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K^+-STIMULATED ATPase IN PURIFIED MICROSOMES OF BULLFROG OXYNTIC CELLS

ALLEN L. GANSER and JOHN G. FORTE

Department of Physiology–Anatomy, University of California, Berkeley, Calif. 94720 (U.S.A.)

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SUMMARY

Differential centrifugation of oxyntic cell homogenates yielded microsomal fractions which contained large amounts of mitochondrial membrane. The presence of marker enzymes (succinate dehydrogenase and cytochrome *c* oxidase) indicated that mitochondrial contamination of crude microsomes ranged from 20 to 60% in different preparations. A discontinuous sucrose density gradient procedure was developed for the routine preparation of purified oxyntic cell microsomes. A K^+-stimulated, Mg^{2+}-requiring ATPase was localized in these purified membranes and coincided with the presence of a K^+-stimulated *p*-nitrophenylphosphatase. Na^+ and ouabain had no effect on the K^+ stimulation of the microsomal ATPase. The apparent activation constant for K^+ was approximately 1 mM at pH 7.5, the optimal pH for stimulation.

An anion-sensitive ATPase has been widely studied in gastric microsomal preparations. We found that the basal microsomal ATPase (*i.e.* without K^+) and the mitochondrial ATPase were inhibited by SCN^- and enhanced by HCO_3^-, however, the K^+-stimulated component of the microsomal ATPase was virtually unaffected by these anions.

INTRODUCTION

The microsomal fraction from gastric mucosa of numerous species contains enzymes thought to be closely related to the acid secretory process. These membranes show K^+-dependent phosphatase activity[1] and they catalyze the hydrolysis of ATP[2]; both activities depend on Mg^{2+}. An interesting feature of the ATPase is that, like acid secretion, it is inhibited by SCN^- and stimulated by HCO_3^-. Since these enzymes are abundant in the microsomal fraction it has been presumed that they are localized on the very specialized endoplasmic reticulum of the oxyntic cell[3,4] (referred to as its tubular membrane system).

However, several questions went unanswered in the earlier work with gastric microsomal systems. Since oxyntic cells generally represent only a fraction of the tissue volume of the gastric mucosa, to what extent did the membranes and consequently the enzymatic activities in the microsomal fraction derive from other

Abbreviations: PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); DCIP, 2,6-dichlorophenolindophenol.

cells? And perhaps more importantly, were the microsomal fractions studied free of mitochondrial particles, which in gastric mucosa[3,5,6] and other tissues[7–9] show high amounts of ATPase activity sensitive to SCN^- and HCO_3^-?

We have studied the enzymatic composition of purified oxyntic cell microsomes so that the findings could more clearly be related to acid secretion. The bullfrog gastric mucosa represents an advantageous, well-studied system. A simple, rapid procedure for isolating pure oxyntic cells is available for this tissue[10] and in these cells the tubular membrane system is present in abundance[3,11]. Furthermore, the histochemical demonstration of large amounts of carbohydrate present on these membranes[12] provided us with a useful marker for this organelle. Thus we employed a sucrose density gradient procedure to obtain a band of microsomal membranes essentially free of mitochondrial contamination. These purified microsomes are the source of the K^+-stimulated phosphatase and furthermore they demonstrate a unique K^+-stimulated ATPase.

METHODS

Cell fractionation

Oxyntic cells were harvested from large bullfrogs (*Rana catesbeiana*) by first removing the mucus-secreting cells of the gastric mucosa according to Forte *et al.*[10] and then scraping off the remaining oxyntic cells with a glass slide. All procedures were carried out at 0–4 °C. The oxyntic cells were homogenized by hand in a loose fitting teflon–glass homogenizer as a 1–2% (w/v) suspension in 220 mM sucrose and 5 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (sodium salt, pH 6.8 at 0–4 °C). 10 up–down strokes were sufficient for complete rupture of the cells. The homogenate was fractionated in a SS-34 Sorvall rotor (*g* values are for R_{max}). A low speed fraction was harvested at $2000 \times g$ for 5 min and rehomogenized and recentrifuged as above. The combined supernatant was centrifuged at $12\,000 \times g$ for 10 min and $40\,000 \times g$ for 90 min to yield the mitochondrial and crude microsomal fractions, respectively. The remaining material was termed the final supernatant.

