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ATPase AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICK AND RAT SMALL INTESTINAL MUCOSA

A.W. SKILLEN and M. RAHBANI-NOBAR

University Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP (U.K.)

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Summary

The alkaline phosphatase and $(Ca^{2+} + Mg^{2+})$-ATPase (ATP phosphohydrolase, EC 3.6.1.3) of chick and rat small intestine have been investigated. The same pH optimum was found for membrane-bound and solubilized alkaline phosphatase, whereas those of the corresponding ATPases differed. The solubilised ATPases had inhibition and activation characteristics similar to those of alkaline phosphatase but markedly different from those of the membrane-bound ATPase. These results suggest that membrane-bound alkaline phosphatase and ATPase are not the same enzyme.

Introduction

It has been suggested that intestinal calcium-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) is identical with alkaline phosphatase [1—3]. The evidence fot his has largely been based on the similar behaviour of the two enzymes towards L-phenylalanine and heat inactivation and their parallel induction in rachitic animals following administration of vitamin D. It is however, not easy to differentiate between true ATPase activity and hydrolysis of ATP by nonspecific alkaline phosphatase. It has been suggested, however, that all ATPases require lipid for their reactivity [4,5] whereas no such requirement has been reported for alkaline phosphatase. The present paper is concerned with the differentiation of the alkaline phosphatase activity from the ATPase activities of the small intestinal mucosa of the chicken and rat.

Materials and Methods

Materials. ATP was obtained from Boehringer Corp. (London) Ltd., Norit A from Norit-Clydesdale Co. Ltd., Glasgow and all other chemicals from BDH

Ltd., Poole, Dorset, Analar grade being used if available.

Methods. White Leghorn chicks, 4 weeks old and 250—350 g rats of both sexes from the Rochester strain of Wistar rats were killed by cervical dislocation. The small intestines were removed and irrigated with ice-cold 0.9% saline.

The brush border membranes were prepared by the method of Forstner et al. [6] except for substitution of EGTA for EDTA. A butanol extract of the membranes was prepared by mixing 7 ml of the membrane suspension with 3 ml butanol for 2 h at 4°C. After 10 min centrifugation at 1000 × *g* the aqueous extract was dialysed for 24 h against 0.1 M Tris-HCl, pH 7.4. The protein content was measured by the method of Lowry et al. [7].

ATPase was measured according to the method of Bais [8] using a reaction mixture containing 50 nmol/l Tris-HCl, 3 mmol/l [γ-^{32}P]ATP and appropriate divalent cations. 0.05 ml of the brush border suspension or its butanol extract, equivalent to 0.5—0.6 mg protein was incubated at 35°C with 1 ml of the reaction mixture for 30 min. The reaction was terminated by addition of 1 ml ice-cold 10% (w/v) trichloroacetic acid containing 10% (w/v) Norit A. After centrifugation at 1000 × *g* for 10 min at 4°C, the ^{32}P in the supernatant was measured in a liquid scintillation counter without the use of a scintillator, the extent of hydrolysis of ATP being calculated from the original cpm (about 20 000) of the 3 mM [γ-^{32}P]ATP. Enzyme activity was expressed in U/l with each unit equivalent to 1 μmol substrate transformed/min.

Alkaline phosphatase activity was measured using a reaction mixture containing 10 mM *p*-nitrophenyl phosphate and 0.5 mM Mg^{2+} in 50 mM Tris-HCl within the pH range 7—9.5 and in 50 mM carbonate/bicarbonate buffer above pH 9.5. 0.05 ml sample was incubated at 35°C with 1 ml of the reaction mixture for 30 min. The reaction was terminated with 5 ml 5 mM EDTA in 0.02 M NaOH and the amount of *p*-nitrophenol released estimated by measuring the absorbance at 405 nm, the enzyme activities being expressed as before.

Results

The ATPase and alkaline phosphatase activities of suspensions of brush border membranes of the small intestinal mucosa of the chicken and the rat and butanol extracts of these are given in Table I. The recovery of ATPases in the butanol extracts was poor and there was negligible activity in the sediment obtained after centrifugation of the butanol extract. The activity-pH relationships of the enzymes are shown in Fig. 1. The membrane-bound ATPases all showed highest activity at pH 8, whereas the solubilised ATPases had highest activity at pH 9. Maximum activity for both membrane-bound and solubilised chick and rat alkaline phosphatases was found at pH 10.

