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Functional Expression of the Colonic H^+,K^+-ATPase α-Subunit

PHARMACOLOGIC PROPERTIES AND ASSEMBLY WITH X^+,K^+-ATPase β-SUBUNITS*

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Juan Codina, Bruce C. Kone‡, Juan T. Delmas-Mata, and Thomas D. DuBose, Jr.§

From the Departments of Internal Medicine and Integrative Biology, The University of Texas Medical School at Houston, Houston, Texas 77030

The functional and pharmacological properties of the α-subunit of the colonic H^+,K^+-ATPase (α_C) were studied in *Xenopus laevis* oocytes. α_C was injected with different rat β-subunits, the β-subunit of the gastric H^+,K^+-ATPase (β_G, the only H^+,K^+-ATPase β-subunit identified in rat), or the β_1-subunit of the Na^+,K^+-ATPase (β_1) (associated with the basolateral Na^+,K^+-ATPase, but also expressed in the epithelial apical membranes of rat distal colon) (Marxer, A., Stieger, B., Quarini, A., Kashgarian, M., and Hauri, H. P. (1989) *J. Cell Biol.* 109, 1057–1069). The effect of the different β-subunits was studied by measuring $^{86}Rb^+$ uptake (a K^+ congener) in the presence or absence of Sch-28080 and ouabain. Significant Na^+-independent $^{86}Rb^+$ uptake was observed only when α_C was coexpressed with one of the β-subunits. The expressed $\alpha_C\beta_1$ and $\alpha_C\beta_G$ complexes were not inhibited by Sch-28080, were only partially sensitive to ouabain (IC_{50} = 400–600 μM, in the presence of external 1 mM KCl), and exhibited comparable K^+ activation kinetics. Coexpression of α_C with epitope-tagged β_G or β_1, followed by immunopurification of the $\alpha\beta$ complexes, confirmed stable assembly of $\alpha_C\beta_G$ and $\alpha_C\beta_1$ complexes. Since the β_1-subunit, but not the α_1-subunit, of Na^+,K^+-ATPase is expressed in the apical membrane of rat colonocytes, our data support the view that, in rat distal colon, the β_1-subunit may play a surrogate role as the β-subunit for the colonic H^+,K^+-ATPase.

The H^+,K^+-ATPase comprises a group of integral membrane proteins that belong to the X^+,K^+-ATPase[1] subfamily of P-type cation-transporting ATPases (1). The X^+,K^+-ATPases, which also include the Na^+,K^+-ATPase isozymes, share a common catalytic cycle, the ability to extrude a cation (Na^+ or H^+, respectively) from the cell in exchange for K^+, and an apparent requirement for heterodimeric structure (2–4). To date, cDNAs encoding homologous H^+,K^+-ATPase α-subunits have been cloned from gastric parietal cells (α_G or HKα1) (5, 6), rat and guinea pig distal colon (α_C or HKα2) (7, 8), toad urinary bladder (α_{bl} or HKα3) (9), and human skin (*ATP1AL1* or HKα4) (10). Although these H^+,K^+-ATPase isoforms share approximately 60–70% amino acid identity, they exhibit distinct kinetic and pharmacological properties when expressed in heterologous systems (9, 11, 12). While the physiological role of the gastric H^+,K^+-ATPase isozyme in mediating gastric acid secretion is widely recognized, the biological roles of the other isoforms have not been clearly established.

Recent molecular biological and biochemical studies indicated that the colonic H^+,K^+-ATPase participates in the chronic adaptation to changes in K^+ homeostasis (7).[2] While K^+ balance is governed principally by the kidney, the colon plays a smaller but highly significant role. During chronic K^+ restriction, active K^+ reabsorption by epithelial cells of the renal collecting duct and distal colon serves to restore K^+ balance (13). K^+-ATPase activities are expressed in these cell types, and these activities are up-regulated during chronic dietary K^+ depletion. The K^+-ATPase activity in the renal collecting duct was reported to be ouabain-resistant and Sch-28080-sensitive, findings compatible with the established properties of α_G (14). However, the fact that expression of α_G mRNA is not significantly altered in the medullary collecting duct during chronic dietary K^+ depletion (15), and that it is not expressed in the distal colon, makes it unlikely that this gene plays a role in K^+ adaptation in either the kidney or the distal colon (16).[2] In contrast, α_C mRNA is principally expressed in the renal collecting duct and surface epithelial cells of the distal colon, and its abundance increases 3–5-fold in the renal medulla during chronic dietary K^+ depletion (17).[2] Thus, α_C has emerged as the candidate gene most likely to mediate K^+ conservation. Consequently, considerable effort has been devoted to define the functional properties of α_C.

