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Alkaline Phosphatase of Basal Lateral and Brush Border Plasma Membranes From Intestinal Epithelium

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The alkaline phosphatases present on isolated brush border and basal lateral membranes of rat duodenal epitheilum were examined by means of a variety of biochemical assays and physical methods. The two alkaline phosphatases have similar pH optima of 9.6–9.8, similar substrate k_m's for p-nitrophenyl phosphate (PNPP) of 71 micromolar, similar responses to the inhibitors 2-mercaptoethanol, theophylline, phenylalanine, and ethylenediaminetetra-acetic acid (EDTA), similar sensitivities to calcium, magnesium, zinc, sodium, and potassium, and similar insensitivities to digestion with trypsin or papain. The two enzymes also exhibit similar molecular weights on SDS-polyacrylamide gels in the range 124,000–150,000, and both enzymes show an R_f value of 0.092 on Triton X-100 polyacrylamide gels, indicating similar intrinsic charges. The V_{max} of the brush border enzyme is ten times greater than that of the basal lateral enzyme, 140 μmoles/mg-h as opposed to 14 μmoles/mg-h. The differences in V_{max} are a reflection of the known distribution of alkaline phosphatase in rat duodenum, there being more alkaline phosphatase activity present on the brush border than on the basal lateral surface. One other major difference was observed between the two enzymes, the stimulation of the basal lateral and not the brush border alkaline phosphatase by SDS, Triton X-100, or cholate. We conclude that the enzymes are very similar to one another and probably perform similar membrane functions.

Key words: alkaline phosphatase, basal lateral membranes, brush border membranes, intestinal epithelium

During the last ten years, significant progress has been made with regard to understanding the functions of intestinal brush border and basal lateral membranes. Both surface membranes have been isolated by conventional centrifugation methods [1–5], and both membranes form vesicles capable of various transport functions [6–9]. Most of the investigations performed on brush border and basal lateral plasma membranes have dealt with the two membranes as nonidentical membranes with mutually exclusive

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enzyme compositions [2, 7, 10]. The brush borders have been believed to contain all of the alkaline phosphatase and sucrase activities, while the basal lateral membranes have been thought to contain all of the Na,K-adenosine triphosphatase (Na,K-ATPase), 5′-nucleotidase, and adenyl cyclase activities [2, 7, 10]. The brush border membranes are capable of sodium-coupled, stereospecific D-glucose transport, while transport in the basal lateral membranes is stereospecific and sodium-independent [6, 7, 9]. Major differences in lipid composition of the two membranes have also been reported [11]. It is clear from these observations that the intestinal cell exerts strict control over the composition and function of each membrane.

It has recently been established that at least two enzyme activities, alkaline phosphatase and guanylate cyclase, are located on both the brush border and basal lateral membranes of intestinal cells. Alkaline phosphatase has been observed on both membranes in histochemical studies [12, 13] and in analytical membrane isolation studies [4, 14]. Guanylate cyclase has also been demonstrated to be present on both isolated brush border and basal lateral membranes [15]. These two enzymes, alkaline phosphatase and guanylate cyclase, must therefore be regulated in a different manner than the majority of the plasma membrane enzymes of the intestinal cell.

We have chosen to compare brush border and basal lateral alkaline phosphatase, in order to determine if the two enzymes are the same or are merely alkaline manifestations of two different enzymes. Alkaline phosphatases from various tissues, including intestine, have previously been shown to differ from one another by several criteria [16–20]. We have compared the two alkaline phosphatases by multiple biochemical and physical criteria, and we find little difference between the two enzymes. It is not unreasonable to assume, therefore, that the two enzymes are performing similar functions.

