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CHEMICAL MODIFICATION OF GASTRIC MICROSOMAL POTASSIUM-STIMULATED ATPase *

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Summary

Selective chemical modification was used to examine amino acid residues that might be critical for the operation of the gastric K^+-stimulated ATPase. Modification of amino groups with the fluorigenic reagent 2-methoxy-2,4-diphenyl-3-dihydrofuranone resulted in selective inhibition of the K^+-stimulated ATPase and H^+-transporting activities of the gastric microsomes, while the Mg^{2+}-ATPase was not affected. Half-maximal inhibition occurred at about 3 μg 2-methoxy-2,4-diphenyl-3-dihydrofuranone/ml at pH 8.5. ATP provided complete protection against inhibition; the apparent K_m for ATP protection was about 50 μM. Nucleotide selectivity for protection was ATP > ADP > ITP > GTP > CTP > AMP. Sodium dodecyl sulfate gel electrophoresis of the reacted microsomes showed that virtually all the fluorescent label was on the M_r 100 000 peptide band, a very small peptide, and aminolipids. In the presence of ATP there was about 75% reduction in the fluorescent label on the M_r 100 000 peptide, but no change in the labeling of the other components. The arginine specific reagent, butanedione, inhibited Mg^{2+}-ATPase and K^+-ATPase activities, with the former being much less reactive. Similar to 2-methoxy-2,4-diphenyl-3-dihydrofuranone, ATP provided complete protection from butanedione treatment. It is concluded that amino and guanidino groups are critical to the function of the K^+-ATPase and may be actually at the ATP binding site.

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Abbreviations: MDPF, 2-methoxy-2,4-diphenyl-3-dihydrofuranone; SDS, sodium dodecyl sulfate; Pipes, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid); Hepes, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid.

Introduction

A preparation of vesicular microsomes isolated from fundic gastric mucosa of several species has been shown to possess Mg^{2+}-dependent, K^+-stimulated ATPase activity [1—3]. Immunocytochemical and other studies have demonstrated that these membranes are largely derived from the oxyntic, or acid-secreting, cells [4,5]. With the use of the pH electrode method [6,7] and fluorescent amino accumulation method [8,9] the ability of K^+-stimulated ATPase to transport H^+ into intravesicular space in exchange for K^+ was demonstrated. It thus appears that this K^+-stimulated ATPase might be the molecular apparatus for gastric H^+ secretion. Elucidation of the molecular architecture and functions of this enzyme should be of considerable interest and may provide further insight to the molecular mechanism of gastric acid secretion.

Total ATP hydrolyitc activity of the gastric microsomes can be divided into two types of ATPase activity: a basal, Mg^{2+}-dependent ATPase activity (Mg^{2+}-ATPase), and the characteristic gastric Mg^{2+}-dependent, K^+-stimulated ATPase activity (K^+-stimulated ATPase). The K^+-stimulated ATPase can be further stimulated by the addition of K^+ ionophores, which have been shown to provide increased access of K^+ to some internal or intramembrane activating site [10,8]. With the use of group-specific chemical modification we demonstrated that amino and sulfhydryl groups are important to the function of the gastric ATPases [9]. A systematic correlation study using amino and SH reagents showed that the K^+-stimulated ATPase, in particular that part stimulated by ionophores, most closely correlated to the H^+ transport activity.

In this report, we have extended the chemical modification study to show that an amino group-specific reagent, 2-methoxy-2,4-diphenyl-3-dihydrofuranone (MDPF), and arginyl group-specific reagents, butanedione and cyclohexanedione, are able to inhibit the gastric K^+-stimulated ATPase activity. We have further demonstrated that ATP provides a complete protective effect against the inhibition, thus suggesting that amino and arginyl groups may actually be at the ATP binding site.

Materials and Methods

Isolation of gastric vesicles. Gastric vesicles were isolated from pig gastric mucosa as described previously [8]. The lightest membrane fraction which layered at the interface between 20 and 27% sucrose was used exclusively in this study.