The effect of Mg^{2+} and Ca^{2+} on the membrane-bound and solubilised intestinal alkaline phosphatases and ATPases of the chick is shown in Fig. 2. Each cation had a marked effect on the activity of the membrane-bound ATPase. The effect of Ca^{2+} on the solubilised ATPase was much less and similar to that found with the membrane-bound and solubilised alkaline phosphatase (Table II). Highest ATPase activity was found when the concentration of Mg^{2+} was the same as that of ATP. When the total divalent cation concentration was kept at 3 mM but the proportion of Ca^{2+} and Mg^{2+} varied, it was found that

TABLE I

ALKALINE PHOSPHATASE AND ATPase ACTIVITIES OF BRUSH BORDER PREPARATIONS OF CHICKEN AND RAT SMALL INTESTINAL MUCOSA AND THEIR BUTANOL EXTRACTS

The enzyme activities are the mean and standard deviation of six assays of three separate preparations. Measurements of Mg^{2+}-ATPase Ca^{2+}-ATPase and (Ca^{2+} + Mg^{2+})-ATPase were made with 3 mM Mg^{2+}, 3 mM Ca^{2+} and 1.5 mM Mg^{2+} plus 1.5 mM Ca^{2+}, respectively, 3 mM ATP being used in all cases.

	Enzyme activity (U/l)		% recovered
	Brush border suspension	Butanol extract	
Chicken			
Alkaline phosphatase	393 ± 70	403 ± 68	102
Mg^{2+}-ATPase	315 ± 52	66 ± 14	21
Rat			
Alkaline phosphatase	345 ± 67	350 ± 71	101
Mg^{2+}-ATPase	273 ± 42	76 ± 17	30
Ca^{2+}-ATPase	228 ± 33	44 ± 7	19
(Ca^{2+} + Mg^{2+})-ATPase	367 ± 20	53 ± 5	14

maximum activity was found when the concentration of the Ca^{2+} was the same of that of ATP. When the total divalent cation concentration was kept at 3 mM but the proportion of Ca^{2+} and Mg^{2+} varied, it was found that maximum activity was found when the concentration of the Ca^{2+} was four times that of the Mg^{2+}. It was clear that the observed activity in the presence of both Ca^{2+} and Mg^{2+} is not given by adding the activity found with Mg^{2+} alone to that found with Ca^{2+} alone (Table III).

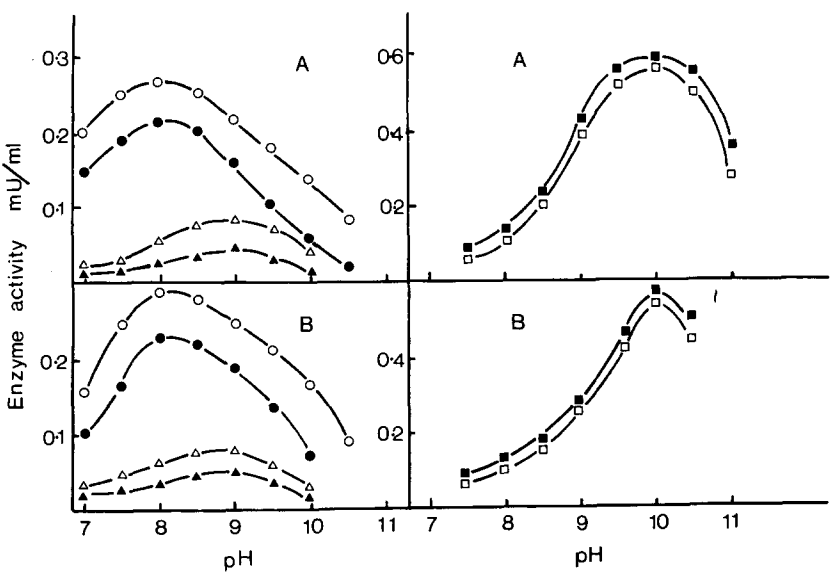

Fig. 1. Activity-pH curves for alkaline phosphatase and ATPase of intestinal mucosa. (A) Chick. (B) Rat. ○, membrane-bound Mg^{2+}-ATPase; ●, membrane-bound Ca^{2+}-ATPase; △, solubilised Mg^{2+}-ATPase; ▲, solubilised Ca^{2+}-ATPase; ■, membrane-bound alkaline phosphatase, and □, solubilised alkaline phosphatase.

