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The β-subunit of the gastric H^+/K^+-ATPase can occur without the α-subunit

G.S. Baldwin

Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, PO Royal Melbourne Hospital, Victoria, 3050, Australia

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The renal Na^+/K^+-ATPase and the gastric H^+/K^+-ATPase both contain α- and β-subunits. We report here the identification and partial purification of a second population of the β-subunit of the gastric H^+/K^+-ATPase, which has no accompanying α-subunit detectable by Coomassie blue staining or by Western blotting with monoclonal antibodies specific for the α-subunit.

Proton pump; ATPase

1. INTRODUCTION

The gastric ATPase produces the acid released by the gastric mucosa. The enzyme catalyses the ATP-dependent counter transport of protons and potassium ions across the tubulo-vesicular membranes of the gastric parietal cell [1]. Until recently the H^+/K^+-ATPase was thought to consist of a single α-subunit of apparent molecular mass 95,000–100,000 [2], which was phosphorylated on an aspartic acid residue during the catalytic cycle [3]. The entire protein sequences of the porcine (1034 amino acids [4]) and rat (1033 amino acids [5]) α-subunits have been deduced from the nucleotide sequences of the corresponding cDNAs. In the past year several groups have independently provided evidence for the existence of an extensively glycosylated β-subunit of the H^+/K^+-ATPase, of apparent molecular mass 60,000–90,000 [6–9]. The sequence of the 290 amino acid core protein of the β-subunit has been deduced from the nucleotide sequence of the corresponding porcine [9] and rat [10] cDNAs. Both α- [11,12] and β-subunits [9] are targets for autoimmune attack in atrophic gastritis and pernicious anaemia.

The α- and β-subunits of the porcine gastric H^+/K^+-ATPase are both related (63% and 33%, respectively) to the corresponding subunits of the porcine Na^+/K^+-ATPase. By analogy with the Na^+/K^+-ATPase whose α- and β-subunits are both essential for ouabain binding and ouabain-sensitive ATPase activity [13], the β-subunit of the H^+/K^+-ATPase is presumably also essential for enzyme activity. In this context it is of particular interest that a population of the β-subunit without any accompanying α-subunit can be isolated from porcine gastric mucosal membranes.

Correspondence address: G.S. Baldwin, Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, PO Royal Melbourne Hospital, Victoria, 3050, Australia.

2. MATERIALS AND METHODS

Concanavalin-A Sepharose and DEAE-Sepharose were from Pharmacia, Uppsala, Sweden. The monoclonal antibodies 1H9 and 2B6, which are specific for the α- and β-subunits of the gastric H^+/K^+-ATPase, respectively, were derived from mice with autoimmune gastritis induced by neonatal thymectomy [14].

Membranes from the mucosa (200 g) of porcine gastric corpus were collected by differential centrifugation between 600 *g* and 27,600 *g* as previously described [15]. All subsequent steps were performed at 4°C. The membranes were washed by homogenization in 100 ml 50 mM Na^+ HEPES, 1 mM EDTA, 1 mM PMSF (pH 7.6) (buffer A), and collected by centrifugation at 27,600 *g* for 30 min. The washed membranes were resuspended in 50 ml 0.4% digitonin, 0.08% Na^+ cholate in buffer A with 6 stroked of a Wheaton glass-glass homogenizer (Type A). The suspension was immediately centrifuged (27,600 *g*, 30 min), and the digitonin/cholate pellet was resuspended in 50 ml 1% Triton X-100 in buffer A with 6 strokes of a Wheaton homogenizer. The suspension was allowed to stand for 30 min, and centrifuged (27,600 *g*, 30 min) to yield the Triton extract (40 ml).

10 ml concanavalin A-Sepharose was equilibrated with 20 ml 50 mM Na^+ HEPES, 1 mM $MnCl_2$, 1 mM PMSF, 0.1% Triton X-100 (pH 7.6) (buffer B) and transferred to a 50 ml tube containing the Triton extract, which had been made 2 mM in $MnCl_2$. The tube was rotated end over end for 60 min and centrifuged at 1000 *g* for 5 min. The supernatant fluid was removed, and the Sepharose was transferred to a column (1.5 × 8 cm), washed with 20 ml buffer B, and eluted with 20 ml of buffer B containing 300 mM α-methylglucopyranoside. 5 ml of eluate were collected immediately and the remaining 15 ml 60 min later.