Biochemical studies found that the apical membranes of surface epithelial cells of distal colon express distinct ouabain-sensitive (18, 19) and ouabain-insensitive (19, 20) K^+-ATPase activities. Since α_C is the only X^+,K^+-ATPase α-subunit known to be expressed in the apical membranes of these cells, these results suggested either the existence of a novel α-subunit, or the possibility that different $\alpha_C\beta$-subunit complexes exhibit different ouabain sensitivities. The latter possibility gained credence with the recent conflicting reports regarding the pharmacological properties of α_C expressed in heterologous systems. Lee *et al.* (11) demonstrated that expression of α_C without an exogenous β-subunit in baculovirus-infected *Spodoptera frugiperda* (Sf-9) cells yielded K^+-ATPase activity that was resistant to high concentrations (1 mM) of ouabain, but inhibited by high concentrations (100 μM) of Sch-28080. Subsequently, Cougnon *et al.* (12) reported that coinjection of *Xenopus laevis*

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§ To whom correspondence should be addressed: Dept. of Internal Medicine, Division of Renal Diseases and Hypertension, The University of Texas Medical, School at Houston, 6431 Fannin, MSB 4.148, Houston, TX 77030. Tel.e: 713-792-5425; Fax: 713-794-1197; E-mail: tdubose@heart.med.uth.tmc.edu.

[1] The abbreviations use are: α_G, α-subunit of the gastric H^+,K^+-ATPase (also called HKα1); α_C, α-subunit of the colonic H^+,K^+-ATPase (also called HKα2); β_G, β-subunit of the gastric H^+,K^+-ATPase; β_1, β_1-subunit of the Na^+,K^+-ATPase; NMDG, *N*-methyl-D-glucosamine; PIPES, 1,4-piperazinediethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; X^+, undesignated cation.

[2] Codina, J., Pressley, T. A., and DuBose, T. D., Jr. (1996) *Am. J. Physiol.*, in press.

oocytes with cRNAs encoding α_C and a β-subunit from toad bladder (β_{bl}) resulted in functional H^+,K^+-ATPase activity that was described as ouabain-sensitive ($IC_{50} \sim 970$ μM at 5 mM external K^+ concentration) but Sch-28080-resistant. Neither study, however, examined the expression of an H^+,K^+-ATPase holoenzyme comprised of α_C and a mammalian β-subunit, nor did they directly establish whether α_C can complex with any of the known β-subunits. These limitations assume considerable importance when one considers that different β-subunits, when coexpressed with the α-subunits of the gastric H^+,K^+-ATPase or the Na^+,K^+-ATPase, may confer unique functional properties on the holoenzyme (21, 22).

Accordingly, we used the oocyte expression system to examine the functional properties of α_C when it is coexpressed with either of two rat X^+,K^+-ATPase β-subunits: the β-subunit of the gastric H^+,K^+-ATPase (β_G), which is expressed in renal collecting duct (4) but not distal colon (23), and the β_1-subunit of the Na^+,K^+-ATPase (β_1), which is expressed in both collecting duct (24) and in apical and basolateral membranes of colonocytes (25) in distal colon. We used this expression system instead of the baculovirus system, because Sf-9 cells exhibit endogenous K^+/H^+ exchange (26) that would likely confound interpretation of K^+ activation kinetics for a heterologously expressed H^+,K^+-ATPase. In addition, we constructed β_G- and β_1-subunits bearing a common c-*myc* epitope to test, in a coimmunoprecipitation assay (27), whether α_C stably assembles with β_G and/or β_1. The results indicate that α_C can interact with both β-subunits, that oligomerization is required for functional activity of the expressed enzymes in this system, and that the inhibitor sensitivities and K^+ activation kinetics of $\alpha_C\beta_G$ and $\alpha_C\beta_1$ holoenzymes are very similar. The ability of β_1 to support functional activity of α_C combined with the colocalization of these subunits in the apical membrane of the colonocytes (25) suggests that $\alpha_C\beta_1$ likely represents at least one of the K^+-ATPase activities expressed in this locale, and that the existence of a "β_C"-subunit need not be necessarily invoked.