Density gradients

Membrane suspensions in homogenization media were layered over continuous sucrose density gradients ranging from 20 to 55% (w/v) sucrose with 5 mM PIPES buffer at pH 6.8, and centrifuged overnight at 25 000 rev./min in a SW 25 Spinco rotor at 2 °C. The bands, as visible to the eye, were collected through a hole made in the bottom of the centrifuge tube. The concentration of sucrose at different levels of the gradient was measured with a refractometer and was used to estimate the density of the membrane bands[13].

From the results of numerous experiments with the continuous gradient, a discontinuous density gradient procedure was developed. For the latter the crude microsomal fraction was layered over 22, 37, 43 and 55% (w/v) sucrose and centrifuged at 0–4 °C for 100 min in a Spinco SW 25 or SW 27 rotor at maximum speeds. The gradient was slowly pumped out through a flow-through cuvette and continuously monitored spectrophotometrically for the presence of membrane particles. The appropriate fractions were collected and stored at 2 °C until use (within 2 weeks of preparation).

Enzyme assays

ATPase activity was measured as the rate of release of P_i. In some cases, in order to increase the linear range of the assay, an ATP-regenerating system was present. This consisted of 4 mM sodium phosphoenol pyruvate and 20 μg pyruvate kinase in $(NH_4)_2SO_4$ solution. The microsomes did not hydrolyze phosphoenol pyruvate under our assays conditions. The specific kind of ATPase assay system is indicated in the appropriate figures. In general, the reaction was carried out in 1.0 ml at 37 °C for 5 or 10 min. It was started by the addition of an equimolar mixture of ATP and Mg^{2+} and stopped by adding 1 ml of ice-cold 10% trichloroacetic acid and cooling on ice. After centrifuging out the protein, a 0.5 or 1.0-ml aliquot was removed for the determination of P_i by the method of Eibl and Lands[14]. The term "basal ATPase" refers to the activity observed when the only activating ion present was Mg^{2+}. We have confirmed earlier observations that gastric microsomal ATPase activity has an absolute requirement for Mg^{2+}.

K^+-stimulated phosphatase was assayed as the rate of release of *p*-nitrophenol from *p*-nitrophenyl phosphate. The reaction media contained 5 mM *p*-nitrophenyl phosphate, 25 mM KCl as activator and 40 mM Tris–HCl (pH 8.5 at 37 °C). Other aspects of the procedure were described previously[15].

The method of Smith[16] was used to assay cytochrome *c* oxidase. There was no evidence of interference by cytochrome *c* reductase.

Succinate dehydrogenase was assayed by the method of Arrigoni and Singer[17]. The reaction media contained 50 mM potassium phosphate buffer (pH 7.6 at 38 °C), 0.05% Triton X-100, 20 mM sodium succinate, 1 mM fresh KCN, 0.05 mM 2,6-dichlorophenolindophenol (DCIP), and 0.1 mM phenazine methosulfate. The enzyme was preincubated for 10 min at 38 °C in the presence of succinate and the reaction started by the rapid addition of DCIP followed by phenazine methosulfate.

All enzyme assay conditions were such that activity was linearly proportional to the amount of protein added. Enzyme assays and protein determinations were run in duplicate and the averages reported. Protein was determined by the method of Lowry *et al.*[18] using bovine serum albumin as a standard.

Gel electrophoresis

Polyacrylamide gel electrophoresis in 1% sodium dodecyl sulfate was carried out by the method of Fairbanks *et al.*[19]. The gels, however, were 7.5% in acrylamide and 0.28% in bisacrylamide.

Difference spectra

Oxidized–reduced difference spectra were taken on a Cary 14 recording spectrophotometer using the light scattering-transmission attachment for dense membrane suspensions. $Na_2S_2O_4$ was used as the reductant.

