Biochimica et Biophysica Acta, 507 (1978) 83—93

BBA 77917

IS THERE A PLASMA MEMBRANE-LOCATED ANION-SENSITIVE ATPase?

III. IDENTITY OF THE ERYTHROCYTE ENZYME WITH $(Ca^{2+} + Mg^{2+})$-ATPase

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(Received July 12th, 1977)

Summary

The characteristics of the anion-sensitive Mg^{2+}-ATPase activity of the rabbit erythrocyte have been studied in a lyophilized ghost preparation.

The enzyme appears to be different from the anion-sensitive Mg^{2+}-ATPase activity of other tissues in many parameters, such as optimal pH, effects of various anions, oligomycin sensitivity and effects of Triton X-100.

The enzyme is insensitive towards inhibition by irreversibly bound 4,4′-diisothiocyano-dihydrostilbene-2,2′-disulfonic acid (H_2DIDS). This excludes a relationship between the enzyme and the "band 3" protein, which is thought to be involved in the anion exchange over the erythrocyte membrane.

From the effects of ethyleneglycol-bis-(β-aminoethylether)-*N,N*′-tetraacetic acid (EGTA), $CaCl_2$, chlorpromazine and ruthenium red it is concluded that the enzyme activity does not represent a separate entity but is part of the $(Ca^{2+} + Mg^{2+})$-ATPase system of the erythrocyte membrane. A reported stimulatory effect of carbonic anhydrase is attributed to a contamination of the carbonic anhydrase preparation by calcium and/or $(Ca^{2+} + Mg^{2+})$-ATPase activator protein.

Introduction

Previous studies by others [1] and ourselves [2—4] have cast serious doubt on the presence of an anion-sensitive Mg^{2+}-ATPase in plasma membranes of various tissues like gastric mucosa [1,2], trout gill [2], kidney [3] and pancreas [4] and thus on its alleged role in anion transport. In these tissues the enzyme

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Abbreviations: H_2DIDS, 4,4′-diisothiocyano-dihydrostilbene-2,2′-disulfonic acid; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N*′-tetraacetic acid.

seems to be located in the mitochondria rather than in the plasma membrane.

A particulate anion-sensitive Mg^{2+}-ATPase activity has, however, also been reported in rabbit erythrocytes [5], which are devoid of mitochondria. Hence, this activity must be located in the plasma membrane. It differs in one respect from the comparable activity in other tissues: it is not inhibited by thiocyanate. In view of the obvious importance of the question whether this enzyme activity can be involved in anion transport, we have decided to make a thorough study of its characteristics.

Methods and Materials

Preparation of erythrocyte ghosts. Erythrocyte ghosts are prepared according to the method of Duncan [5] with slight modifications. Blood from New Zealand white rabbits, freshly obtained by heart puncture, is immediately mixed with 0.1 volume 3.8% sodium citrate, is cooled to 0°C, is filtered over four layers of surgical gauze, and is centrifuged for 15 min at 1400 × *g*. After removal of plasma and "buffy coat" by aspiration, the erythrocytes are washed three times with isotonic (310 mosM) sodium phosphate buffer at pH 8.0 [6], and hemolyzed in 20 mM imidazole · HCl (pH 7.5). The membranes are centrifuged for 25 min at 30 000 × *g*, are washed once with 4 mM $MgCl_2$, 1 mM EDTA, 20 mM imidazole · HCl (pH 7.5), two or three times with 2 mM $MgCl_2$, 20 mM imidazole · HCl (pH 7.5) and finally in double-distilled water. The membranes are resuspended in double-distilled water, lyophilized, and stored in closed tubes at −18°C. For each assay a sample is resuspended in double-distilled water to a protein concentration of approx. 5 mg/ml.

Assay of anion-sensitive ATPase. For reasons to be explained in Results, media different from those used previously [2] are chosen.