EXPERIMENTAL PROCEDURES

Materials—The α_C (7) and α_G (5) cDNAs were gifts from Dr. G. Shull (University of Cincinnati). The β_1 cDNA (28) was a gift from Dr. T. A. Pressley (Texas Tech University). The β_G cDNA was cloned as described below. The expression vector pAGA#2 was a gift from Dr. L. Birnbaumer (University of California at Los Angeles) (29). mAb 9E10 was purified from culture supernatants of hybridoma myc 1–9E10.2 (American Type Culture Collection, Rockville, MD). Restriction enzymes were from Promega Biotech Inc. and New England Biolabs (Beverly, MA). T7 Cap Scribe was from Boehringer Mannheim. The *X. laevis* oocytes were prepared and injected in Dr. L. Parent's laboratory (Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX). Oligonucleotides were synthesized by Genosys (The Woodlands, TX). The remaining reagents were of the best available quality.

Cloning of cDNA Encoding the Gastric H^+,K^+-ATPase β-Subunit—The complete open reading frame of β_G was generated by a polymerase chain reaction using cDNA prepared from total rat stomach RNA and primers based on the published sequence (23), according to the conditions previously described by our laboratory (16). The sense oligonucleotide (5′-ATTCCATGGCAGCCCTGCAGGAGAAG-3′) contained an *Nco*I site (underlined), and the antisense oligonucleotide (5′-TACGGTCGACTTACTTCTGTATTGTGAGCTT-3) contained a *Sal*I site (underlined) to facilitate subcloning into pAGA#2. The correct sequence was verified by direct sequencing (30) of one of the clones.

cRNA Synthesis and Protein Expression in Xenopus Oocytes—Encoding DNAs for rat α_G-, α_C-, β_G-, and β_1-subunits were subcloned into pAGA#2. The recombinant molecules were linearized with *Hin*dIII or *Xho*I as appropriate, and capped cRNAs were synthesized using T7 RNA polymerase and T7 Cap Scribe (Boehringer Mannheim) according to the manufacturer's methods. Parametric studies demonstrated that full-length cRNAs were generated for each gene. Stage V–VI oocytes obtained from *X. laevis* were injected with 10 ng of cRNA or an equivalent volume of water and incubated at 19 °C in modified Barth's medium (31). Three days later, $^{86}Rb^+$ uptake was measured according to the procedure described by Modyanov *et al.* (1). Briefly, the oocytes were equilibrated in solution A (90 mM NaCl (in some instances 90 mM NMDG was used), 1 mM $MgCl_2$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 5 mM $BaCl_2$, and 10 mM PIPES, pH 7.4) for 15 min. Subsequently oocytes were preincubated in the presence or absence of inhibitors (ouabain or Sch-28080) in buffer A for 15 min. The oocytes were then incubated in buffer A containing 5–10 $\times$ 10^6 cpm $^{86}Rb^+$ and 1–5 mM KCl in the presence or absence of inhibitors. All incubations were performed at room temperature, and 1 μM ouabain was added to all the buffers to inhibit the activity of endogenous *X. laevis* Na^+,K^+-ATPase (1). After 15 min, the reaction was stopped by aspirating the bulk of the radioactivity and washing three times with 4 ml of buffer A at 4 °C. Finally, the oocytes were disrupted by pipetting up and down, transferred to scintillation vials, and counted. The quantities of $^{86}Rb^+$ uptake were then calculated. The blanks (no oocyte) of the experiment were routinely less than 200 cpm.

