylation capacities of purified Sf9 membranes. Only cells expressing both H,K-ATPase subunits exhibit a K^+- as well as SCH 28080-sensitive phosphorylation activity indicating that the in vitro produced protein is capable of performing the same conformational changes as native H,K-ATPase. We therefore conclude that both subunits are involved in formation of a functional enzyme.

SCH 28080- and K^+- sensitive phosphorylation capacities of Sf9 membranes containing both H,K-ATPase subunits are low compared to purified enzyme (1 pmol/mg vs. 2 nmol/mg). Since estimates from ELISA experiments and Coomassie blue staining indicate that at least 5% of the membrane proteins in the infected cells are the two subunits of H,K-ATPase, this indicates that large amounts of produced subunits do not form a functional enzyme. This defect can be explained by several options. First it is yet unknown if all proteins produced are correctly folded in the Sf9 membranes, which is of course a prerequisite for the formation of an active enzyme. Secondly it is unknown if all α-subunits produced are also involved in formation of an α,β heteroduplex molecule. Thirdly it could be possible that once some functional H,K-ATPase has been produced, the activity of the in vitro produced enzyme interferes with Sf9 cell metabolism, because of cellular alkalanization, inhibiting the formation of more functional enzyme. This option is currently being investigated. Fourthly it might be that the glycosylation pattern of the expressed β-subunit, which is clearly different from that of the native subunit, is not optimal for full activity. Lastly it might be that native H,K-ATPase is not active as a single α,β-oligomer but as a dimer or polymer of equimolar amounts of both α- and β-subunits [30,31]. If the latter is true, successful formation of large amounts of a functional H,K-ATPase in vitro would be even more dependent on the expression system used and difficult to establish. Nevertheless, because baculovirus infected Sf9 cells are capable of forming a functional H,K-ATPase we conclude that the baculovirus expression system can provide a useful method for studying the characteristics of gastric H,K-ATPase.

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