Each of the media contains one major anion: HCO_3^-, Cl^-, SCN^-, acetate, azide or SO_3^{2-}. After addition of 20 μl enzyme preparation to 300 μl medium, the incubation medium has the following final composition: 30 mM imidazole, 2 mM $MgCl_2$, 2 mM Na_2ATP (neutralized with imidazole before addition), 10^{-4} M ouabain and 50 mM $NaHCO_3$, NaCl, NaSCN, NaN_3 or acetate or 27.5 mM Na_2SO_3. The pH of the medium is adjusted to 7.0 with the corresponding acid, except in the case of SCN^- or azide where acetic acid is used. After incubation at 37°C the reaction is stopped by addition of 1 ml ice-cold 8.6% trichloroacetic acid. Blanks are run at 0°C. The liberated phosphate is determined as previously described [2].

When oligomycin in ethanolic solution is applied, controls containing the same amount of ethanol ($<2\%$, v/v) are included. Preincubation for 15 min at 0°C and 5 min at 37°C in the absence of ATP precedes the incubation in this case.

The effect of Triton X-100 on the enzyme activity is tested by treating the lyophilized preparation, resuspended in 30 mM imidazole · HCl (pH 7.0) with various Triton X-100 concentrations for 60 min at 0°C after which aliquots are taken to measure ATPase activity.

Protein determination. Protein concentrations are estimated by the method of Lowry et al. [7]. Bovine serum albumin serves as standard.

Materials. Na_2ATP, Na_3CTP, Na_3GTP, Na_3ITP, Na_3UTP, Na_2AMP, ADP and carbonic anhydrase (bovine erythrocyte) are obtained from Boehringer (Mannheim, G.F.R.), ruthenium red ($Ru(NH_3)_4(OH)Cl \cdot 2H_2O$) from Fluka AG (Buchs SG, Switzerland), chlorpromazine from Specia (Amstelveen, The Netherlands), Triton X-100 from The British Drug Houses Ltd. (Poole, U.K.) and acetazolamide from Sigma (St. Louis, Mo., U.S.A.). Oligomycin (Sigma, St. Louis, Mo., U.S.A.) consists of 15% oligomycin A and 85% oligomycin B (average molecular weight, 400).

Dichlorphenamide is a gift LaBaz B.V. (Maassluis, The Netherlands), and the ionophore A-23187 from Eli Lilly and Company (Indianapolis, U.S.A.). H_2DIDS (4,4′-diisothiocyano-dihydrostilbene-2,2′-disulfonic acid) is synthesized by Prof. Dr. H. Fasold and is a gift from Prof. Dr. H. Passow (Max-Planck-Institut für Biophysik, Frankfurt a/M, G.F.R.).

All other materials are from E. Merck (Darmstadt, G.F.R.) and are of analytical grade.

Results

Effect of HCO_3^-

With the Tris-buffered media used in our previous studies [2] no stimulation of the erythrocyte Mg^{2+}-ATPase in the HCO_3^- medium compared to the Cl^- medium can be detected between pH 7 and 9. Since Duncan [5] reported such stimulation in a medium buffered with imidazole, we have replaced Tris by 30 mM imidazole. In this medium the activity in the HCO_3^- medium is higher than that in the Cl^- medium.

In Fig. 1 the pH dependence of the Mg^{2+}-ATPase activity in the Cl^- and HCO_3^- media is presented. The activity in the Cl^- medium decreases almost linearly with decreasing pH, whereas the activity in HCO_3^- medium remains fairly constant down to pH 7, and then begins to decrease. Further experiments have been carried out at pH 7.0.

Effect of other anions

The effects of various anions are shown in Table I and compared with their effects on the ATPase activity in gastric mucosa microsomal fraction [2]. Only HCO_3^- raises the enzyme activity above that in Cl^- medium. The inhibition of the enzyme activity by SCN^- is rather small in contrast to the effects of this ion in gastric mucosa [2], which is in agreement with the findings of Duncan [5]. Sulfite, which strongly stimulates the anion-sensitive Mg^{2+}-ATPase activity from other tissues [2,8—10], slightly inhibits the erythrocyte enzyme. Azide, which strongly inhibits the enzyme activity from gastric mucosa [2,11,12], is ineffective here.