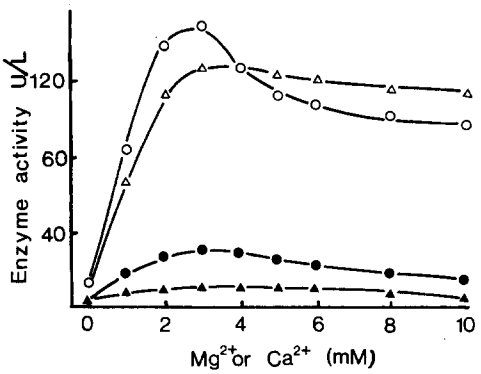

Fig. 2. Effect of Ca^{2+} and Mg^{2+} on chick intestinal ATPase. Membrane-bound enzyme: ○, with Mg^{2+}; △, with Ca^{2+}. Solubilised enzyme: ●, with Mg^{2+}; ▲, with Ca^{2+}.

L-Phenylalanine, a known inhibitor of alkaline phosphatase, was found to have no effect on the activity of membrane-bound ATPases but reduced that of the solubilised enzymes by 62—67%. This degree of inhibition is of the same order as that found with the membrane-bound and solubilised alkaline phosphatases (Table IV).

Using the rat small intestinal mucosa it was found that arsenate did not inhibit the activity of the membrane-bound ATPase at pH 8, whereas the activity of the solubilised ATPase at pH 8 and that of the solubilised and membrane-bound alkaline phosphatases were all inhibited by more than 80% (Table V). With inorganic phosphate and the chicken enzymes a similar pattern was observed, the behaviour of the solubilised ATPase being more like that of alkaline phosphatase than the membrane-bound ATPase (Table VI).

TABLE II

EFFECT OF Ca^{2+} ON THE MEMBRANE-BOUND AND SOLUBLE Mg^{2+}-ATPase AND ALKALINE PHOSPHATASE ACTIVITIES OF CHICK INTESTINE

ATPase was measured at pH 8 using 3 mM ATP and 3 mM Mg^{2+}, alkaline phosphatase at pH 10 using 10 mM p-nitrophenyl phosphate and 0.5 mM Mg^{2+}. Each figure represents the mean of duplicate estimations of three preparations. In no instance did the percent inhibition observed differ from the mean value by more than 2%.

Ca^{2+} (mmol/l)	ATPase activity (% of that in absence of Ca^{2+})		Ca^{2+} (mmol/l)	Alkaline phosphatase activity (% of that in absence of Ca^{2+})	
	Membrane bound	Solubilised		Membrane bound	Solubilised
0	100	100	0	100	100
0.03	97	97	2	102	103
0.15	91	95	4	106	104
0.3	87	93	6	100	102
1.5	82	95	8	101	99
3.0	79	90	10	99	101
15	56	88			

TABLE III

EFFECT OF VARYING Ca^{2+} AND Mg^{2+} ON THE ATPase ACTIVITY OF pH 8.0 OF BRUSH BORDER MEMBRANE PREPARATIONS OF CHICK SMALL INTESTINAL MUCOSA

ATPase was measured at pH 8 using 3 mM ATP. The calculated activity is the sum of that found with the appropriate concentration of each divalent cation alone. The results show the mean and range from duplicate estimations of three separate preparations.

mmol/l		Enzyme activity (U/l)	
Mg^{2+}	Ca^{2+}	Observed	Calculated
3	0	162 (142—185)	—
2.4	0.6	142 (124—158)	198 (192—201)
1.8	1.2	166 (149—192)	232 (224—251)
1.2	1.8	191 (163—175)	226 (212—248)
0.6	2.4	202 (175—226)	198 (186—215)
0	3	146 (130—169)	—

TABLE IV

L-PHENYLALANINE INHIBITION OF MEMBRANE-BOUND AND SOLUBLE ALKALINE PHOSPHATASES AND ATPase OF SMALL INTESTINAL MUCOSA

Percent inhibition in the presence of 20 mM L-phenylalanine. The results are the mean values and range from duplicate estimations using three separate preparations.