Subunit Assembly Assay—A human c-*myc* epitope tag, recognized by human-specific mAb 9E10 (32), was fused to the amino terminus of β_G and β_1 by inserting a double-stranded oligonucleotide adapter (sense 5′-CATGGAGCAAAAGCTGATCTCCGAGGAGGACCT-3′; antisense, 5′-CATGAGGTCCTCCTCGGAGATCAGCTTTTGCTC-3′) that contained an initiation ATG followed by nucleotides encoding the c-*myc* peptide (EQKLISEEDL), into each β-subunit. The resultant recombinant molecules were termed c-*myc*-β_G and c-*myc*-β_1. The addition of the correct nucleotide sequence was confirmed by DNA sequencing. Oocyte proteins were metabolically labeled by coinjection of 0.345 μCi [^{35}S]methionine with the α and c-*myc*-β-subunit cRNAs. Three days after the injection, the oocytes were lysed, and the proteins were extracted by incubating for 30 min at 4 °C in the presence of 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 1% Triton X-100 (buffer B) (27). The insoluble material was removed by centrifugation at 10,000 $\times$ g for 10 min at 4 °C. The extracted proteins from 5–10 oocytes were pooled and incubated with 2 μg of mAb 9E10 for 4 h at 4 °C, followed by addition of 10 μl (packed volume) of protein A/G plus agarose (Santa Cruz Biotechnology). After 2 h of vigorous shaking, the resin was washed six times with buffer B. The bound protein was extracted from the resin with Laemmli sample buffer containing 10% β-mercaptoethanol and separated on SDS-10% polyacrylamide gels. The gels were then fixed in 7% glacial acetic acid, 25% methanol and impregnated with 2,5-diphenyloxazole. The gels were dried and exposed to Kodak XAR-5 film with intensifying screens at −70 °C for 1–3 days. The coprecipitation of an α-subunit with the c-*myc*-β_G- or c-*myc*-β_1-subunit using this protocol was interpreted as subunit assembly in accordance with previous studies of Na^+,K^+-ATPase $\alpha\beta$ assembly (27).

Data Analysis—Quantitative data are presented as means ± S.E. and were tested for significance by analysis of variance. $p < 0.05$ was taken as significant.

RESULTS

Functional Properties of $\alpha_C\beta_G$ or $\alpha_C\beta_1$ Expressed in Xenopus Oocytes—Sets of oocytes were injected with cRNAs for α_C, α_G, β_G, β_1, α_C plus β_G, or α_C plus β_1. The controls were injected with water alone. $^{86}Rb^+$ uptake was measured 3 days later in the presence of 5 mM KCl. When the oocytes were injected with one subunit only, there was no effect on $^{86}Rb^+$ uptake compared to water-injected controls (Fig. 1). However, when oocytes were coinjected with α_C plus β_G or α_C plus β_1 there was a significant increase in $^{86}Rb^+$ uptake compared to any group injected with one subunit alone or the control group. This $^{86}Rb^+$ uptake was independent of the presence of Na^+ in the incubation medium; equimolar replacement of external Na^+ by NMDG did not alter the $^{86}Rb^+$ uptake in any group. This result established that the $^{86}Rb^+$ uptake was not mediated by the Na^+,K^+-ATPase.

To assess the K^+-activation kinetics of the $\alpha_C\beta_G$ and $\alpha_C\beta_1$ complexes, $^{86}Rb^+$ uptake was assayed in α_C plus β_G- and α_C plus β_1-expressing oocytes in the presence of increasing concentrations of KCl in the uptake buffer (Fig. 2). External K^+ activated $^{86}Rb^+$ uptake in these oocytes in a concentration-dependent, saturable manner. Lineweaver-Burk transformation of the kinetic data revealed $K_{½}$ values for K^+ activation of 1.4 mM when the oocytes expressed the $\alpha_C\beta_G$ complex and 1.8 mM when the $\alpha_C\beta_G$ complex was expressed. Both values are slightly higher than the value (1.2 mM) reported for Sf-9 cells express-

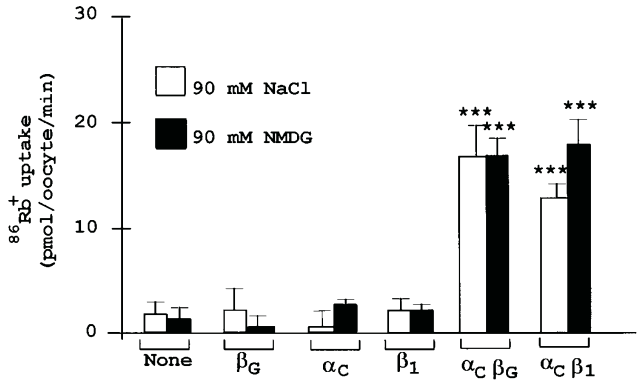

FIG. 1. **^{86}Rb$^+$ uptake in oocytes expressing $\alpha_C\beta_G$ or $\alpha_C\beta_1$ complexes.** Oocytes were injected with cRNAs encoding β_G, α_C, β_1, α_C plus β_G, α_C plus β_1, or water alone. Three days later, ^{86}Rb$^+$ uptake was measured in the presence of 5 mM KCl and either 90 mM NaCl (*open bars*) or 90 mM NMDG (*closed bars*). Data are means of 10–12 oocytes in each group. ***$p < 0.001$ *versus* any group injected with one subunit alone or the control group.