Substrate specificity

The substrate specificity of the enzyme activity is shown in Table II. Only GTP and ITP can replace ATP to a certain extent. The high activity with ADP and especially AMP in Cl^- medium may be due to the presence of a separate 5′-nucleotidase activity in the erythrocyte membrane. The latter activity is inhibited by EDTA [13], and so here the HCO_3^- may complex a divalent cation required for the 5′-nucleotidase activity.

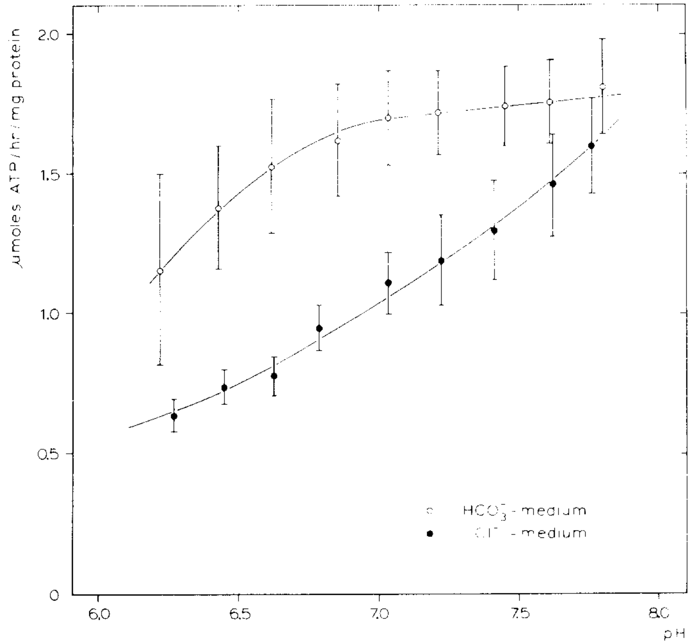

Fig. 1. Effect of pH on the activity of anion-sensitive Mg^{2+}-ATPase of rabbit erythrocyte ghosts in HCO_3^- medium (○) and Cl^- medium (●). The pH values of the media are adjusted with the corresponding acid, except for values below pH 6.7 where HCl is used in both cases. Means of three experiments with standard errors.

Effect of oligomycin

The effect of oligomycin on the enzyme activity is shown in Fig. 2. The inhibition is weak with a pI_{50} below 5.6 (pI_{50} is the negative logarithm of the molar inhibitor concentration at half-maximal inhibition). For the anion-sensitive Mg^{2+}-ATPase activity from rabbit gastric mucosa, rabbit kidney and rat pancreas, we have found pI_{50} values of 7 or above [2—4]. This indicates that the erythrocyte enzyme is much less sensitive towards inhibition by oligomycin.

TABLE I

EFFECTS OF VARIOUS ANIONS ON ANION-SENSITIVE Mg^{2+}-ATPase FROM ERYTHROCYTE GHOSTS COMPARED TO THEIR EFFECTS ON MICROSOMAL ANION-SENSITIVE Mg-ATPase FROM RABBIT GASTRIC MUCOSA

Average relative activities are presented with S.E. for four experiments, with the activity in Cl^- medium set at 1.00.

Major anion	Relative activity	
	Erythrocyte ghosts	Gastric mucosa *
SO_3^{2-}	0.79 ± 0.06	2.59 ± 0.16
SCN^-	0.85 ± 0.04	0.28 ± 0.02
Azide	0.95 ± 0.04	0.10 ± 0.01
Acetate	0.96 ± 0.02	1.38 ± 0.06
Cl^-	≡1.00	≡1.00
HCO_3^-	1.27 ± 0.09	1.54 ± 0.04

* Taken from ref. 2.