RESULTS

Cell fractionation

The distribution of various enzymatic activities among the cell fractions in a typical experiment is presented in Fig. 1. Notice that the K^+-stimulated *p*-nitro-

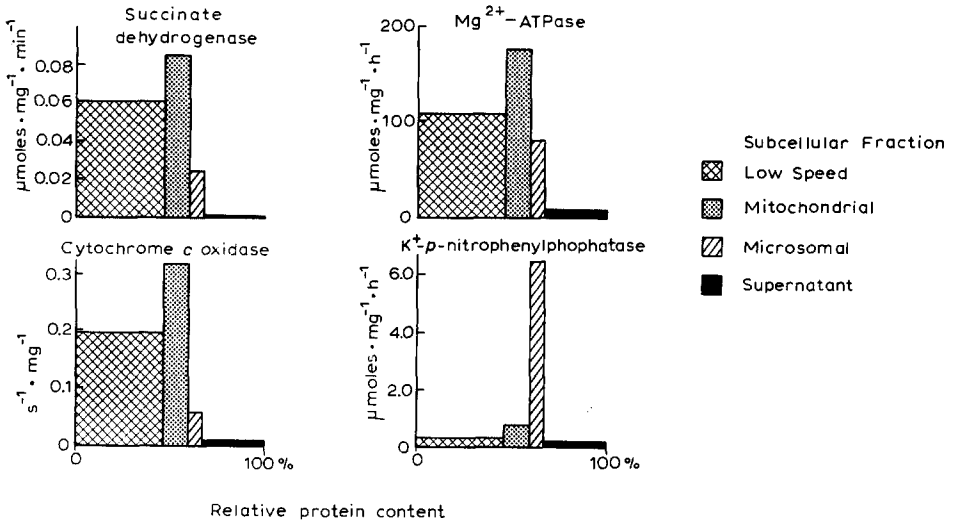

Fig. 1. The distribution of enzymes and protein among the subcellular fractions of bullfrog oxyntic cells. The ATPase reaction media contained: 40 mM Tris–acetate (pH 8.4), 22 mM sucrose, 5 mM Na_2ATP, 5 mM magnesium acetate, and the ATP regenerating system. Other assay procedures were as described in the Methods. Protein recovery was 90% and the recovery of enzymes ranged from 75 to 90%.

phenylphosphatase is very well localized in the crude microsomes which contain about 85% of the activity recovered in all the fractions. While the Mg^{2+}–ATPase activity is not well localized in any one fraction, it does follow closely the pattern of the two mitochondrial enzymes succinate dehydrogenase and cytochrome *c* oxidase, which suggests that most of the ATPase is associated with mitochondrial membranes. However, the microsomal fraction appears to have slightly more ATPase activity than can be accounted for by mitochondrial contamination. As noted by others[3,5,6], we have also observed that the sensitivity of ATPase to SCN^- and HCO_3^- is virtually the same in the nuclear, mitochondrial and microsomal fractions.

Mitochondrial contamination of the microsomal fraction varies considerably from one preparation to another. Assuming the mitochondrial fraction to contain only mitochondria and using the specific activities of succinate dehydrogenase and cytochrome *c* oxidase, we calculate the contamination of crude microsomes in different preparations to range from 20 to 60%. This high degree of mitochondrial contamination is also apparent from oxidized–reduced difference spectra of the fractions: the spectra of the crude microsomal fraction from frog mucosa always shows large amounts of the mitochondrial cytochromes. When the microsomal fraction from a particular preparation shows very high ATPase activity, the mitochondrial contamination is proportionately as high.

As seen in Fig. 1, the low-speed fraction has unexpectedly high levels of mitochondrial enzymes. This happens even when the fraction is prepared at a lower centrifugal force, *i.e.* at $600 \times g$. Because very little K^+–*p*-nitrophenylphosphatase sediments in these low-speed fractions, the presence of mitochondrial enzymatic activities can not arise from some kind of generalized contamination such as whole cells.

The $40\,000 \times g$ supernatant fraction was further centrifuged at $109\,000 \times g$ for 90 min to yield a "light" microsomal fraction. Since the "light" microsomes showed low K^+-*p*-nitrophenylphosphatase activity and low ATPase activity they were not pursued further.

Density-gradient purification of the microsomal fraction

Centrifugation of the microsomal fraction on continuous sucrose density gradients for 16 h yielded two distinct bands of membranes; Band I at 34.2% sucrose (density at 0 °C of 1.136) and Band II just below 50.5% sucrose (density at 0 °C of 1.198). Some material is also detected above and between these bands in two diffuse zones. When the mitochondrial fraction was applied to the density gradient, one principal band formed in the 1.198 density region and very little material was found in the region of 1.136 density.