METHODS

Membrane Isolation

Brush border and basal lateral plasma membranes were isolated from rat duodenal epithelium by previous established methods [4, 5, 14]. Routinely, purification factors of (35–45)-fold and (15–20)-fold were obtained for isolated brush border and basal lateral membranes, respectively, relative to initial homogenate. Purification factors were determined by measuring the specific activity of specific marker enzymes relative to the initial homogenate; sucrase and Na,K-ATPase were used as specific marker enzymes for brush border and basal lateral membranes, respectively. It has been previously demonstrated that the alkaline phosphatase of purified basal lateral membranes is definitely not due to brush border contamination [4]; ratios of alkaline phosphatase:sucrase activity were 2.8 in the basal lateral membranes and 0.8 in brush border membranes. After the final isolation step, the membranes were stored in buffer containing 10 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM sodium azide, and 10 mM $MgCl_2$. The $MgCl_2$ was necesary to preserve alkaline phosphatase activity, which remained stable for at least 2 weeks. Both brush border and basal lateral membranes were isolated from the same intestinal homogenates.

Alkaline Phosphatase Assay

Alkaline phosphatase activity was measured in 50 mM Tris-maleate buffer, pH 9.0, 5 mM $MgCl_2$, 0.25 mM $CaCl_2$, 0.2 mM $ZnCl_2$, and 5 mM p-nitrophenyl phosphate (PNPP), at 23°C. Most reactions were performed in 0.5 ml volumes, and the reaction was initiated

by the addition of approximately 1 μg brush border membrane or 10 μg basal lateral membrane. The reaction was terminated by the addition of 3.5 ml of 1N NaOH after 10–20 min, and the absorbance measured at 410 nm. All experiments were run in duplicate, and data points represent the means of 4–10 determinations. The percentage error within and between experiments was less than 10%. Enzyme activities were calculated with an extinction coefficient of 15,400 $M^{-1} \times cm^{-1}$. Variations in the assay conditions are as follows:

pH optimum: The reaction mixture was adjusted to the appropriate pH with 1 N NaOH or 1 N HCl. The use of other buffers was avoided because of possible inhibition of enzyme activity.

Kinetics: Experiments were performed in 1 ml reaction volume to prevent major changes in substrate concentrations.

2-mercapthoethanol, L-phenylalanine, and theophylline: The inhibitors were added directly to the reaction mixture in concentrations of 0–0.4% (v/v), 0–15 micromolar, or 0–10 mM, for 2-mercaptoethanol, theophylline, and L-phenylalanine, respectively.

Ethylenediaminetetraacetic acid (EDTA) inhibition and reactivation: EDTA was added to brush border and basal lateral membranes to a 2.5 mM excess of EDTA over $MgCl_2$, and samples were assayed for up to 10 min after EDTA addition. For reactivation, membranes were pretreated with a 2.5 mM excess of EDTA for 30 min before assay. The reaction mixture in the EDTA inhibition experiments consisted only of 50 mM Tris-maleate, pH 9.0, and 5 mM PNPP. The reaction mixture in the reactivation experiments consisted of 50 mM Tris-maleate, pH 9.0, 5 mM PNPP, and $ZnCl_2$, $MgCl_2$, or $CaCl_2$ in a concentration range of 0–0.5 mM.

Cation sensitivity: Membranes were pelleted and resuspended in magnesium-free storage buffer. Sodium dodecyl sulfate (SDS) was added to the membranes to a concentration of 1%, to prevent artifacts due to rate-limiting influx or efflux of ions into or out of the membrane vesicles. Activity was then measured in 50 mM Tris-maleate, pH 9.0, 5mM PNPP, and a concentration of $ZnCl_2$, $MgCl_2$ or $CaCl_2$ of 0–1.0 mM. The concentration range for NaCl or KCl was 0–100 mM.

Detergent effects: Assays were performed in normal reaction mixture plus SDS, Triton X-100, or cholate in a concentration range of 1–10 mM. For the SDS samples, the appropriate SDS blanks had to be run simultaneously owing to interference at 410 nm; this was not necessary with Triton X-100 or cholate.

Proteolytic digestion: Membranes were digested with papain or trypsin with a 1:10 ratio of protease:membrane protein (mg:mg) and samples were taken at 15-min intervals until 60 min.