		% inhibition (range)	
		Brush border suspension	Butanol extract
Chicken			
Mg^{2+}-ATPase	pH 8	0 (0— 2)	65 (60—67)
Alkaline phosphatase	pH 8	70 (67—72)	81 (79—84)
	pH 10	81 (78—82)	93 (87—95)
Rat			
Mg^{2+}-ATPase	pH 8	1 (0— 3)	62 (58—66)
Ca^{2+}-ATPase	pH 8	0 (0— 2)	64 (59—65)
$(Ca^{2+} + Mg^{2+})$-ATPase	pH 8	1 (0— 2)	67 (61—70)
Alkaline phosphatase	pH 8	73 (70—76)	82 (79—84)
	pH 10	84 (81—86)	93 (91—95)

TABLE V

ARSENATE INHIBITION OF MEMBRANE-BOUND AND SOLUBLE ALKALINE PHOSPHATASES AND ATPases OF SMALL INTESTINAL MUCOSA OF THE RAT

Percent inhibition in the presence of 10 mM arsenate. The results are the mean values and range from duplicate estimations using three separate preparations.

		% inhibition (range)	
		Brush border suspension	Butanol extract
Mg^{2+}-ATPase	pH 8	1 (0— 2)	88 (85—91)
Ca^{2+}-ATPase	pH 8	2 (0— 3)	90 (86—93)
$(Ca^{2+} + Mg^{2+})$-ATPase	pH 8	0 (0— 1)	90 (88—91)
Alkaline phosphatase	pH 8	71 (67—72)	79 (77—82)
	pH 10	81 (78—83)	88 (85—92)

TABLE VI

INHIBITION OF MEMBRANE-BOUND AND SOLUBILISED ALKALINE PHOSPHATASE AND Mg^{2+}-ATPase OF CHICK INTESTINE BY INORGANIC PHOSPHATE

ATPase was measured at pH 8 using 3 mM ATP and 3 mM Mg^{2+}. The results show percent inhibition and are the mean values and range (in parentheses) from duplicate estimations of three separate preparations.

Phosphate concentration (mM)	Brush border suspension		Butanol extract	
	ATPase	Alkaline phosphatase	ATPase	Alkaline phosphatase
1	7 (6— 9)	20 (19—22)	23 (22—25)	28 (25—29)
5	15 (14—17)	33 (30—34)	35 (31—36)	44 (40—45)
10	17 (16—19)	48 (46—49)	44 (43—45)	55 (51—56)
25	25 (23—28)	63 (60—65)	50 (47—52)	75 (70—77)

TABLE VII

(Ca^{2+} + Mg^{2+})-ATPase CONCENTRATIONS OF DIVALENT CATIONS AND SUBSTRATE USED IN DETERMINATION OF ENZYME ACTIVITY

ATP (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	pH	Ref.
5	5	5	7.4	6
5	40		7.4	9
5	5	0.5	7.4	2
2	25	2	7.4	10
5	10	5	7.4	3
5	40	20	7.4	1
2	2	5	8.5	11
2	2	20	8.5	11
3	1.5	1.5	8.0	present study

Discussion

A number of reports have been made claiming that, at least in the intestinal mucosa, alkaline phosphatase and the Ca^{2+}-ATPases are one and the same enzyme [1—3]. Ca^{2+}-ATPase is normally differentiated from Mg^{2+}-ATPase by measuring the latter activity, adding Ca^{2+} and estimating the ATPase activity in the presence of both cations. Those suggesting this identity of alkaline phosphatase with ATPase have, in general, carried out their investigations at pH 7.4. Whilst they may have been justified in doing this by considering the in vitro pH optimum of the enzyme as non-physiological, the Ca^{2+} concentrations used of up to 40 mM are certainly well above those likely to occur in the intestinal mucosa. The varying conditions used by others are listed in Table VII. It is clear there is no agreement about what constitutes Ca^{2+}-ATPase or (Ca^{2+} + Mg^{2+})-ATPase.

We have compared the activity-pH curves of brush border membrane-bound alkaline phosphatase and ATPase of chick and rat intestinal mucosa with those given by butanol extracts of these membranes. Solubilisation with butanol did not have any significant effect on the alkaline phosphatase activity but reduced

that of ATPase to less than one-third of that in the original suspension. Russell et al. [3] reported a similar loss in the ATPase activity of a preparation of intestinal mucosa after butanol extraction. The membrane-bound and solubilised alkaline phosphatases showed similar activity-pH curves, highest activity being found at pH 10. The activity-pH curves using either MgATP or CaATP as substrate with the membrane-bound enzyme were markedly different from those with the butanol-extracted enzyme. This suggested to us that butanol extraction was inactivating the ATPase and that the hydrolysis of ATP by the butanol extract was largely the result of nonspecific hydrolysis by alkaline phosphatase.