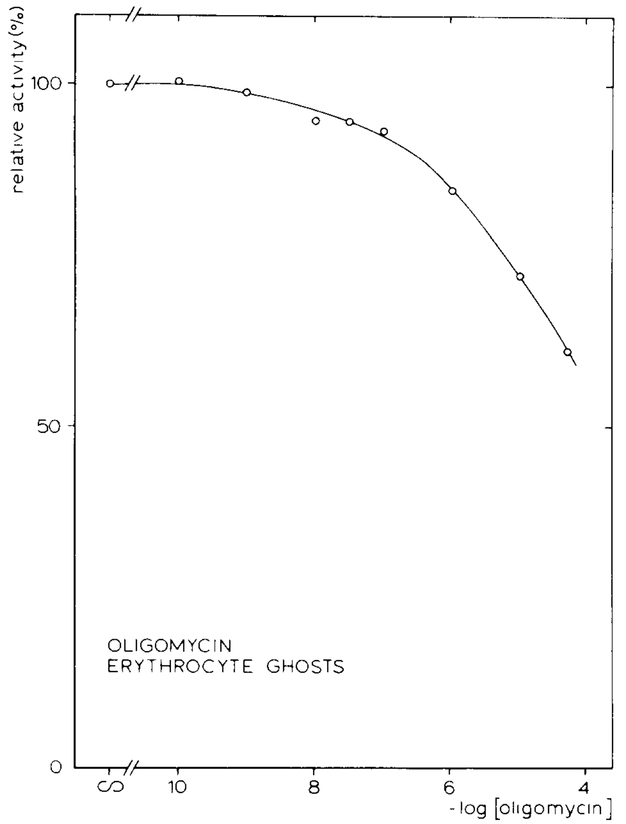

Fig. 2. Effect of oligomycin on the relative Mg^{2+}-ATPase activity in HCO_3^- medium. Mean ratios over the activity without added oligomycin (≡100%) are shown for two experiments.

Effect of Triton X-100

The effect of the non-ionic detergent Triton X-100 on the enzyme activity is shown in Fig. 3. The enzyme activity in both media appears to be relatively unaffected by preincubation with up to 1 mg/ml Triton X-100, but decreases sharply at higher concentrations. At a Triton X-100/protein ratio of 3 the activity is almost abolished. Approx. 25% of the membrane protein but no anion-

TABLE II

SUBSTRATE SPECIFICITY OF ANION-SENSITIVE Mg^{2+}-ATPase FROM ERYTHROCYTE GHOSTS

Relative activities (activity for ATP set at 1.00) are presented with S.E. for three experiments.

Substrate	HCO_3^-	Cl^-
ATP	≡1.00	≡1.00
AMP	0.02 ± 0.01	0.83 ± 0.17
ADP	0.20 ± 0.10	0.37 ± 0.16
GTP	0.40 ± 0.02	0.39 ± 0.04
CTP	0.17 ± 0.04	0.17 ± 0.07
UTP	0.22 ± 0.03	0.18 ± 0.02
ITP	0.39 ± 0.01	0.35 ± 0.04

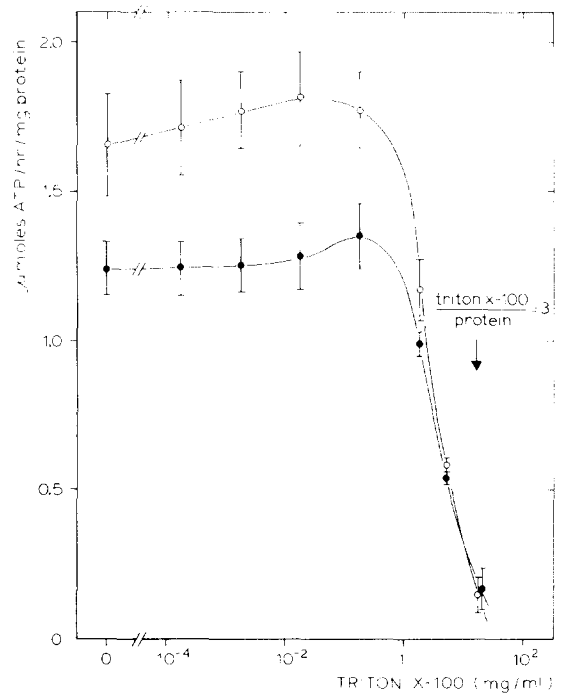

Fig. 3. Effect of preincubation with Triton X-100 on the Mg^{2+}-ATPase activity in HCO_3^- medium (○) and Cl^- medium (●). Means of five experiments with standard errors.

sensitive Mg^{2+}-ATPase activity is solubilized at this Triton X-100/protein ratio (not shown). This is in contrast to the findings for the anion-sensitive Mg^{2+}-ATPase activity from Necturus oxyntic cells [14] and dog [8] or rabbit gastric mucosa (our unpublished results), where a Triton X-100/protein ratio of 3 gives optimal solubilization of the enzyme activity.