10 ml DEAE-Sepharose was equilibrated with 20 ml 50 mM Na^+ HEPES, 1 mM EDTA, 1 mM PMSF, 0.1% Triton X-100, pH 7.6 (buffer C), and transferred to a 50 ml tube containing the concanavalin A-Sepharose eluate, which had been made 2 mM in EDTA. The tube was rotated end over end for 60 min and centrifuged at 1000 *g* for 5 min. The supernatant fluid was decanted, and the Sepharose was transferred to a column (1.5 × 8 cm) and washed with 20 ml buffer C. The column was then successively eluted with 20 ml aliquots of buffer C containing 75, 150 and 500 mM NaCl. The run through and

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wash, and the fraction eluting with buffer C containing 75 mM NaCl, were concentrated to 1 ml and 1.3 ml, respectively, with an Amicon ultrafiltration cell (Model 8050, Amicon Corp., Danvers, MA).

Samples from column fractions were subjected to electrophoresis on SDS-10% polyacrylamide gels, and either visualised by staining with Coomassie blue or transferred to immobilin-P membranes (Millipore Corp., Bedford, MA) with a Sartoblot II blotting apparatus (Sartorius GmbH, Göttingen, FRG) according to the maker's instructions. Membranes were shaken in 200 ml 10% skim milk powder in phosphate-buffered saline containing 0.1% Tween-20 and 0.02% Na^+ azide (buffer D) for 30 min, and sealed in a plastic bag containing 20 ml of a 1 in 500 dilution of antibody in buffer D. The bag was shaken for 16 h at 4°C. The membranes were then washed in 3 × 100 ml buffer D (20 min, 4°C), and shaken in 20 ml of a 1 in 3000 dilution of goat anti-mouse immunoglobulin conjugated to alkaline phosphatase (Biorad, Richmond, CA) for 60 min at 4°C. After washing in 3 × 100 ml 10 mM Tris, 150 mM NaCl, pH 7.5 (10 min, 20°C), bound antibody was detected as described by Leary and coworkers [16].

3. RESULTS

A population of the β-subunit of the gastric H^+/K^+-ATPase was partially purified from Triton extracts of porcine gastric mucosal membranes which had been initially extracted with 0.4% digitonin/0.08% cholate to remove peripheral membrane proteins. The apparent M_r of the β-subunit of the gastric H^+/K^+-ATPase complex decreased from 60,000–90,000 to 35,000 following treatment with *N*-glycanase [9], and as expected for such an extensively glycosylated protein, an efficient purification was achieved by chromatography on concanavalin A-Sepharose (Fig. 1). The β-subunit was purified further by chromatography on DEAE-Sepharose (Fig. 1).

The partially purified β-subunit did not appear to be accompanied by any α-subunit. Thus neither the concentrated DEAE-Sepharose run through and wash (Fig. 2A), nor the concentrated DEAE-Sepharose 75 mM NaCl eluate (Fig. 2D), contained any trace of the 92 kDa α-subunit of the gastric H^+/K^+-ATPase, which could be readily detected by Coomassie blue staining in samples of the gastric H^+/K^+-ATPase prepared from gastric microsomal membranes by the method of Ray [18] (Fig. 2E). The β-subunit itself was not detected by Coomassie blue staining, presumably because of its extensive glycosylation. The 78 kDa protein detected in the DEAE-Sepharose fractions has previously been shown to bind gastrin, and is a candidate for the gastric parietal cell gastrin receptor [19].

The hypothesis that a population of β-subunit with no associated α-subunit had been purified was confirmed by Western blotting. A monoclonal antibody (2B6) specific for the β-subunit [9,14] reacted strongly with both the concentrated DEAE run through and the gastric H^+/K^+-ATPase preparation (Fig. 2C). In contrast, a monoclonal antibody (1H9) specific for the α-subunit [9,14] reacted with the gastric H^+/K^+-ATPase preparation only (Fig. 2B). Further evidence that the β-subunit population described here was different from the β-subunit of the H^+/K^+-ATPase complex was provided by the difference in M_r of the two glycoproteins. Thus the apparent M_r of the β-subunit of the gastric H^+/K^+-ATPase was 55,000–90,000 (Fig. 2C, track 4), while the apparent M_r of the free β-subunit was 65,000–95,000 (Fig. 2C, track 2). Both preparations yielded a 35 kDa core protein after treatment with *N*-glycanase (data not shown).