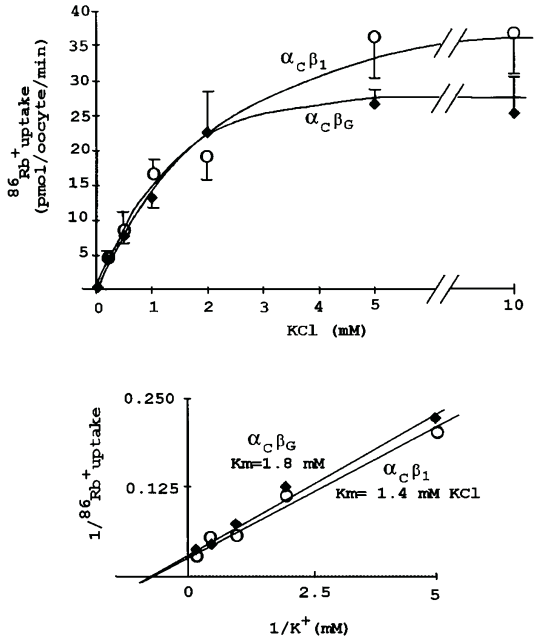

FIG. 2. **K$^+$-dependent activation of ^{86}Rb$^+$ uptake in oocytes coexpressing α_C and β_G- or β_1-subunit.** *Top panel*, oocytes were coinjected with α_C and β_G (*diamonds*) or with α_C and β_1 (*circles*). Three days later, ^{86}Rb$^+$ uptake was measured in the presence of 90 mM NaCl at the indicated external KCl concentrations. *Bottom panel*, the Lineweaver-Burk plot of the same data. Each point on the plot represents the data obtained from 8–10 oocytes. *Straight lines* were fitted by method of least squares. Correlation coefficients (R) were 0.9 for both $\alpha_C\beta_G$ and $\alpha_C\beta_1$.

ing the α_C alone (11). The Hill coefficient for K^+ was 1.0 in both cases (data not shown), indicating no cooperativity in the K^+ effect.

Pharmacological Properties of α_C Coexpressed with Different β-Subunits—In the presence of 1 mM external K^+, ouabain inhibited ^{86}Rb$^+$ uptake of oocytes expressing $\alpha_C\beta_G$ or $\alpha_C\beta_1$ in a dose-dependent manner, with IC_{50} values of ~390 and ~640 μM, respectively (Fig. 3). In contrast, Sch-28080, at concentrations up to 500 μM, failed to inhibit ^{86}Rb$^+$ uptake of oocytes coexpressing α_C β_G or $\alpha_C\beta_1$ (Fig. 4). In positive control experiments, 10 μM Sch-28080 abolished ^{86}Rb$^+$ uptake of oocytes coinjected with α_G plus β_G cRNAs, in agreement with previous reports (33).

Assembly of $\alpha_C\beta_G$ and $\alpha_C\beta_1$ Complexes—Since the ^{86}Rb$^+$ uptake experiments indicated that α_C must be coexpressed with β_G and β_1 for holoenzyme function, we sought to establish directly that stable $\alpha_C\beta_G$ and $\alpha_C\beta_1$ complexes were formed. The human c-*myc* epitope was added to the amino terminus of β_G (c-*myc*-β_G) and of β_1 (c-*myc*-β1) (27). Oocytes were injected with α_C, c-*myc*-β_G, c-*myc*-β_1, α_C plus c-*myc*-β_G, or α_C plus c-*myc*-β_1. The Triton X-100 extracts were immunoprecipitated with mAb 9E10 (27), and samples were analyzed by SDS-PAGE and fluorography. Fig. 5 demonstrates that mAb 9E10 did not immunoprecipitate α_C when it was expressed alone (without a β-subunit). However, when α_C cRNA was coinjected with c-*myc*-β_G cRNA, the two subunits were coprecipitated, indicating stable assembly. As predicted from the ^{86}Rb$^+$ uptake data, stable assembly between α_C and c-*myc*-β_1 was also evident when the oocytes were coinjected with cRNAs for these subunits (Fig. 5).