Effect of H_2DIDS

Next, we have tested the effect of H_2DIDS, which is a well-known inhibitor of the anion transport over the erythrocyte membrane [15,16]. Treatment of intact cells with 12.5 μM H_2DIDS for 25 min at 37°C results in approx. 80% inhibition of sulphate equilibrium exchange [15], but does not affect the anion-sensitive Mg^{2+}-ATPase activity of erythrocyte ghosts prepared from these cells (Table III). In both the HCO_3^- medium and the Cl^- medium no or even a slight stimulatory effect of H_2DIDS is observed. Also the apparent stimulation of the enzyme activity in both media by $CaCl_2$ (see below) is unaffected by H_2DIDS. This suggests that no inter-relationship exists between the enzyme activity and the "band 3" protein, which is thought to be responsible for the anion exchange over the erythrocyte membrane [16].

Effect of ouabain

Another possibility, which must be considered, is that the enzyme activity would not represent a separate entity but reflect a side-effect of the $(Na^+ + K^+)$-ATPase or $(Ca^{2+} + Mg^{2+})$ATPase activities in the erythrocyte membrane which

TABLE III

EFFECT OF IRREVERSIBLY BOUND H_2DIDS ON ANION-SENSITIVE Mg^{2+}-ATPase ACTIVITY IN RABBIT ERYTHROCYTE GHOSTS

Results are expressed in μmol ATP · h^{-1} · mg^{-1} protein (means of two experiments). $CaCl_2$ was tested at a concentration of 100 μM. H_2DIDS was tested by reacting the intact cells at 10% hematocrit in isotonic phosphate buffer at pH 7.4 for 25 min at 37°C with or without 12.5 μM H_2DIDS. After this, the cells were washed and a lyophilized ghost preparation was prepared as described in Methods and Materials.

Medium	$-H_2$DIDS		$+H_2$DIDS	
	HCO_3^-	Cl^-	HCO_3^-	Cl^-
$-CaCl_2$	1.47	1.24	1.50	.1.32
$+CaCl_2$	2.02	1.51	2.11	1.57

are thought to represent separate and distinct enzymes [17]. When the ouabain concentration was raised from 10^{-4} to 10^{-3} M in the assay media, no effect was seen on the anion-sensitive Mg^{2+}-ATPase activity. This result, in addition to the absence of K^+ in the assay media, excludes a relationship between the enzyme activity and (Na^+ + K^+)ATPase activity.

Relation with (Ca^{2+} + Mg^{2+})ATPase

The enzyme could, however, very well be related to the (Ca^{2+} + Mg^{2+})-ATPase activity of the erythrocyte membrane (Fig. 4). With increasing concentrations of $CaCl_2$ the enzyme activity increases in both the HCO_3^- medium and the Cl^- medium, whereas the ratio of these activities remains fairly constant. The lowered activity in the presence of EGTA and the small increase between 1 and 10 μM Ca^{2+} may indicate the presence of some Ca^{2+} in assay medium or membrane preparation. The slight decrease in the ratio of the activities in the HCO_3^- and Cl^- media at higher Ca^{2+} concentrations may be due to precipitation or complexation of calcium in the HCO_3^- medium. There thus appears to be a strict coupling between the ATPase activities in both media at increasing calcium concentration. The activity is inhibited by ruthenium red and even stronger by chlorpromazine (Table IV), which are both thought to be rather specific inhibitors of erythrocyte (Ca^{2+} + Mg^{2+})ATPase activity [18]. Addition of 100 μM EGTA, a calcium-chelating agent, also lowers the activity. The HCO_3^--stimulated part of the ATPase activity is inhibited 38, 94 and 73% by ruthenium red, chlorpromazine and EGTA, respectively. When 100 μM $CaCl_2$ is added to the media, the enzyme activity increases in both media and is inhibited again by ruthenium red and chlorpromazine.