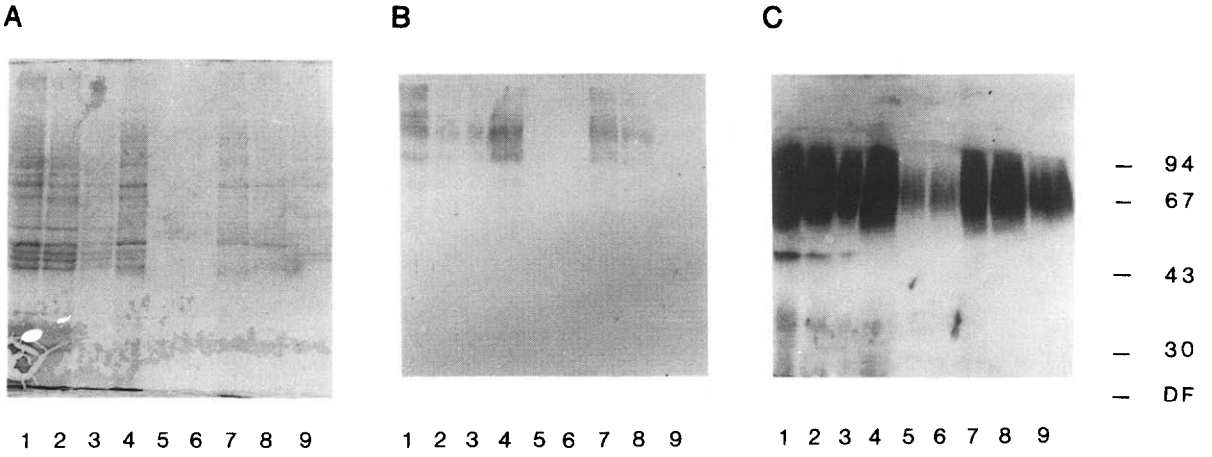

Fig. 1. Partial purification of the β-subunit of the gastric H^+/K^+-ATPase. The β-subunit of the gastric H^+/K^+-ATPase was purified from Triton extracts of porcine gastric mucosal membranes as described in the Materials and Methods section. Samples (1 μl, Triton extract, conanavalin A-Sepharose run through and wash; 25 μl, all other fractions) were reduced with dithiothreitol and run on 3 parallel 10% polyacrylamide gels. One gel (A) was stained with Coomassie blue, while the other gels were Western blotted to Immobilon membranes. The blots were probed with monoclonal antibodies specific for the α- (B) and β- (C) subunits of the gastric H^+/K^+-ATPase. The figures on the right represent the sizes of molecular mass markers in kDa, and DF represents the dye front. 1, Triton extract; 2, concanavalin A-Sepharose run through; 3, concanavalin A-Sepharose wash; 4, concanavalin A-Sepharose eluate; 5, DEAE-Sepharose run through; 6, DEAE-Sepharose wash; 7, DEAE-Sepharose 75 mM NaCl eluate; 8, DEAE-Sepharose 150 mM NaCl eluate; 9, DEAE-Sepharose 500 mM NaCl eluate.