TABLE I

ENZYMATIC PROPERTIES OF THE PRINCIPAL BANDS FORMED WHEN MICROSOMES ARE APPLIED TO A CONTINUOUS DENSITY GRADIENT

Gradient band	*Cytochrome c oxidase* ($s^{-1} \cdot mg^{-1}$)	*K^+-p-nitrophenyl-phosphatase* ($\mu moles \cdot mg^{-1} \cdot h^{-1}$)	*Mg^{2+}-ATPase*** ($\mu moles \cdot mg^{-1} \cdot h^{-1}$)
Band I ($\rho^* = 1.136$)	0.014	7.6	15.6
Band II ($\rho^* = 1.198$)	0.573	0.7	89.6

* Density at 0 °C.
** Assay conditions as in Fig. 1.

The membranes in these two bands have very different enzymatic properties as shown in Table I. Band II is high in cytochrome *c* oxidase and ATPase activities, which indicates that these are mitochondrial membranes. Band I shows very little cytochrome *c* oxidase and is relatively low in ATPase; however it is high in K^+-*p*-nitrophenylphosphatase.

Polyacrylamide gel electrophoresis demonstrated a pronounced difference in the basic protein constituents between gradient Bands I and II. As shown in Fig. 2, Band II from the crude microsomal fraction gives essentially the same electrophoretic pattern as the principal mitochondrial band. Consistent with the enzyme marker studies, Band II material represents a mitochondrial contamination in the crude microsomal fraction. On the other hand, Band I is not only distinct in protein staining pattern, but is the only material which contains large amounts of glycoprotein. Thus both the marker enzyme studies and the gel electrophoresis data demonstrate that microsomes in gradient Band I have been freed of mitochondrial contamination.

Based on these results a discontinuous density gradient procedure was developed for the routine preparation of purified microsomes. The distribution of protein

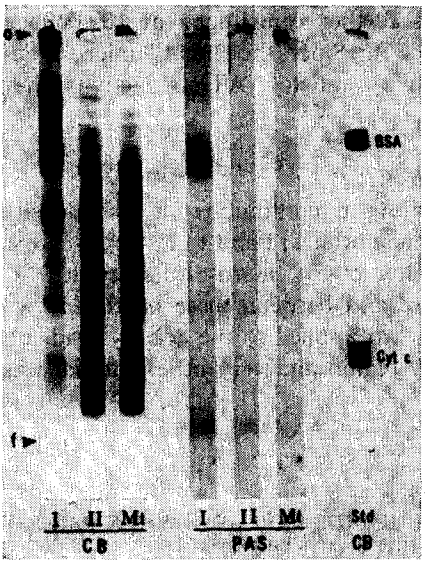

Fig. 2. Electrophorograms of density gradient Bands I and II and of mitochondria (Mt). Gels were run in the dodecyl sulfate system, stained for protein with coomassie blue (CB) and for carbohydrate with periodic acid– Schiff reagent (PAS)[19]. 75 μg of membrane protein were applied to each gel. Bovine serum albumin (BSA) and cytochrome *c* (Cyt c) were run as standard (Std) molecular weight markers. The origin is indicated by "o" and the ion front by "f".

TABLE II

DISTRIBUTION OF PROTEIN AND ENZYMES OF THE CRUDE MICROSOMAL FRACTION ON A DISCONTINUOUS DENSITY GRADIENT

Portion of gradient (% *sucrose, w/v*)	*Protein* (*mg*)	*Cytochrome c oxidase* ($s^{-1} \cdot mg^{-1}$)	*K^+–p-nitrophenyl-phosphatase* ($\mu moles \cdot mg^{-1} \cdot h^{-1}$)	*Mg^{2+}–ATPase** ($\mu moles \cdot mg^{-1} \cdot h^{-1}$)
22%	4.9	0.012	5.2	20.9
22–37% interface (purified microsomes)	9.8	0.021	10.2	30.9
37%	4.1	0.097	6.4	27.9
37–43% interface	2.6	0.106	1.8	101.8
43%	1.2	0.289	1.3	111.6
43–55% interface (purified mitochondria)	3.3	0.455	1.9	92.0
Recovery	107%	84%	74%	68%
Crude microsomal fraction**	24.2	0.137	8.6	74.5

* The ATPase reaction media contained: 40 mM Tris–acetate (pH 8.4), 5 mM Na_2ATP, 5 mM magnesium acetate and 22–150 mM sucrose depending on the portion of the gradient.