Enzyme assays. ATPase activity was determined by measuring the liberation of inorganic phosphate from ATP according to the method of Sanui [11]. The basal Mg^{2+}-stimulated ATPase activity (Mg^{2+}-ATPase) was measured in 1.0 ml of reaction medium consisting of 1 mM $MgSO_4$, 1mM ATP and 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (Pipes, pH 6.5). K^+-stimulated ATPase activity was determined as the activity in the basal medium together with 140 mM KCl, minus the activity in the basal medium. K^+-stimulated ATPase activity was measured both in the absence and presence of 10 μM valinomycin. Typically, reaction tubes contained about 35 μg microsomal

protein and the reaction carried out for 20 min at room temperature (20—23°C).

K^+-stimulated *p*-nitrophenylphosphatase was assayed by the liberation of *p*-nitrophenol which absorbs characteristically at 410 nm. The reaction medium used consisted of 4 mM $MgSO_4$, 10 mM Tris (pH 7.5), 5 mM *p*-nitrophenylphosphate and 10 mM KCl. Again, the assay was usually carried out for 20 min at room temperature.

Reaction with MDPF. Gastric vesicles (approx. 0.35 mg protein/ml) in a medium containing 0.25 M sucrose and 37 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (Hepes) at pH 8.5 were reacted with small aliquots of MDPF dissolved in acetone to a final reagent concentration of 5—10 μg/ml. The final concentration of acetone never exceeded 0.5% (v/v). The reaction was initiated by injecting MDPF solution through a microsyringe into a vortexed reaction mixture and allowed to react for various periods of time at room temperature (20—23°C). The reaction was terminated by 10-fold (or greater) dilution in the reaction media prepared for the determination of ATPase or *p*-nitrophenylphosphatase activities.

Reaction with butanedione. Gastric vesicles (1.11 mg protein/ml) in a medium containing 28 mM Hepes and 28 mM sodium borate (pH 7.5) were reacted with a small aliquot of 2,3-butanedione to a final reagent concentration of 12 mM. The reaction was allowed to proceed at room temperature for various periods of time and terminated by 25-fold dilution into the enzyme assay medium.

Assay of H^+ transport activity. H^+ transport activity of the gastric vesicles was measured by the acridine orange uptake method. The uptake of acridine orange driven by the H^+ gradient across vesicles results in the quenching of its fluorescence. Thus H^+ transport activity can be monitored by the change of the fluorescence intensity of acridine orange [8]. Specific experimental conditions will be listed in appropriate figure legends.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis. MDPF-labeled gastric vesicles were solubilized in 3% sodium dodecyl sulfate (SDS) and separated electrophoretically in 7.5% polyacrylamide gel made up in 0.1% SDS. The fluorescent bands were visualized and recorded photographically with an ultraviolet light source before staining with Coomassie Blue to reveal the protein bands.

Miscellaneous. Protein concentration was determined by the method of Lowry et al. [12]. MDPF was a gift from Dr. Weigele of Hoffman-La Roche Inc. 2,3-Butanedione was from Sigma Chemical Co. All other chemicals were of highest purity available commercially.

Results

Modification of amino residues in gastric vesicles

Time course of inactivation. MDPF and fluorescamine are two fluorigenic reagents recently developed by Weigele et al. [13,14] for the labeling and quantitation of primary amines includes amino acids, peptides and proteins. These reagents are non-fluorescent but react with primary amino groups at an alkaline pH to yield highly fluorescent products; the excess of reagent

TABLE II

EFFECT OF DIVALENT METAL IONS ON $^{32}P_i$-PHOSPHOLIPID BREAKDOWN IN ISOLATED ERYTHROCYTE MEMBRANE

Erythrocyte membranes pre-labelled with $^{32}P_i$ were re-incubated in a final volume of 0.25 ml buffer containing 20 mM Tris-HCl (pH 7.45) for 30 min at 37°C, with $CaCl_2$ in the presence or absence of 1 mM $MgCl_2$. The values are expressed as percent of radioactivity (cpm) associated with control membranes (unincubated membranes without any additions) and are the means of four determinations, ±S.D. Radioactivity associated with controls, in cpm/mg non-Hb protein, was 6800, 3600 and 3100 for triphosphoinositide, diphosphoinositide and phosphatidate, respectively. One set of pre-labelled membranes was boiled at 100°C for 2 min and cooled before re-incubation.