The relative amounts of $\alpha_C\beta_1$ and $\alpha_C\beta_G$ coprecipitated in this assay were roughly comparable. The immunoprecipitated β_G and β_1 were in the core glycosylated (narrow band at ~50 kDa) and fully glycosylated (broad band at 60–70 kDa) forms. Both the core and fully glycosylated β_1-subunits migrated more rapidly than the corresponding glycosylated forms of β_G on SDS-PAGE, as observed by others (34). The explanation for such differences in mobility is unknown. A band that migrated near α_C (labeled *?* in Fig. 5) was also immunoprecipitated from oocytes injected with cRNA for β_G or β_1, regardless of whether α_C cRNA was coinjected. Presumably this band represents the endogenous α_1-subunit of the Na^+,K^+-ATPase.

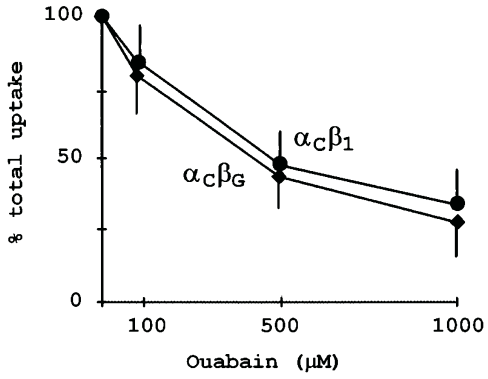

FIG. 3. **Effect of ouabain concentration on ^{86}Rb$^+$ uptake in oocytes expressing $\alpha_C\beta_G$ or $\alpha_C\beta_1$ complexes.** ^{86}Rb$^+$ uptake in oocytes coexpressing α_C and β_G or α_C and β_1 was determined in the presence of 1 mM KCl with addition of the indicated ouabain concentrations. Symbols as in Fig. 1. Eight to 10 oocytes were used for group.

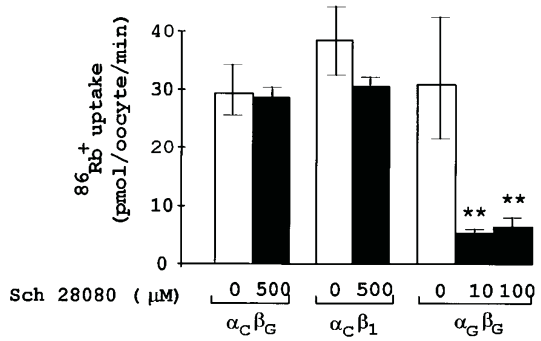

FIG. 4. **Effect of Sch-28080 concentration on ^{86}Rb$^+$ uptake in oocytes expressing $\alpha_C\beta_G$, $\alpha_C\beta_1$, or $\alpha_G\beta_G$ complexes.** ^{86}Rb$^+$ uptake, in oocytes coexpressing α_C and β_G, α_C and β_1, or α_G and β_G was determined in the presence of 1 mM KCl, 90 mM NaCl and the indicated concentrations of Sch-28080. **$p < 0.01$ *versus* no Sch-28080. *Open bars*, no inhibitor; *closed bars*, incubated in presence of Sch-28080. Ten oocytes were used in each group.