Relation with carbonic anhydrase

Since carbonic anhydrase was reported to stimulate the anion-sensitive Mg^{2+}-ATPase activity of renal brush border membranes [19], we have tested its effects on the erythrocyte activity. The erythrocyte enzyme appears to be stimulated somewhat by the addition of carbonic anhydrase (Table V). However, the stimulation is not prevented by acetazolamide or dichlorphenamide, which are both strong inhibitors of carbonic anhydrase activity [20]. Furthermore, the effect is exerted also by denatured (boiled) carbonic anhydrase and it

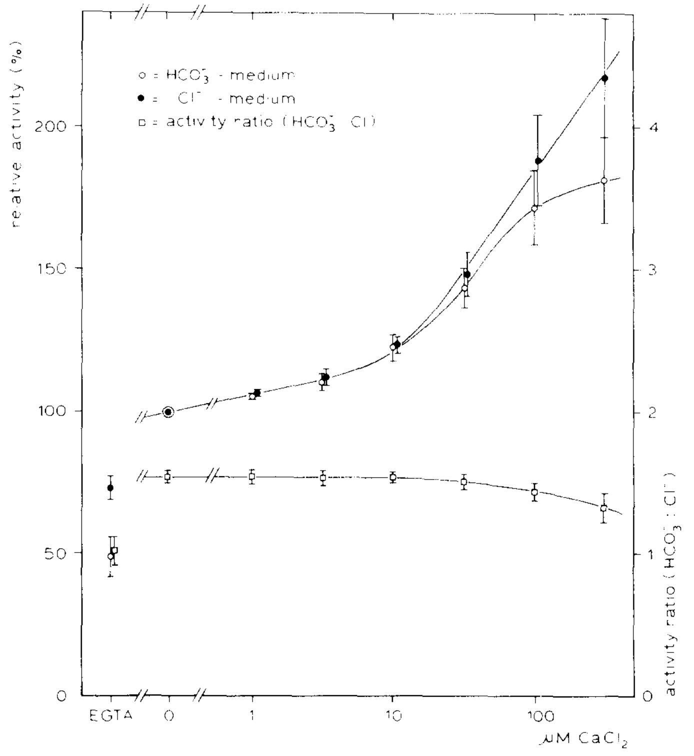

Fig. 4. Effect of CaCl$_2$ on the Mg^{2+}-ATPase activity in HCO$_3^-$ medium (○) and in Cl$^-$ medium (●) and on the ratio of ATPase activity in both media (□). EGTA is tested at a concentration of 100 µM. Mean ratios over the activity in the absence of added CaCl$_2$ (≘100%), with standard errors, are shown for five experiments.

is abolished by addition of EGTA. Bovine serum albumin has no effect on the anion-sensitive Mg^{2+}-ATPase activity (not shown), which suggests that the stimulation is not due to a general "protein" effect. Zinc, a cofactor of carbonic anhydrase, is ineffective and at higher concentrations even inhibitory.

TABLE IV

EFFECTS OF VARIOUS AGENTS ON ANION-SENSITIVE ATPase ACTIVITIES IN ERYTHROCYTE GHOSTS

CaCl$_2$, ruthenium red, chlorpromazine and EGTA are tested at 10^{-4} M. Specific activity is expressed in µmol ATP · h^{-1} · mg^{-1} protein with the standard error; *n* is the number of experiments.

CaCl$_2$ added	Agent	Specific activity		Difference HCO$_3^-$ — Cl$^-$	Ratio HCO$_3^-$/Cl$^-$	*n*
		HCO$_3^-$ medium	Cl$^-$ medium			
—	Control	1.07 ± 0.06	0.81 ± 0.04	0.26 ± 0.04	1.32 ± 0.04	6
—	Ruthenium red	0.90 ± 0.06	0.75 ± 0.04	0.16 ± 0.03	1.21 ± 0.03	6
—	Chlorpromazine	0.58 ± 0.02	0.56 ± 0.02	0.02 ± 0.02	1.03 ± 0.04	6
—	EGTA	0.81 ± 0.06	0.75 ± 0.06	0.07 ± 0.02	1.09 ± 0.03	5
+	Control	1.73 ± 0.08	1.24 ± 0.06	0.49 ± 0.03	1.39 ± 0.02	6
+	Ruthenium red	1.39 ± 0.07	1.23 ± 0.10	0.16 ± 0.04	1.14 ± 0.04	6
+	Chlorpromazine	1.08 ± 0.03	0.98 ± 0.05	0.10 ± 0.03	1.19 ± 0.09	6