Phosphatide	Ca^{2+} (10 μM)		Ca^{2+} (100 μM)			EGTA (2 mM)
	Mg^{2+} absent	Mg^{2+} present	Mg^{2+} absent	Mg^{2+} present	Mg^{2+} present 100°C, 2 min	
Triphosphoinositide	54 ± 3	62 ± 5	31 ± 2	29 ± 2	68 ± 6	74 ± 6
Diphosphoinositide	53 ± 3	57 ± 3	26 ± 2	31 ± 2	57 ± 5	57 ± 5
Phosphatidate	70 ± 8	70 ± 6	59 ± 6	62 ± 7	69 ± 8	69 ± 8

dent breakdown of these lipids. When the pre-labelled membranes were heated at 100°C for 2 min before the addition of calcium and magnesium and subsequently re-incubated, the breakdown of both triphosphoinositide and diphosphoinositide was greatly reduced (Table II).

Two types of pathologic erythrocyte, one with a higher-than-normal cellular calcium content (sickle cells) [12], the other with increased permeability to calcium (pyropoikilocytes) [13], were tested for the pattern of incorporation of $^{32}P_i$ into their membrane polyphosphoinositides and phosphatidic acid components. Table III shows that incorporation of the label into all the lipid components of pyropoikilocytic red cell membranes was greater in comparison to normal erythrocyte membranes. With sickle cells, incorporation of the label into both the diphosphoinositide and phosphatidate was greater than that into the control erythrocytes. On the other hand, incorporation into the triphosphoinositide was slightly less. In general, the normal $^{32}P_i$ incorporation pattern

TABLE III

DISTRIBUTION OF $^{32}P_i$ INCORPORATION INTO THE PHOSPHOLIPIDS OF NORMAL AND PATHOLOGIC ERYTHROCYTES

Incorporation of $^{32}P_i$ into normal and pathologic erythrocytes was carried out in solution K (pH 7.45) at 30% hematocrit for 3 h at 37°C. Reticulocyte-free cells labelled with $^{32}P_i$ were washed and erythrocyte membranes prepared. Radioactivity associated with each of the lipid fractions was determined, The mean values (±S.D.) of four determinations are indicated in the table and are expressed as percent of radioactivity (cpm/mg non-Hb membrane protein) in the corresponding lipids from normal erythrocyte membranes. Radioactivity associated with normal membrane triphosphoinosotide, diphosphoinositide and phosphatidate was 7400, 2900 and 2100 cpm/mg non-Hb protein, respectively. These values are the means of three determinations.

Phosphatide	Normal	Hereditary pyropoikilocytes	Sickle cells
Triphosphoinositide	100	123 ± 10	73 ± 10
Diphosphoinositide	100	225 ± 32	254 ± 14
Phosphatidate	100	587 ± 31	196 ± 20

TABLE I

ATP PROTECTION AGAINST MDPF INHIBITION OF H^+ GRADIENT FORMATION AS MONITORED BY ACRIDINE ORANGE UPTAKE

Gastric microsomal membranes were suspended in 0.25 M sucrose and 7.5 mM Hepes buffer, pH 8.5, to a protein concentration of 0.7 mg/ml. 0.85 ml aliquots were removed and (i) stored with no treatment (control); (ii) treated with 8.6 μg/ml MDPF, and (iii) treated with 8.6 μg/ml MDPF plus 2.5 mM ATP. After 10 and 20-min periods of preincubation, 0.2 ml samples were removed and added to 1.8 ml of assay medium containing 150 mM KCl, 1 mM $MgSO_4$, 50 μM EDTA, 20 mM Pipes buffer (pH 7.0), and 2 μM acridine orange. Acridine orange uptake was monitored by the fluorescence quenching method [8] after the addition of 1 mM ATP. Values are reported as the means ± S.E. (n = 4) of the percent fluorescence quenched after a 6 min incubation at room temperature. For all samples, a reference of no H^+ gradient (100% fluorescence) was subsequently established by adding 2.5 μg/ml of the ionophore, nigericin.