Polyacrylamide Gel Electrophoresis

Slab polyacrylamide gels were run using the system of Laemmli [21], at a constant current of 25 mA. Samples were solubilized in 0.125 M Tris/HCl, pH 6.8, 10% glycerol, 10% 2-mercaptoethanol, 0.0001% bromophenol blue, and either 2.5% SDS or 5% Triton X-100. Samples that were to be stained for alkaline phosphatase activity were not treated with 2-mercaptoethanol. The gels were stained for protein according to Cleveland et al [22] and for alkaline phosphtase activity according to the napthol AS-BI method [23]. Protein standards used were myosin, β-galactosidase, phosphorylase b, bovine serum albumin (BSA), and ovalbumin. For molecular weight determinations, the molecular

weights of the standards were plotted versus their respective mobilities according to Weber and Osborn [24]; linear regression analysis gave a correlation coefficient of 0.985.

Chemicals

All chemicals were obtained from Sigma (St. Louis) with the exception of electrophoresis protein standards, which were obtained from Bio Rad (Richmond, California).

RESULTS

pH Optima and Kinetics

Our initial comparison and characterization of brush border and basal lateral alkaline phosphatases was to establish pH optima of the two enzymes and to establish the kinetic parameters of each enzyme. A plot of PNPP hydrolysis versus pH is presented in Figure 1, and it can be seen that the two normalized pH curves are essentially identical. The pH optimum for each enzyme is 9.6–9.8, and this result is close to the pH optimum of 9.5 found for membrane-bound calf intestinal alkaline phosphatase [25]. The pH optima curves establish that, by definition, both the brush border and basal lateral membrane contain an alkaline phosphatase. This is particularly important with regard to the basal lateral membrane, which also contains acid phosphatase and Na, K-ATPase activities [4]. Figure 1 establishes that the basal lateral alkaline phosphatase activity is not merely a "spillover" of an unrelated phosphatase activity with optimum activity at neutral or acid pH.

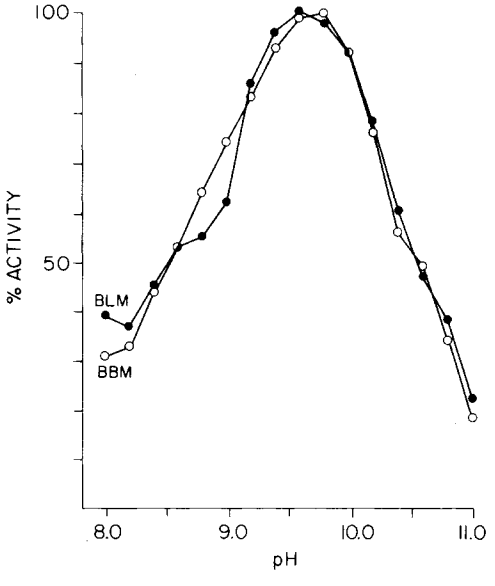

Fig. 1. pH optima of brush border and basal lateral alkaline phosphatases. Alkaline phosphatase activity was measured as described in Materials and Methods at various pH's, and the results are plotted as percentage of the maximum observed activity, in order to give a more precise comparison of the pH optima. On an absolute scale, the specific activity of the brush border enzyme was approximately ten times higher than that of the basal lateral enzyme. BBM, brush border membrane alkaline phosphatase; BLM, basal lateral membrane alkaline phosphatase.