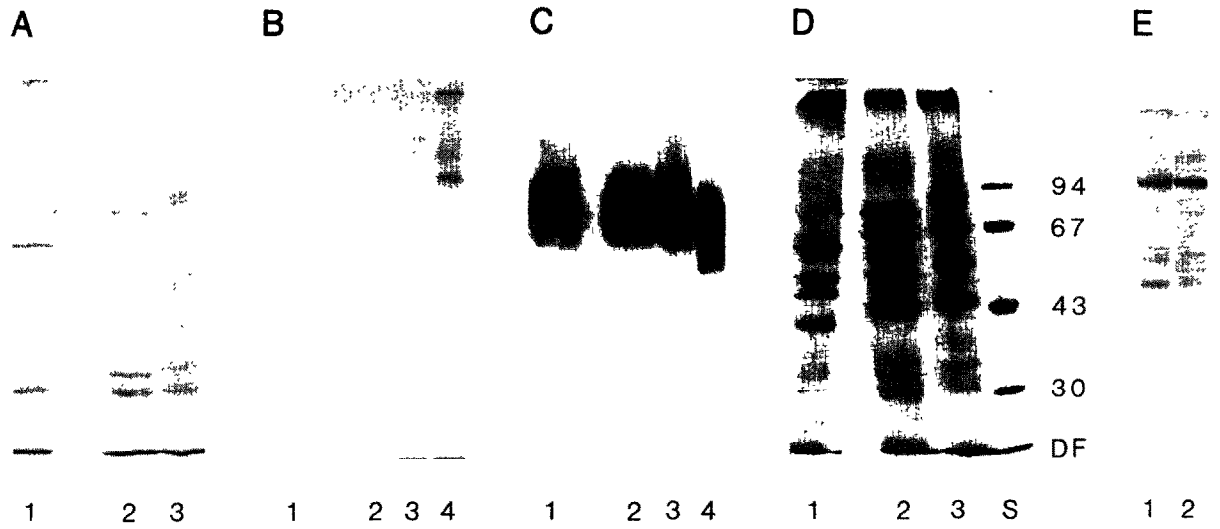

Fig. 2. Absence of the α-subunit from the β-subunit preparations. Aliquots of the concentrated DEAE-Sepharose run through and wash (A, B, C, 12 μg protein), the concentrated DEAE-Sepharose 75 mM NaCl eluate (D, 42 μg protein), and a gastric microsomal ATPase preparation (E, 3.3 μg protein) were treated with an equal volume of Laemmli loading buffer without (track 1) or with (track 2) 50 mM dithiothreitol. A third aliquot (track 3) of both concentrated DEAE-Sepharose fractions was reduced and carboxymethylated with iodoacetic acid as previously described [17], and treated with Laemmli loading buffer containing 50 mM dithiothreitol. All samples were run in parallel on 10% polyacrylamide gels. Gels A, D and E were stained with Coomassie blue, while gels B and C were Western blotted to Immobilon membranes. Reduced samples of the gastric microsomal H^+/K^+-ATPase (track 4, 2.5 μg) were also electrophoresed on gels B and C as positive controls. The blots were probed with monoclonal antibodies specific for the α-subunit (B) or the β-subunit (C) of the gastric H^+/K^+-ATPase. The sizes of molecular mass markers (track S) are given in kDa, and DF represents the dye front.

4. DISCUSSION

In this paper evidence is presented that a population of the β-subunit of the gastric H^+/K^+-ATPase exists without any associated α-subunit. This separation of subunits is not due to removal of the α-subunit during pre-extraction of the membranes with digitonin/cholate, since similar results are obtained when membranes are extracted with Triton alone (data not shown). Although a 78 kDa gastrin-binding protein co-purifies with the β-subunit during chromatography on concanavalin A-Sepharose and DEAE-Sepharose, separation of the two proteins on tomato lectin-Sepharose rules out the possibility of a strong association between the two proteins (data not shown).

Previous immunohistochemical studies suggest that the β-subunit of the Na^+/K^+-ATPase also exists in two populations [20]. In the basolateral membranes of epithelial cells from rat distal colon β-subunit is detected together with α-subunit. In contrast, the apical membranes of the same cells contain β-subunit, but no detectable α-subunit. Similar studies on canine gastric mucosa with the monoclonal antibodies 1H9 and 2B6 co-localize the β-subunit of the H^+/K^+-ATPase together with the α-subunit on the tubulo-vesicular membranes of the parietal cell [9]. However, the evidence presented in this paper for a population of the β-subunit of the H^+/K^+-ATPase with no accompanying α-subunit in porcine gastric mucosa suggests either that the subcellular localization of the β-subunit differs in different species, or that the α- and β-subunits are present in the tubulo-vesicular membranes in unequal amounts.

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