The effect of Mg^{2+} and Ca^{2+} on the ATPase activity at pH 8 of the membrane-bound and solubilised enzymes was also markedly different (Fig. 2). Each cation was able to produce marked activation of the membrane-bound ATPase. Their effect on the solubilised ATPase was much less and similar to that found with the membrane-bound and soluble alkaline phosphatase. When the [Mg^{2+}] was optimal, i.e. equal to the ATP concentration, Ca^{2+} inhibited the activity of the membrane-bound ATPase much more than that of the corresponding solubilised enzyme (Table II). Ca^{2+} had little effect on either the membrane-bound or solubilised alkaline phosphatase activities when Mg^{2+} were present. The relative proportions of Ca^{2+} and Mg^{2+} used in the estimation of the (Ca^{2+} + Mg^{2+})-ATPase is important. When the sum of the concentrations of the Ca^{2+} and Mg^{2+} was the same as that of ATP and the proportions of the divalent cations altered, it was found that the observed activity was less than that calculated by summing the activities found with the appropriate concentration of each cation. This suggests that the interaction between the two cations is complex and that the Ca^{2+}-ATPase activity cannot be simply the additional activity observed when Ca^{2+} are added.

L-Phenylalanine did not inhibit the membrane-bound ATPases but reduced that of the solubilised enzymes by 62—67%. This degree of inhibition is of the same order as that of the membrane-bound and solubilised alkaline phosphatases (Table IV). Similarly, arsenate did not inhibit the membrane-bound ATPase, whereas the activity of the solubilised ATPase at pH 8 and that of the solubilised and membrane-bound alkaline phosphatase were all inhibited by more than 80% (Table V). Inorganic phosphate also inhibited the membrane-bound and soluble ATPases to differing extents, that of the latter being similar to that found with the membrane-bound and soluble alkaline phosphatase (Table VI).

Our results do not support the view that the membrane-bound (Ca^{2+} + Mg^{2+})-ATPase is identical with alkaline phosphatase. Butanol extraction results in loss of most of the former activity but does not alter that of the latter. L-Phenylalanine, arsenate and phosphate all inhibit the ATPase activity of butanol extracts and both membrane-bound and solubilised alkaline phosphatase but have no effect on the membrane-bound ATPase. It is possible that alkaline phosphatase acts as an ATPase when it is an integral part of the membrane. Nevertheless, it appears that both enzymes have distinct roles in vivo. Transport of calcium across the intestinal mucosa is considered to be under the influence of the (Ca^{2+} + Mg^{2+})-ATPase [12] and is not inhibited by L-phenylalanine [11]. In contrast, uptake of phosphate is reduced in the presence of L-phenylalanine [13,14] and could therefore likely be under the control of alkaline phosphatase.

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References

1 Haussler, M.R., Nagode, L.A. and Rasmussen, H. (1970) Nature 228, 1199—1201
2 Norman, A.W., Mirchoff, A.K., Adams, T.H. and Spielvogel, A. (1970) Biochim. Biophys. Acta 215, 348—359
3 Russell, R.G.G., Monod, A., Bonjour, J.P. and Fleisch, H. (1972) Nat. New Biol. 240, 126—127
4 Martonosi, A., Donky, J.R., Pucell, A.G. and Halpin, H. (1971) Arch. Biochem. Biophys. 144, 529—540
5 Coleman, R. (1973) Biochim. Biophys. Acta 300, 1—30
6 Forstner, G.G., Sabesin, S.M. and Isselbacher, K.J. (1968) Biochem. J. 106, 381—390
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
8 Bais, R. (1975) Anal. Biochem. 63, 271—273
9 Martin, D.L., Melancon, M.J., Jr. and De Luca, H.F. (1969) Biochem. Biophys. Res. Commun. 35, 819—823
10 De Wolff, F.A. (1975) Eur. J. Pharmacol. 33, 71—79
11 Holdsworth, E.S. (1970) J. Membrane Biol. 3, 43—53
12 Omdahl, J.L. and De Luca, H.F. (1973) Physiol. Rev. 53, 327—372
13 Moog, F. and Glazier, H.S. (1972) Comp. Biochem. Physiol. 424, 321—336
14 Wasserman, R.H. and Taylor, A.N. (1973) J. Nutr. 103, 586—599