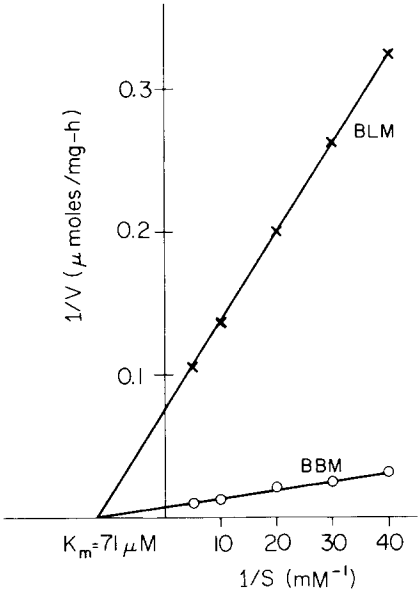

Fig. 2. Substrate kinetics of brush border and basal lateral alkaline phosphatases. Alkaline phosphatase activity was measured as described in Materials and Methods at various PNPP concentrations, and the resulting lines were determined by least-squares analysis. Correlation coefficients for the brush border and basal lateral enzymes were 0.99 and 1.00, respectively. V is the rate of PNPP hydrolysis, and S is the PNPP concentration.

Substrate kinetics, presented in Figure 2, support the idea that the two alkaline phosphatases are similar. The K_m's of the alkaline phosphatases are identical, having a value of 71 micromolar at pH 9.0, and this similarity in K_m is striking when compared to the almost tenfold difference observed in V_{max}. The difference in V_{max}'s is consistent with the known distribution of alkaline phosphatase, the majority of activity being found on the brush border membrane [4].

Inhibitors

Alkaline phosphatases from different tissues have typically shown different responses to various inhibitors [16, 17]. In particular, L-phenylalanine is a specific inhibitor of intestinal alkaline phosphatase, and we have also found theophylline to be a particularly potent inhibitor. 2-Mercaptoethanol has been shown to dissociate alkaline phosphatase dimers to monomers [26, 27], and we have found the same effect in preliminary studies on purified calf alklaine phosphatase. The effect of these three inhibitors on brush border and basal lateral alkaline phosphatase is shown in Figure 3, and all the inhibitors used reduce alkaline phosphatase activity to below 50%. The inhibition by 2-Mercaptoethanol (Fig. 3a) is presumably due to the dissociation of active dimer into an inactive monomer. It is also possible that one or more disulfide bridges are essential to the active site of the enzyme, and that inhibition of the enzyme and dissociation into monomers are not related; we cannot differentiate between these two effects at the present time. Theophylline inhibits alkaline phosphatase at micromolar concentrations (Fig. 3b), while L-phenylalanine, an uncompetitive inhibitor of alkaline phosphatase [28], is effective in the millimolar range (Fig. 3c). The relationship of theophylline and L-phenylalanine to