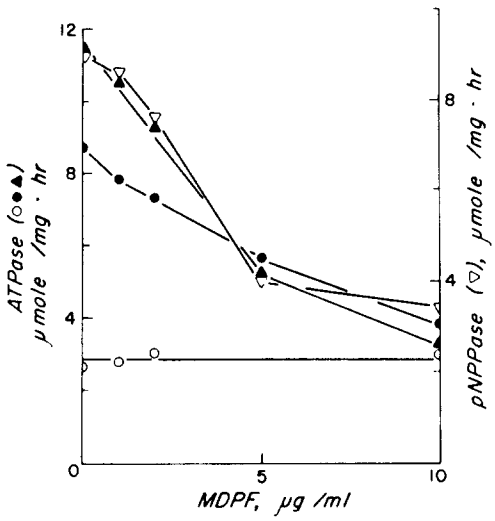
Time of preincubation with MDPF (min)	% fluorescence quench		
	Control (no MDPF)	MDPF	MDPF + ATP
10	25.5 ± 0.65	15.5 ± 0.96	24.5 ± 1.04
20	29.3 ± 0.25	5.7 ± 1.6	25.3 ± 0.48

and marked inhibition of vesicular H^+ transport, measured by quenching of acridine orange fluorescence, as compared to the control without MDPF. Similar to the ATPase activity, ATP effected nearly complete protection of the H^+ transport activity from the inhibition by MDPF.

Effects of reagent concentration and pH on inactivation. Fig. 2 shows the effect of MDPF concentration on the inhibition of various components of the ATPase activity. No inhibition was observed on the Mg^{2+}-ATPase activity for MDPF concentrations as high as 10 μg/ml. On the other hand, the stimulation of the K^+-ATPase activity by valinomycin was eliminated at a reagent concentration of 5 μg/ml and all K^+-stimulated ATPase activity was abolished at about 10 μg MDPF/ml. Inhibition of the K^+-stimulated *p*-nitrophenylphosphatase generally followed that of the K^+-stimulated ATPase when MDPF was below 5 μg/ml. However, there was a residual component of K^+-stimulated *p*-nitrophenylphosphatase which was very resistant and was not inhibited even up to 10 μg MDPF/ml.

The dependence of the inhibition by MDPF on the pH of reaction medium is shown in Fig. 3. There was essentially no inhibition of any component of the ATPase below pH 8.0. However, the effectiveness of the reagent was strikingly increased above pH 8.0. At pH 8.5 the inhibition of the valinomycin stimulation of K^+-ATPase was complete. There was, however, no inhibition on the Mg^{2+}-ATPase even when the pH was raised to 9.0.

The effects of ATP on the MDPF reaction. The protective effect of ATP against MDPF inhibition was demonstrated in Fig. 1. This effect might be explained if MDPF were reacting with those amino groups which are responsible for ATP binding. By virtue of its attachment to these sites, ATP should be able to shield them from reacting with MDPF and thus should protect against the inhibition. Therefore we might expect fewer amino groups to be reacted with MDPF in the presence of ATP. This is indeed the case as shown in Fig. 4. The extent of the MDPF reaction was followed by measuring the increase in fluorescence intensity. As can be seen from the figure there was