TABLE V

EFFECTS OF A CARBONIC ANHYDRASE PREPARATION ON ANION-SENSITIVE Mg^{2+}-ATPase ACTIVITIES IN ERYTHROCYTE GHOSTS

Specific activities are expressed in μmol ATP · h^{-1} · mg^{-1} protein with the standard error for five experiments in both cases. Acetazolamide and EGTA were tested at a concentration of 10^{-3} and 10^{-4} M, respectively.

Agent	Specific activity		Difference HCO_3^- — Cl^-	Ratio HCO_3^-/Cl^-
	HCO_3^- medium	Cl^- medium		
Control	1.57 ± 0.03	1.20 ± 0.03	0.37 ± 0.01	1.31 ± 0.02
Carbonic anhydrase, 200 units/ml	2.99 ± 0.24	1.93 ± 0.06	1.06 ± 0.19	1.54 ± 0.08
Carbonic anhydrase + acetazolamide *	3.35 ± 0.29	2.13 ± 0.14	1.23 ± 0.18	1.57 ± 0.06
Acetazolamide	1.60 ± 0.07	1.24 ± 0.05	0.35 ± 0.05	1.29 ± 0.05
Control	1.41 ± 0.12	1.08 ± 0.09	0.34 ± 0.04	1.31 ± 0.02
Carbonic anhydrase, 100 units/ml	2.10 ± 0.16	1.41 ± 0.06	0.69 ± 0.13	1.48 ± 0.09
Carbonic anhydrase denaturated	1.99 ± 0.15	1.34 ± 0.09	0.66 ± 0.08	1.49 ± 0.05
Carbonic anhydrase + EGTA	1.02 ± 0.06	0.91 ± 0.04	0.11 ± 0.04	1.12 ± 0.05

* Dichlorphenamide, another inhibitor of carbonic anhydrase (n = 2) or higher concentrations of acetazolamide (5 mM) do not abolish the carbonic anhydrase effect either.

Hence, the stimulatory effect might be due to contamination of the commercial carbonic anhydrase preparation with either calcium or an activator of erythrocyte (Ca^{2+} + Mg^{2+})ATPase [21,22]. The first possibility, a stimulatory effect of calcium, cannot fully explain the carbonic anhydrase effect, since the activity ratio also increases (Table V) which is in conflict with the negligible effect of $CaCl_2$ on this ratio (Fig. 4). The second possibility, stimulation by an activator in the carbonic anhydrase preparation, is not ruled out by the persistence of the effect upon boiling the preparation, since the activator protein seems to be relatively heat stable [21]. On the other hand, simultaneous addition of carbonic anhydrase and EGTA abolishes the stimulation by carbonic anhydrase (Table V). Thus both calcium and the activator protein could, either together or separately, be responsible for the observed effect.

Effect of ionophore

The question arises, whether the anion-sensitivity of the (Ca^{2+} + Mg^{2+})-ATPase activity of the erythrocyte membrane is a direct effect of HCO_3^- on the enzyme, or whether HCO_3^- acts by increasing the Ca^{2+} permeability of membrane vesicles. Although vesicle formation is rather improbable in the lyophilized preparation, we have tested the effect of the calcium ionophore A-23187. At $2 \cdot 10^{-5}$ M this substance is without effect on the enzyme activity in both the HCO_3^- medium and the Cl^- medium, with or without added 100 μM $CaCl_2$. This makes a direct effect of HCO_3^- on the (Ca^{2+} + Mg^{2+})ATPase more likely.