** Represents the microsomal membranes, collected by differential centrifugation, prior to application to the density gradient. Values indicate the total amount of protein or the specific activity of the material applied to the gradient and were used to determine the percent recovery.

and enzyme markers when the crude microsomal fraction is applied to this gradient is given in Table II. The purified microsomes (formerly Band I) collect at the 22–37% step and mitochondrial material (formerly Band II) concentrates at the 43–55% interface. The discontinuous and continuous gradient methods give comparable degrees of purification of the microsomes. While the ATPase activity remaining in the purified microsomes is much lower than in the original fraction, it is however higher than expected from mitochondrial contanimation and may therefore be endogenous to these purified microsomal membranes.

K^+-stimulated ATPase

Since the microsomes are the source of the K^+-stimulated *p*-nitrophenylphosphatase and since earlier studies with less purified gastric microsomal ATPase demonstrated some K^+ stimulation[1,15], the effects of K^+ were investigated. It may be seen from Table III that microsomal ATPase was markedly stimulated by K^+, whereas Na^+ alone was virtually without effect. Combinations of Na^+, K^+ and ouabain did not alter the K^+ effect. The lack of effect of Na^+ and ouabain on the microsomal enzyme clearly distinguishes it from (Na^+–K^+)-ATPase activity. Microsomal ATPase activities, basal and K^+-stimulated, were completely dependent on Mg^{2+}. As demonstrated in Table III, K^+-stimulated ATPase activity is not present in mitochondria.

A comparison of the pH optima for microsomal and mitochondrial enzyme activities is shown in Fig. 3. Whereas the peak activity for the mitochondrial enzyme occurred at pH 8, the K^+-stimulated activity in the microsomes has a broad maximum around pH 7.5.

The kinetic effect of various concentrations of K^+ on the microsomal ATPase is shown in Fig. 4. The K^+-stimulated activity (the total activity *minus* that without K^+) at 25 mM K^+ in six different assays (three preparations) ranged from 7.5 to 20 μmoles $P_i \cdot mg^{-1} \cdot h^{-1}$. The apparent activation constant for K^+ was approximately 1.0 mM.

TABLE III

DEMONSTRATION OF A K^+-STIMULATED ATPase IN PURIFIED MICROSOMES

In addition to the reagents shown, the reaction media contained: 40 mM Tris–PIPES (pH 7.5), 2 mM Tris–ATP, 2 mM $MgCl_2$, and 38 μg of microsomal protein or 13 μg of mitochondrial protein.

Additions	*Purified microsomes* (μ*moles* $P_i \cdot mg^{-1} \cdot h^{-1}$)	*Purified mitochondria* (μ*moles* $P_i \cdot mg^{-1} \cdot h^{-1}$)
Basal Mg^{2+}–ATPase	19.6	84.6
25 mM KCl	36.0	85.5
25 mM KCl + 0.1 mM ouabain	36.6	—
60 mM NaCl	20.4	—
25 mM KCl + 60 mM NaCl	35.2	—
25 mM KCl + 60 mM NaCl + 0.1 mM ouabain	34.1	—

The effects of SCN^- and HCO_3^- on basal and K^+-stimulated microsomal ATPase are given in Table IV. As previously noted for gastric mitochondrial and crude microsomal fractions[3,5,6], 25 mM SCN^- markedly inhibited (82%) the basal Mg^{2+}–ATPase of the purified microsomes. However, the K^+stimulation (as judged by comparing the NaSCN and KSCN activities) appeared to be very