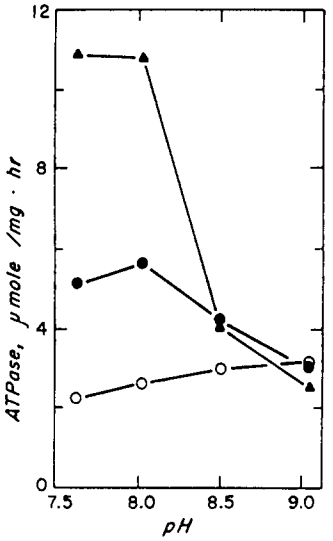


Fig. 2. Effect of MDPF concentration on the ATPase and *p*-nitrophenylphosphatase activities. Gastric microsomes (0.35 mg protein/ml) were reacted with various concentrations of MDPF, as indicated, for 12 min at room temperature. The reaction was terminated by 10-fold dilution into the assay medium for ATPase of *p*-nitrophenylphosphatase activity. ATPase activity was measured in the presence of Mg^{2+} alone (○), Mg^{2+} plus 140 mM KCl (●), and Mg^{2+}, 140 mM KCl plus 10 μM valinomycin (▲). Also shown is K^+-*p*-nitrophenylphosphatase activity (▽).

Fig. 3. Effect of pH on the inhibition of ATPase by MDPF. Gastric microsomes were reacted with 5 μg/ml MDPF for 10 min. The reaction medium consisted of 0.25 M sucrose and 23 mM 4-(2-hydroxyethyl)-1-piperazinepropanesulfonate buffer at the various pH as indicated. ATPase activity is shown for Mg^{2+} alone (○), Mg^{2+} plus 140 mM KCl (●), and Mg^{2+}, 140 mM KCl plus 10 μM valinomycin (▲).

about 25% less reaction in the presence of ATP; the specific sites of MDPF inhibition would be included within this 25%.

It was of interest to determine the effect of ATP on the fluorescent labeling pattern of various protein bands and lipids. Gastric vesicles were labeled with MDPF in the presence and absence of ATP. The vesicles were then solubilized with SDS and electrophoretically separated on SDS-polyacrylamide gel (Fig. 5). The labeled bands were identified by the green fluorescence under ultraviolet illumination and recorded photographically. Densitometry was performed on the film negative to produce the quantitative pattern shown in Fig. 5. The Coomassie Blue-stained electrophoretogram reveals a major protein band of M_r 100 000, which has been shown to contain the K^+-stimulated ATPase [3,15], and many other minor bands. Only two peptide bands are significantly labeled by MDPF in the absence of ATP (Fig. 5). One is the ATPase band; the other is of very low molecular weight which migrates just behind the lipid band. In addition there is label associated with the lipid band (aminolipid). In the presence of ATP (Fig. 5) there is a large decrease in the amount of label on the ATPase band but no significant change occurred on either the low molecular weight peptide or the lipid band. Integration of the area under the trace showed that there was a 71% reduction in the M_r 100000 protein band in the presence of ATP. When the total area (which corresponds to total amount of label) of the two traces were compared there was a 30% reduction in the