Discussion

The purpose of our study was to determine whether the erythrocyte anion-sensitive Mg^{2+}-ATPase activity can be a plasma membrane-located anion transport system. In our previous studies of various tissues we could only detect a mitochondrial localization of the enzyme in all tissues studied, viz. gastric mucosa [2], gill [2], kidney [3] and pancreas [4], thus making a role of the enzyme in anion transport across the plasma membrane in these tissues highly unlikely. However, since erythrocytes do not contain mitochondria, the reported HCO_3^--stimulated Mg^{2+}-ATPase activity in this cell [5] could still play a role in such a process.

The absence of HCO_3^- stimulation in the presence of Tris buffer and the pH behaviour emphasize that this enzyme activity differs quite markedly from that in gastric mucosa [2] and kidney [19]. The erythrocyte activity does not only differ from the others by being relatively insensitive towards inhibition by SCN^-, as previously reported by Duncan [5] and recently by Izutsu et al. [23], but also by the slight effects of sulfite and azide. Its substrate specificity resembles that of the gastric mucosal anion-sensitive Mg^{2+}-ATPase [2], except for the high AMPase activity in the Cl^- medium. It has a much lower sensitivity towards oligomycin compared to the enzyme activity from other tissues [2—4]. Triton X-100 strongly inhibits the erythrocyte activity at the concentration, which optimally solubilizes the gastric mucosal enzyme [8,14]. It is obvious that the erythrocyte enzyme differs in many ways from other anion-sensitive Mg^{2+}-ATPase activities.

The ineffectiveness of H_2DIDS appears to exclude a relationship between the anion-exchange protein in the erythrocyte membrane and the enzyme activity. Ouabain, a known inhibitor of $(Na^+ + K^+)$ATPase activity, is also without effect, indicating that the activity does not represent an anion sensitivity of the $(Na^+ + K^+)$ATPase system. The inhibition of the enzyme activity by chlorpromazine, ruthenium red and EGTA and its stimulation by Ca^{2+} strongly suggest that it is a part of the $(Ca^{2+} + Mg^{2+})$ATPase system of the erythrocyte membrane. Increasing concentrations of $CaCl_2$ result in a parallel increase of the enzyme activity in both the HCO_3^- medium and the Cl^- medium, indicating a direct relationship between these two activities.

Finally, the activity increase evoked by carbonic anhydrase, which was thought to form a part of the anion transport system, can be attributed to a contamination of the carbonic anhydrase preparation. Boiled carbonic anhydrase also stimulates the enzyme activity, whereas acetazolamide and dichlorphenamide do not abolish the carbonic anhydrase effect. EGTA also abolishes the effect, whereas Zn^{2+}, a cofactor of carbonic anhydrase, is ineffective. The ineffectiveness of the Ca^{2+} ionophore A-23187 suggests that the HCO_3^- stimulation is not caused by an increase in calcium permeability of membrane vesicles, but is rather a direct effect of HCO_3^- on the $(Ca^{2+} + Mg^{2+})$ATPase activity, such as reported by Ahlers and Günther [24] for $(Ca^{2+} + Mg^{2+})$ATPase from *Escherichia coli* in the presence of Cl^-.

We must therefore conclude that the HCO_3^--stimulated Mg^{2+}-ATPase of the rabbit erythrocyte membrane does not only greatly differ in its properties from the anion-sensitive Mg^{2+}-ATPase found in other tissues, but that it actually forms part of the $(Ca^{2+} + Mg^{2+})$ATPase activity in the erythrocyte membrane and is not a separate enzyme. A role of this activity in anion transport across this membrane appears very improbable. In other tissues with anion-sensitive Mg^{2+}-ATPase activity, the enzyme does not seem to be located in the plasma membrane, but rather in the mitochondria, which also precludes a role of the enzyme in anion transport.

Acknowledgements

The skilful technical assistance of Mrs. Carolien van Dreumel-Hermens is gratefully acknowledged. We thank Professor Dr. H. Passow (Max-Planck-Institut für Biophysik, Frankfurt a/M, G.F.R.) for the gift of H_2DIDS, which was synthesized by Professor Dr. H. Fasold. This work was supported in part by the Netherlands Organisation for Basic Research (Z.W.O.) through the Netherlands Foundation for Biophysics.

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