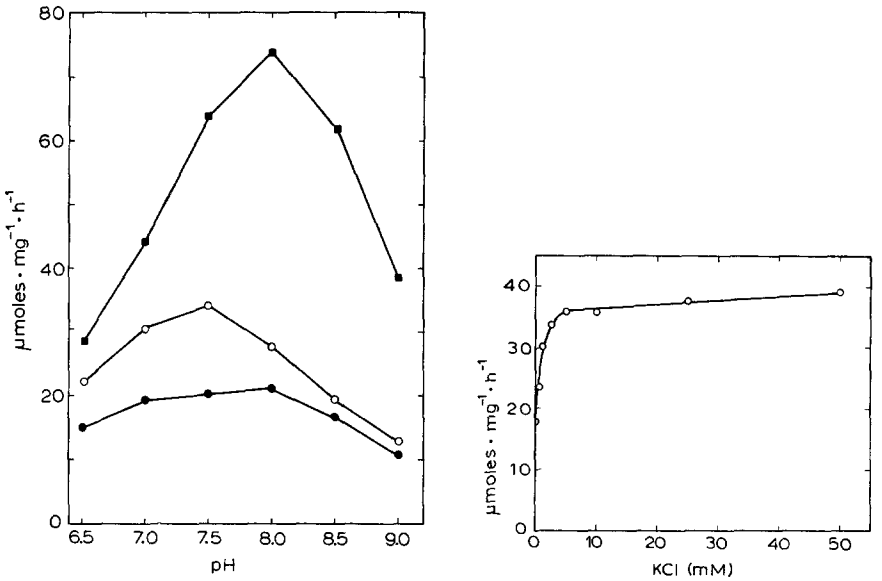

Fig. 3. pH profiles for purified microsomal and mitochondrial ATPases. Circles represent purified microsomal activity with (○) and without (●) 25 mM KCl; boxes indicate purified mitochondrial ATPase activity. The buffer for pH 6.5–7.5 was 40 mM PIPES (final concentration in the reaction media) brought to the pH with Tris base; for pH 7.5–9.0, it was 40 mM Tris titrated with PIPES. The reaction media also contained 2 mM Na_2ATP and 2 mM $MgCl_2$. The values were adjusted to be equal at pH 7.5.

Fig. 4. Effect of K^+ on microsomal ATPase activity. The reaction media contained 40 mM Tris–HCl (pH 7.5), 2 mM Na_2ATP, 2 mM $MgCl_2$, 9 mM sucrose and 38 μg of purified microsomal protein.

TABLE IV

ANION SENSITIVITY OF PURIFIED MICROSOMAL ATPase

Data is expressed as percent of basal Mg^{2+}–ATPase activity. Assay conditions as described in Fig. 4. Ion additions were at 25 mM. Where bicarbonate was added, 5% CO_2–95% O_2 was slowly bubbled through the reaction media; this maintained the pH at 7.5 ± 0.1. The basal ATPase control for the HCO_3 test system was bubbled with room air.

	Basal ATPase	*NaCl*	*KCl*	*NaSCN*	*KSCN*	*$NaHCO_3$*	*$KHCO_3$*
Tris buffer system	100	101	134	19	56		
HCO_3^- test system	100					113	150

little affected by SCN^-. When testing the effects of HCO_3^- on the ATPase we elevated the partial pressure of CO_2 in order to maintain the pH at 7.5, the optimum for K^+ stimulation. As seen in Table IV, the gradient-prepared microsomes show only small stimulatory effects in the presence or absence of K^+.

An earlier report from another laboratory[5] as well as our own observations using crude gastric microsomes, have shown that the sulfhydryl reagent *N*-ethylmaleimide has little to no effect on microsomal ATPase activity. The results of experiments comparing the effects of *N*-ethylmaleimide on basal Mg^{2+}- and K^+-stimulated activities of the ATPases and phosphatases of gradient-purified membranes are presented in Fig. 5. The inhibitory effects of *N*-ethylmaleimide were relatively small on the Mg^{2+}–ATPase activities of purified microsomes and mitochondria. On the other hand, with the microsomes there was a marked inhibition of the K^+-stimulated ATPase and a 44% reduction of the K^+-stimulated phosphatase by 0.1 mM *N*-ethylmaleimide.