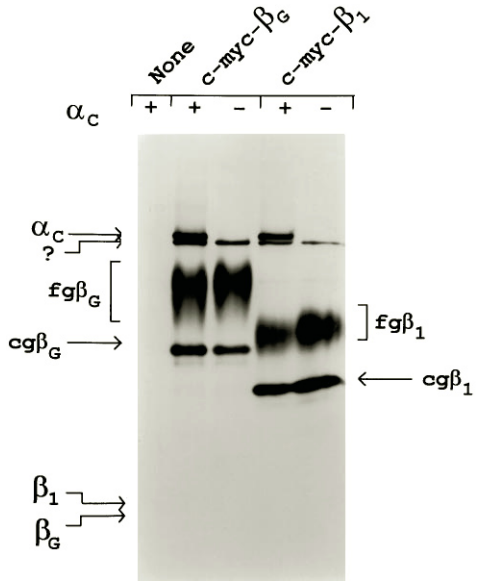

FIG. 5. **Assembly of α_C plus c-*myc*-β_G and α_C plus c-*myc*-β_1 complexes.** Oocytes were injected with α_C, c-*myc*-β_G, α_C plus c-*myc*-β_G, β_1, or α_C plus c-*myc*-β_1 in the presence of 0.345 μCi of [^{35}S]methionine. The Triton X-100 extracted proteins were immunoprecipitated with mAb 9E10 and protein A/G PLUS-agarose and analyzed by SDS-PAGE and fluorography as described under "Experimental Procedures." Representative fluorographs are shown. Eight to 10 oocytes were used in each group. *c-myc-*β_G, β-subunit of the gastric H^+,K^+-ATPase with the human c-*myc* epitope at the amino terminus; *c-myc-*β_1, β_1-subunit of the Na^+,K^+-ATPase with the human c-*myc* epitope at the amino terminus; *cg*β_G, core glycosylated c-*myc*-β_G; *cg*β_1 core-glycosylated c-*myc*-β_1; *fg*β_G, fully glycosylated c-*myc*-β_G; *fg*β_1, fully glycosylated c-*myc*-β_1; β_G, expected position of c-*myc*-β_G core protein; β_1, expected position of c-*myc*-β_1 core protein.

DISCUSSION

The colonic H^+,K^+-ATPase plays a central role in the regulation of K^+ absorption by the colon (11, 18, 35, 36) and kidney (37). However, the functional and pharmacologic properties of this isoform, and its requirements for association with a β-subunit remain ambiguous. We used the oocyte expression system to study the properties of α_C interacting with differing β-subunits. The rat β_G-subunit was selected for study because it is the only known mammalian H^+,K^+-ATPase β-subunit, and it is coexpressed with α_C in the renal collecting duct (4, 38). The rat Na^+,K^+-ATPase β_1-subunit was chosen because immunoreactivity for this protein was found in the apical membrane of rat colonocytes (25), a membrane domain in which Na^+-independent K^+-ATPase activity, but not Na^+,K^+-ATPase activity or α_1-subunit immunoreactivity, was observed. We therefore hypothesized that both β_G and β_1 would support α_C functional activity.

In agreement with the majority of studies of X^+,K^+-ATPases (9, 12, 23), our $^{86}Rb^+$ uptake data provide clear evidence that α_C requires a β-subunit for functional activity in the oocyte expression system. Cougnon *et al.* (12), using α_C coexpressed in oocytes with an amphibian β-subunit, reached a similar conclusion. As predicted from our $^{86}Rb^+$ uptake data, α_C was coprecipitated with either the β_G- or β_1-subunit in the assembly assay, confirming the formation of stable heterodimers. The fact that comparable amounts of $\alpha_C\beta_1$ and $\alpha_C\beta_G$ complexes were coprecipitated in the assembly assay (Fig. 5) suggests that the assembly efficiency and/or stability of these heterodimers are quite similar. Using an identical assembly assay, Lemas *et al.* (27) found that the Na^+,K^+-ATPase α_1-subunit can assemble with β_1 or β_G, and they identified a 26 amino acid region of the Na^+,K^+-ATPase α_1 sufficient to allow stable interaction with these β-subunits. In addition, Jaisser *et al.* (22) found that β_1 could support functional activity of α_G. Our data, therefore, extend the range of potential $\alpha\beta$ pairs to include $\alpha_C\beta_G$ and $\alpha_C\beta_1$, and lend further support to the concepts that X^+,K^+-ATPase α-subunits contain a conserved assembly domain for β-subunit association, and that there is no remarkable α/β isoform selectivity in the assembly process.

Previous studies suggested that the different β-subunit isoforms could confer different K^+-activation kinetics on the Na^+,K^+-ATPase. Coexpression of the *Bufo* Na^+,K^+-ATPase α_1-subunit with the *Bufo* Na^+,K^+-ATPase β_1-subunit, Na^+,K^+-ATPase β_3-subunit, or rabbit H^+,K^+-ATPase β_G-subunit in *Xenopus* oocytes resulted in different K^+-activation kinetics for the various holoenzymes. The Na^+,K^+-ATPase α_1/H^+,K^+-ATPase β_G enzyme performed as a Na^+,K^+ pump with a much lower apparent affinity for K^+, both in the presence and absence of external Na^+, compared to the Na^+,K^+-ATPase α_1/Na^+,K^+-ATPase β_1 and Na^+,K^+-ATPase α_1/Na^+,K^+-ATPase β_3 pumps (39). In the present report, however, $\alpha_C\beta_1$ and $\alpha_C\beta_G$ enzymes exhibited comparable K^+-activation kinetics. Thus the ability of different β-subunits to alter this functional property may be restricted to specific X^+,K^+-ATPase α-subunits or to hybrid ion pumps of specific species.