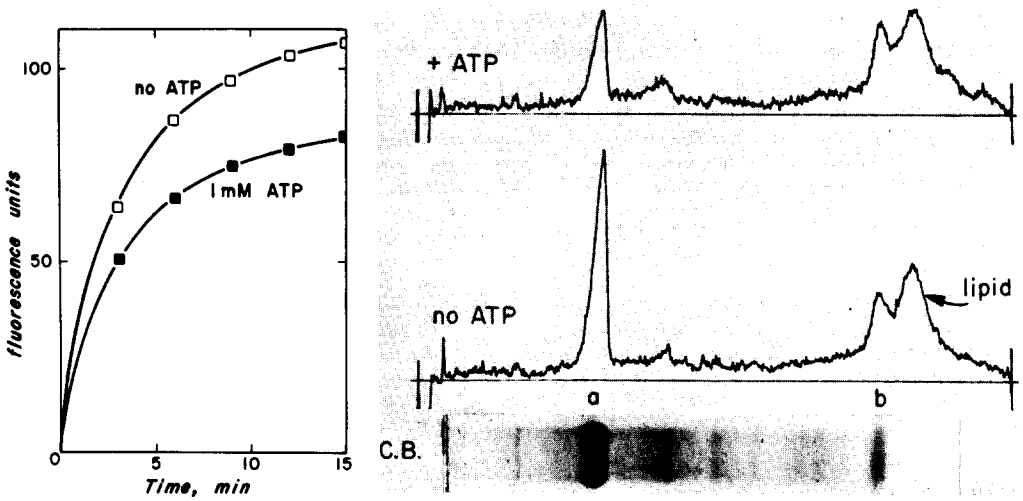


Fig. 4. Effect of ATP on the time course of MDPF reaction. Gastric microsomes (0.39 mg protein/ml) were reacted with 5 μg/ml MDPF at pH 8.5 in room temperature with (■) or without (□) 1 mM ATP. The reaction was terminated at the indicated times by 10-fold dilution into 76% ethanol containing 0.8 mM citrate (pH 4.0). The extent of the MDPF reaction was determined by measuring the fluorescence intensity at 480 nm with the excitation set at 380 nm.

Fig. 5. SDS-polyacrylamide gel electrophoretogram of MDPF-labeled gastric microsomes. Microsomes (0.36 mg protein/ml) were reacted with 7.5 μg/ml MDPF at pH 8.5 for 11 min, with or without 1 mM ATP. The reaction was terminated by 4-fold dilution into 50 mM cold Pipes buffer (pH 6.7). The reacted microsomes were harvested by centrifugation and dissolved in 3% SDS. 120 μg of dissolved microsomes were applied to a 7.5% polyacrylamide slab gel and developed in 0.1% SDS. The fluorescent bands were visualized by ultraviolet light; densitometric tracings are shown for microsomes treated with or without ATP (±ATP). Protein bands were revealed by Coomassie Blue staining (C.B). The major bands labeled by MDPF were the 100 000 M_r band (a), a low molecular weight peptide band (b) and aminolipids (lipid) which do not stain by Coomassie Blue.

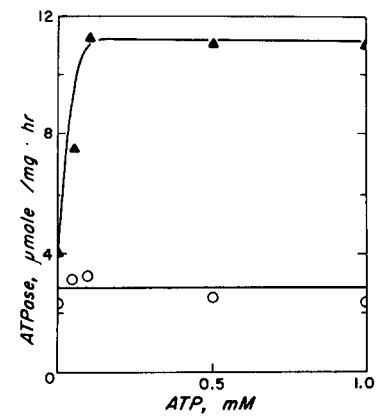


Fig. 6. Effect of ATP concentration on the protection from MDPF inhibition. Gastric microsomes (0.2 mg/ml) were reacted with 5 μg/ml MDPF at pH 8.5 in room temperature for 6 min with various concentrations of ATP. The reaction was terminated by dilution and ATPase activity measured in the presence of Mg^{2+} alone (○) and in the presence of Mg^{2+}, 140 mM KCl plus 10 μM valinomycin (▲).

TABLE II

RELATIVE EFFECT OF VARIOUS NUCLEOTIDES IN PROTECTING GASTRIC ATPase FROM MDPF

Gastric microsomes were pretreated for 10 min with 5 μg/ml MDPF as described in Materials and Methods, either in the presence of 0.5 mM nucleotide or without nucleotide. The reaction was terminated by dilution in the ATPase reaction medium. Values are given as the mean ±S.E. (n = 4). K^+-stimulated ATPase activity was measured with 140 mM KCl and 10^{-5} M valinomycin.

	Mg^{2+}-ATPase ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)	K^+-stimulated ATPase	
		$\mu mol \cdot mg^{-1} \cdot h^{-1}$	% of untreated
Untreated	2.1 ± 0.08	7.0 ± 0.38	100
MDPF-treated			
No nucleotide	2.6 ± 0.01	1.7 ± 0.35	24
ATP	2.0 ± 0.06	7.1 ± 0.64	101
ADP	2.1 ± 0.02	5.2 ± 0.25	74
AMP	2.3 ± 0.03	2.4 ± 0.23	34
CTP	2.4 ± 0.12	2.9 ± 0.43	41
GTP	2.4 ± 0.02	3.6 ± 0.74	51
ITP	2.4 ± 0.04	4.8 ± 0.55	69

presence of ATP which is close to what was observed by the direct fluorescence method shown in Fig. 4. We therefore conclude that the reduction in the total fluorescence in the presence of ATP is due entirely to the reduction in the amount of label on the M_r 100 000 protein band.