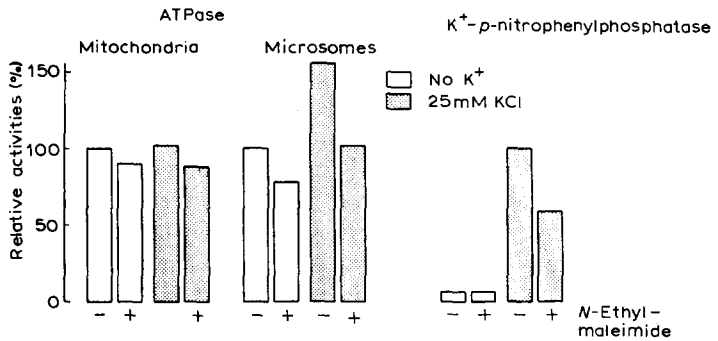

Fig. 5. Effect of *N*-ethylmaleimide on basal and K^+-stimulated ATPase and phosphatase activities of mitochondria and purified microsomes. Membranes were reacted with 0.1 mM *N*-ethylmaleimide at room temperature in the respective enzyme reaction media prior to the addition of substrate. Consult Fig. 4 for the ATPase assay conditions; where K^+ was not present, 25 mM NaCl was added to maintain ionic strength. There was 8 μg of mitochondrial and 34 μg of microsomal protein in the ATPase assay and 11 μg of microsomal protein in the K^+–*p*-nitrophenylphosphatase assay.

DISCUSSION

We have shown that the microsomal fraction prepared from bullfrog oxyntic cells by differential centrifugation contains large amounts of mitochondrial membrane. By applying sucrose density gradient techniques we have been able to isolate microsomal membranes essentially free of mitochondria. As the microsomes are purified the specific activity of ATPase diminishes; however, the procedure clearly makes manifest a K^+-stimulated component of the ATPase.

In previous studies on amphibian gastric mucosa, where rigorous purification procedures were not performed, little or no stimulation by K^+ was observed. For instance, Forte *et al.*[1] and Limlomwongse and Forte[15] found only about 10% stimulation by 5–10 mM K^+. Kasbekar and Durbin[2] and Sachs *et al.*[5], when testing for (Na^+–K^+)-ATPase, report finding no stimulation in the presence of K^+. When interpreting results obtained from crude fractions, the degree and signi-

ficance of mitochondrial contamination must be considered. Because contaminating mitochondrial activity can make up so much of the background Mg^{2+}–ATPase (see Fig. 1 and Table II) and is insensitive to K^+ (see Table III), the effect of K^+ could be obscured. In addition, the effects of K^+ have usually been tested at pH 8.3–8.4 while we find that the sensitivity to K^+ is optimal around pH 7.5. At higher pH values the percent stimulation decreases. Thus, the purity of our membranes and the pH of the assay have made it possible to describe a K^+–ATPase in the microsomes of bullfrog oxyntic cells.

In the microsomes from rabbit gastric mucosa, Tanisawa and Forte[20] reported that K^+ can increase ATPase activity as much as 70%. They used 50 mM KCl and assayed at pH 7.4. In addition, these authors studied the incorporation of ^{32}P from [γ-^{32}P]ATP into a phosphorylated membrane component. The phosphorylated intermediate was profoundly influenced by K^+, but neither Na^+ nor ouabain altered the incorporation of ^{32}P under any conditions. These properties of the rabbit gastric microsomal ATPase make it similar to the one we describe here for the bullfrog.

Previous work with either bullfrog or rabbit gastric microsomes has shown rather little (Na^+–K^+)-ATPase[2,5,15,20]. Our work extends these observations to the purified microsomal preparation. However, the demonstration of (Na^+–K^+)-stimulated activity may depend on the species of animal studied. For instance, in dog gastric mucosa the purified microsomes are reported to have abundant (Na^+–K^+)-ATPase[21]. Since the separate effects of Na^+ and K^+ were not reported and in view of the present results, it would be interesting to know whether the dog (Na^+–K^+)-ATPase activity is simply a K^+-sensitive ATPase. A more thorough recent study of the effects of Na^+, K^+ and ouabain was carried out by Hansen *et al.*[22] on the ATPase in lyophilized, total homogenate preparations of lizard gastric mucosa. For this system approximately 12% of total ATPase activity appeared to be a typical (Na^+–K^+)-activated, ouabain-sensitive ATPase. By further treatment with NaI they induced some relative enhancement of (Na^+–K^+)-stimulated activity, but no attempts to localize the activity in a particular membrane fraction were reported.