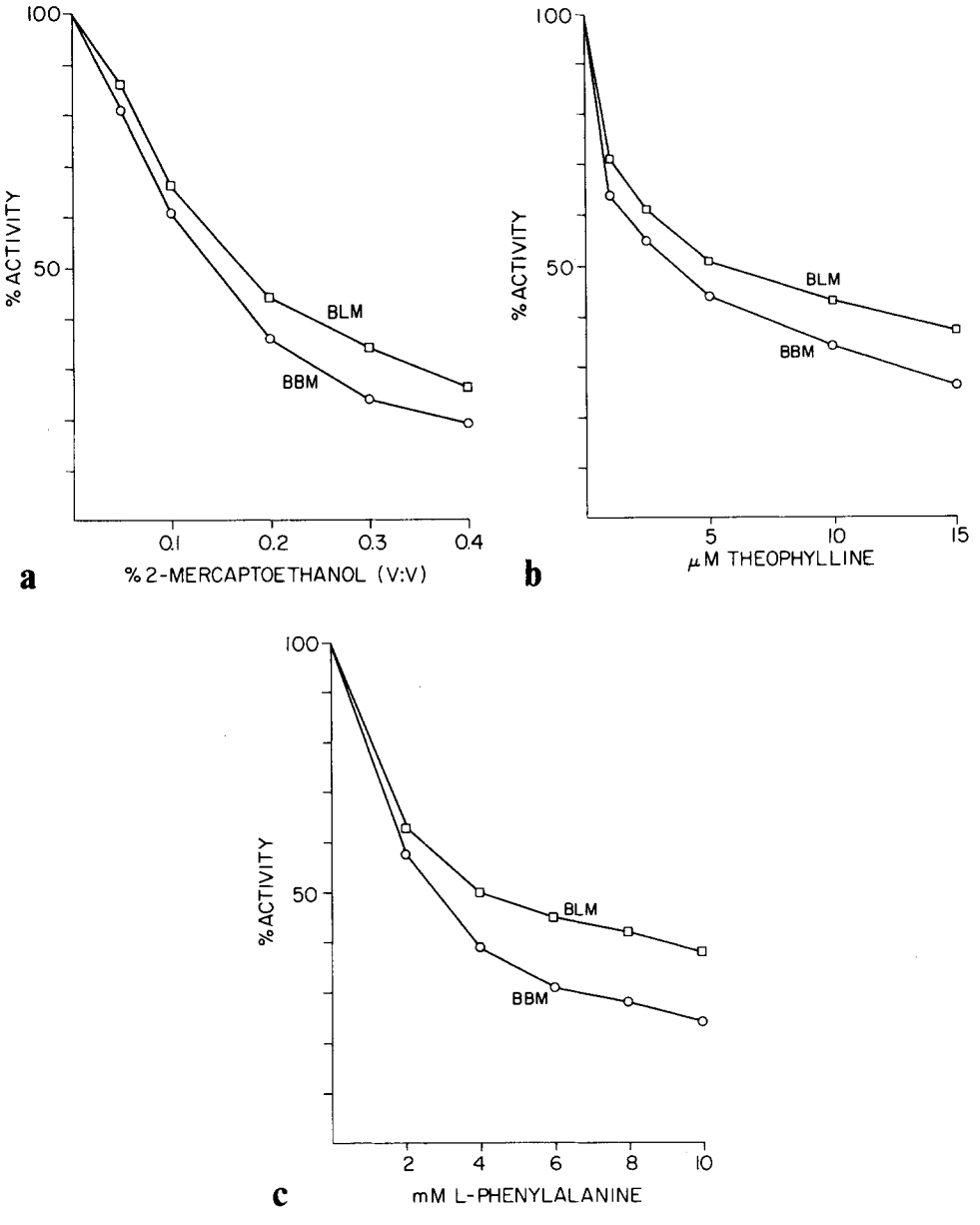

Fig. 3. Effect of inhibitors on brush border and basal lateral alkaline phosphatases. Alkaline phosphatase activity was measured according to Materials and Methods. a: Effect of 2-mercaptoethanol; b: effect of theophylline; c: effect of L-phenylalanine. Inhibitor concentrations represent concentrations present in the assay mixture.

enzyme function is currently unknown, primarily because the physiologic functions of alkaline phosphatases are currently unknown. Both alkaline phosphatases respond to the inhibitors in a similar manner, although the brush border enzyme is slightly more sensitive to the inhibitors used than is the basal lateral enzyme.

EDTA Inhibition and Subsequent Reactivation

The interaction of alkaline phosphatase with divalent cations is relatively complex; magnesium stimulates enzyme activity, yet removal of zinc from the enzyme causes a rapid loss of activity [29]. Alkaline phosphatases are typically sensitive to treatment with chelating agents, and only zinc is effective in reactivating chelator-treated alkaline phosphatase [29]. Brush border and basal lateral alkaline phosphatases both exhibit the expected inhibition upon EDTA treatment (Fig. 4a), and reactivation of the inhibited enzyme is specific for zinc (Fig. 4b). Both calcium and magnesium are relatively ineffective in reactivating the EDTA-treated enzyme (Fig. 4b). The two alkaline phosphatases show similar responses on a qualitative level to EDTA inhibition and zinc reactivation, yet on a quantitative level the brush border enzyme shows a more rapid inhibition and a more complete zinc reactivation than does the basal lateral enzyme.