Specificity of the protection by ATP. Fig. 6 shows the dependence of the protective effect on the ATP concentration. About 50 μM ATP is enough to give half-maximal protection. This is about the same as the K_m of ATP for the K^+-stimulated ATPase reaction reported previously [9] and is consistent with the interpretation that the protective effect of ATP is due to its binding to the active site. Table II shows the effectiveness of various nucleotides in protecting ATPase from the inhibition by MDPF. As noted above there was little effect of MDPF on the basal, Mg^{2+}-ATPase activity, and preincubation with the nucleotides did not alter this result. ATP was the most effective nucleotide in protecting K^+-stimulated ATPase from inhibition by MDPF, with the following sequence for the several nucleotides: ATP > ADP > ITP > GTP > CTP > AMP.

Modification of arginyl residues

Butanedione and cyclohexanedione are two reagents that have been shown to react specifically with arginyl residues in a variety of biological systems under appropriate conditions [16,17]. These two reagents form a reversible intermediate with the guanidino group which can be stabilized by borate ions [18], thus borate has been included in the modification mixture. Although both reagents showed similar effects on our system, butanedione was more potent. We therefore report here only the results obtained using butanedione as a specific probe for arginyl residues in our system.

The time course for alteration of gastric ATPase activity after reaction with 12 mM butanedione is shown in Fig. 7A. Modification of arginyl residues produced only slight reduction in Mg^{2+}-ATPase activity over the 20 min period of treatment, while the inhibitory effects on K^+-stimulated ATPase activity

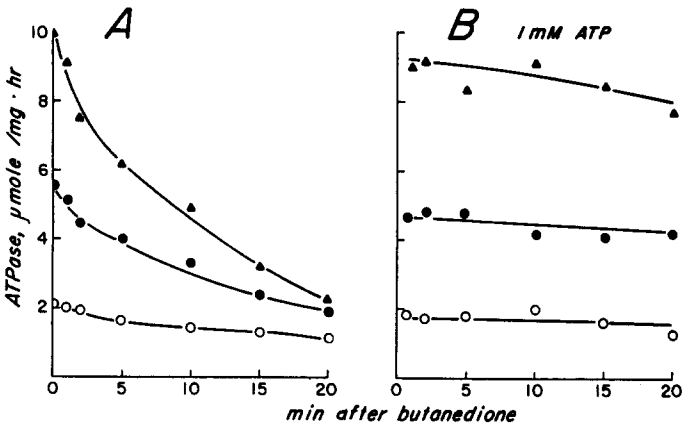


Fig. 7. Time course for inhibition of ATPase activity by 2,3-butanedione and the protective effect of ATP. Gastric vesicles were reacted as described in Materials and Methods with 12 mM butanedione in the absence (A) and presence (B) of 1 mM ATP. The reaction was terminated at the indicated times by dilution into the ATPase assay medium. ATPase activities were measured in the presence of Mg^{2+} alone (○), Mg^{2+} plus 140 mM KCl (●), and Mg^{2+}, 140 mM KCl plus 10 μM valinomycin (▲).

were much more striking. After 20 min of treatment with 12 mM butanedione K^+-stimulated ATPase activities, both in the absence and presence of valinomycin, were reduced to less than 25% of the enzyme rates measured for control preparations. As shown in Fig. 7B, in the presence of 1 mM ATP the microsomal ATPase activities were relatively resistant to inhibition by butanedione.