One aspect of acute interest in gastric microsomal ATPase stems from its anion sensitivity, namely HCO_3^- stimulation and SCN^- inhibition. The fact that the mitochondrial ATPase is similarly sensitive to these anions[3,5–9] has complicated specification of the origin of "gastric ATPase". In crude microsomal fractions from bullfrog the ATPase activity is stimulated about 40% at pH 8.4 by 20 mM $NaHCO_3$[2,6]. In other species, Necturus[23] and dog [24], the stimulation ranges from 32 to 48%. The Mg^{2+}–ATPase of the purified microsomes reported here still appears to be sensitive to HCO_3^- although we find only about 13% stimulation. Furthermore, the K^+-stimulated component of the ATPase is virtually unaffected by HCO_3^- (*cf.* Table IV). Note that our studies were carried out at pH 7.5 which is the optimum for K^+ stimulation. Consequently, in order to maintain the desired conditions of pH and HCO_3^- concentration (25 mM), it was necessary to raise the partial pressure of CO_2.

In studies by others[21,23,24], where large stimulations by HCO_3^- (up to 175%) were observed at the nominal pH of 7.4, it is not certain to what extent the activation was induced by the anion or by an alkaline pH shift which would occur upon

addition of 20 mM HCO_3^- at atmospheric CO_2 tension. However, it is unlikely that the pH shift can account for all the stimulation these authors observed and the difference in species must also be considered as a contributing factor.

Although purified microsomal and mitochondrial preparations can clearly be distinguished on the basis of stimulation by K^+, there are some properties that the basal Mg^{2+}–ATPases have in common. Both activities are inhibited by SCN^- and stimulated somewhat by HCO_3^-. Both enzymes are also relatively insensitive to *N*-ethylmaleimide. These similarities may indicate that the two enzymes have some mechanism or function in common; perhaps those already suggested by other authors[3,21].

The fact that K^+ stimulates both the ATPase and the phosphatase of gastric microsomes raises the question of the relationship between these two enzymes. Both enzymic functions have a number of features in common. They are purified together. HCO_3^- and SCN^- have very little effect on the K^+-stimulated ATPase and Forte *et al.*[1] found that these anions are also without effect on the K^+-stimulated phosphatase. In addition, *N*-ethylmaleimide greatly inhibits the K^+-stimulated components of the ATPase and the phosphatase while having little effect on the basal activities. Recently, a phosphorylated intermediate was demonstrated to be part of the ATP utilizing system in purified bullfrog microsomes[25]. Furthermore, this intermediate was sensitive to K^+ and had properties similar to the one present in rabbit gastric microsomes[20]. These data are all consistent with the hypothesis that both K^+-stimulated enzyme functions of the gastric microsomes are part of a single enzyme system, perhaps analogous to the ATPase and phosphatase activities of the (Na^+–K^+)-transport ATPase[26].

Specification of the cellular origin of the purified microsomes is important for correlating the enzymatic activities of these membranes with the acid secretory process. Electron micrographs[1–3,23,24] have shown that the microsomal fraction and gradient-purified microsomes contain smooth vesicular and tubular membranous structures presumably derived from the tubular membrane system of the intact cell. The presence or absence of mitochondria has been monitored by Forte *et al.*[1] and very recently by Sachs *et al.*[21] using marker enzymes. In our work any significant mitochondrial contamination has been removed from the microsomes. The enrichment of glycoprotein in the purified microsomes correlated well with the localization of K^+-stimulated phosphatase and ATPase. Since abundant glycoprotein has been shown to be associated with the tubular membrane system of the oxyntic cell[12], it is reasonable to conclude that this organelle was the major membrane source of our microsomal preparation. However, the possible presence of Golgi, rough endoplasmic reticulum and plasma membrane has not been rigorously excluded.

The requirement for K^+ for gastric acid secretion has been well established[27–30]. It was concluded[30] that the K^+-requiring site is probably intracellular. Since there are several enzymes within the cell which require K^+, the observation that a particular reaction requires K^+ is not conclusive evidence that it is directly involved in H^+ transport. However, the unique demonstration of a K^+-regulated system of ATP utilization, as described here, presents a very interesting catalytic system to pursue in elucidating the molecular mechanism of HCl secretion.

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