Cation Sensitivity

The responses of brush border and basal lateral alkaline phosphatases, without prior EDTA treatment, to magnesium, calcium, and zinc are shown in Figure 5, and the responses of the two enzymes are similar. The presence of zinc causes a slight stimulation

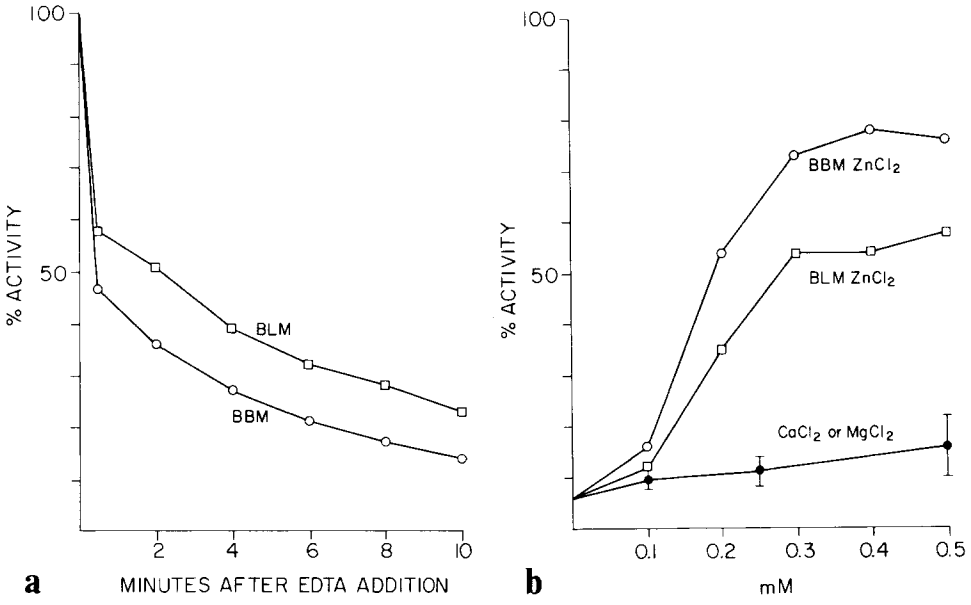

Fig. 4. a: Inhibition of brush border and basal lateral alkaline phosphatases with EDTA. EDTA treatment is described in Materials and Methods. b: Reactivation of EDTA-treated alkaline phosphatases by divalent cations. EDTA-treated brush border and basal lateral alkaline phosphatases were treated as described in Methods, and results were plotted relative to the activity measured in untreated membranes. The results for $CaCl_2$ and $MgCl_2$ reactivation of both brush border and basal lateral alkaline phosphatases fall within the given range bars.