Discussion

The use of the chemical modification method has led to the conclusion that arginyl and amino groups are involved in the binding of negatively charged substrate or cofactors in a wide variety of soluble enzymes [16,17,19]. Recent studies on ATPase systems indicated that an arginyl residue is at the ATP binding site of bicarbonate-sensitive ATPase in *Escherichia coli* [20], mitochondrial ATPase [21] and $(Na^+ + K^+)$-ATPase [22]. In the case of Ca^{2+}-ATPase from the sarcoplasmic reticulum, both amino and arginyl groups have been shown to be involved [23,24]. Similarly, evidence has been presented in this study to indicate that both of these chemical groups are involved in the ATP site of the gastric K^+-stimulated ATPase. This widespread occurrence of positively charged amino acid residues at the binding site for negatively charged substrate suggests that ionic interaction between the enzyme and the substrate should play a critical role in the catalysis.

Using the fluorigenic amino reagent, MDPF, the present results show the importance and sensitivity of amino groups for gastric K^+-stimulated ATPase activity. This is generally consistent with our previous study using imidoesters [9]. The demonstration that ATP was able to protect against the inhibition by MDPF suggested that the sensitive amino group is at or near the ATP site. However, at the present time we cannot rule out the possibility that the protective effect of ATP is due to a conformational change which might render some amino groups (not necessarily at the binding center) inaccessible to attack by

MDPF. Conversely, the reaction of MDPF with the amino groups may block this conformational change and, by this mechanism, inhibit the ATP binding and/or enzyme activity.

Application of the guanidino group-specific reagent, 2,3-butanedione, showed that arginine groups are also critical to gastric microsomal ATPase activity. As with the MDPF treatment, ATP is also a relatively good protective agent from butanedione inhibition suggesting that arginyl residues are in close proximity to ATP binding sites, or that some ATP-induced conformational change reorients the critical reactive group.

Both MDPF and butanedione were considerably more effective in reducing K^+-stimulating enzyme activities (with and without valinomycin) than in altering basal, Mg^{2+}-ATPase activity. Similar differential inhibitory effects have been noted previously for other inhibitors [9,25]. Taken together with observed differences in pH optima and energy of activation [9] we can conclude that the Mg^{2+}-ATPase is a distinct enzyme from the K^+-stimulated ATPase. Moreover, from the studies of Sachs' group showing partial separation by free-flow electrophoresis [4,7], the enzymes are likely to be associated with separate membrane components.

The sequence reported here for protection of the K^+-stimulated ATPase by nucleotides against MDPF inactivation (i.e. ATP > ADP > ITP > GTP > CTP ▷ AMP) is similar to what we have seen for protection against reaction of amino groups with fluorescamine or guanidyl groups with butanedione. This nucleotide sequence clearly differs from that reported by Sachs et al. [7] for K^+-activated nucleotide hydrolysis (ATP > CTP > GTP > ADP > ITP), and which we have recently confirmed (unpublished observation). For the latter cases, the enzyme turnover for nucleotides was measured, whereas in the case of protection from the amino group reagent the important phenomenon is most likely the relative binding affinity of the nucleotide to the critical site(s). For instance, the Sachs group also reported no hydrolysis of β,γ-methylene ATP, although this analog of ATP did act as a competitive inhibitor for the ATPase [7].

The relatively specific nature of the MDPF reaction with the gastric K^+-ATPase is apparent from the fluorescence traces of the SDS gels. Only two of the protein bands and the aminolipids showed significant labeling by MDPF. In the presence of ATP there was a marked (70%) reduction of fluorescent label only on the M_r 100 000 protein band. The fact that ATP did not alter the labeling pattern of the other two bands suggests that this 'protective' effect may be specific for the critical ATP binding sites on the K^+-ATPase within M_r 100 000 band. It therefore seems feasible to use MDPF as a specific label for the K^+-ATPase. In view of the high quantum yield of the probe it may represent a convenient way to follow the K^+-ATPase distribution through further purification and/or membrane reconstitution processes. This possibility is presently under investigation in our laboratory.

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