Structure-function studies of the Na^+,K^+-ATPase α-subunit indicated that amino acids in several transmembrane regions (H1, H2, H5, and H6), the first extracellular loop, and the third extracellular loop (specifically Cys^{104}, Tyr^{108}, Gln^{111}, Asp^{121}, Asn^{122}, and Tyr^{308}, Phe^{786}, Leu^{793}, Thr^{797}, Phe^{863}, Arg^{880}), contributed to ouabain sensitivity (40, 41). Comparison of the amino acid composition of α_C with the Na^+,K^+-ATPase α-subunits indicates that most of these amino acids are conserved, with the notable exception that Tyr^{108} and Phe^{786} of the Na^+,K^+-ATPase α-subunit are substituted with Phe and Tyr in α_C. The specific role of these two amino acids in conferring relative ouabain-insensitivity to α_C has not yet been studied. It has also been suggested that Phe^{124} and Asp^{137} (rat α_G sequence) (6) are required to confer Sch-28080-sensitivity to α_G, but the α_C and α_G sequences are identical at these two positions. Given evidence that α_C is Sch-28080-insensitive (12) (present report), sequences other than these two amino acids must be implicated in Sch-28080 binding affinity to the α-subunit. One candidate motif for conferring Sch-28080 sensitivity is the sequence GDLT (amino acids 131–134 of α_G), which is conserved in the known α_G-subunits (all of which are Sch-28080-sensitive) of amphibians, birds and mammals, but is absent from α_C (6). Pharmacologic analysis of chimeric α_G/α_C molecules and site-directed mutants of α_C should clarify this question.

Functional studies have suggested the presence of both ouabain-sensitive and -insensitive components of K^+-ATPase activity in rat distal colon (11, 18, 19, 20, 42) and kidney (43). In addition, a polyclonal antibody directed against the amino terminus of α_C inhibited both ouabain-sensitive and -insensitive components of K^+-ATPase activity in apical membranes prepared from distal colon, and it specifically labeled only the apical membrane of the distal colon epithelium (11). Since this antibody did not label the basolateral membrane, where the Na^+,K^+-ATPase resides in these cells, and did not inhibit Na^+,K^+-ATPase activity in membranes from rabbit renal medulla, this antibody does not appear to cross-react with the Na^+,K^+-ATPase α_1-subunit. Moreover, whereas 1 mM ouabain failed to inhibit K^+-ATPase activity in α_C-expressing Sf-9 cells, it dramatically inhibited (by 75%) K^+-ATPase activity of colonic apical membranes. Integration of these data with the present results supports the idea that, in the apical membrane of the distal colon epithelium, $\alpha_C\beta_1$ holoenzymes contribute the Sch-28080-insensitive and relatively ouabain-insensitive com-

ponent of K^+-ATPase activity, and that a novel X^+,K^+-ATPase α-subunit, bearing an amino terminus antigenically similar to α_C, mediates the more ouabain-sensitive fraction of Na^+-independent K^+-ATPase activity. Given the fact that the ouabain sensitivities of $\alpha_C\beta_G$ and $\alpha_C\beta_1$ were virtually indistinguishable, it seems less likely that a novel β-subunit could confer ouabain sensitivity on α_C. If α_C indeed interacts with β_1 *in vivo*, it is logical to predict that these two subunits will be coordinately up-regulated in the colonocyte apical membrane during chronic K^+ deprivation. Studies are presently under way in our laboratory to test this hypothesis.

In conclusion, the present studies show that both β_G and β_1 can interact with α_C, each creating a functional H^+,K^+-ATPase that is Sch-28080-insensitive and only partially sensitive to ouabain. The functional and pharmacological properties of $\alpha_C\beta_G$ and $\alpha_C\beta_1$ holoenzymes are quite similar. Our data support the view that, in the rat distal colon, β_1 may play a surrogate role as the β-subunit for the colonic H^+,K^+-ATPase.

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