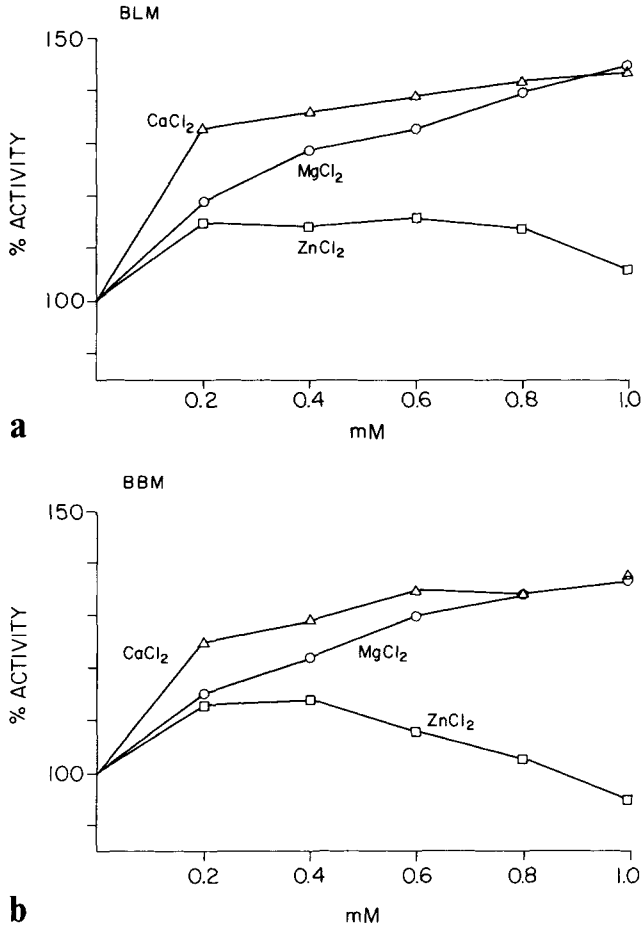

Fig. 5. Effect of divalent cations on basal lateral (a) and on brush border (b) alkaline phosphatase activity. Alkaline phosphatase activity was measured in the presence of $CaCl_2$, $MgCl_2$, or $ZnCl_2$, as described in Materials and Methods.

of activity at 0.2 mM, followed by a gradual decrease to 105% and 95% of original activity at 1 mM concentration for the basal lateral and brush border enzyme, respectively. Magnesium causes a gradual stimulation of enzyme activity, with a stimulation to 145% of original activity for basal lateral and 137% of original activity for brush border alkaline phosphatase with 1 mM $MgCl_2$. Surprisingly, calcium caused the same degree of stimulation as magnesium at 1 mM concentration, and calcium is more stimulatory than magnesium at the lower concentrations used. This calcium stimulation of alkaline phosphatase has not been previously demonstrated, and is interesting in light of previous investigations that have indicated a possible relationship between intestinal alkaline phosphatase and calcium ATPase [30, 31].

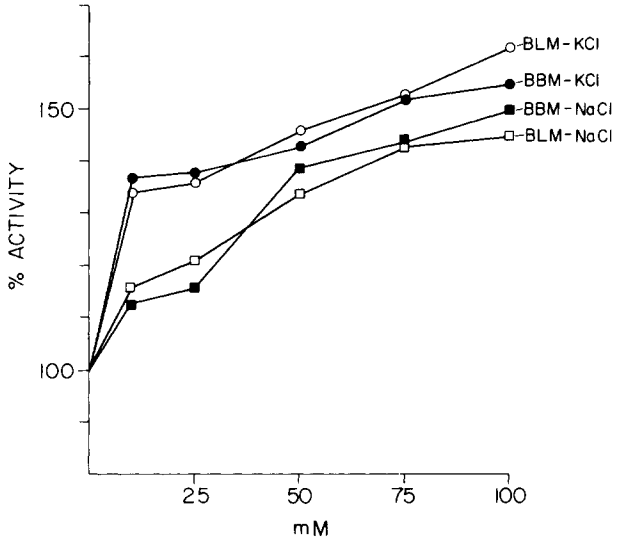

Fig. 6. Effect of monovalent cations on brush border and basal lateral alkaline phosphatases. Alkaline phosphatase activity was measured in the presence of NaCl or KCl, as described in Materials and Methods.

The two alkaline phosphatases are also stimulated by the presence of KCl or NaCl (Fig. 6), although much more KCl or NaCl is necessary to achieve stimulation comparable with that found with 1 mM $CaCl_2$ or $MgCl_2$ (Fig. 5). Both enzymes show a greater stimulation with KCl than NaCl, and that difference is more pronounced at lower concentrations. These results conclusively rule out any contribution of Na,K-ATPase (often measured as K-stimulated PNPPase) to the basal lateral alkaline phosphatase activity, because the brush border alkaline phosphatase shows a similar sensitivity to KCl and NaCl, yet the brush border membrane is largely devoid of Na,K-ATPase [1–4].

Detergent Effects

Many membrane-bound enzymes are inhibited by detergent solubilization of the membrane, yet alkaline phosphatase from calf thymus shows little inhibition by Triton X-100 or SDS [32]. We have examined the effect of Triton X-100, SDS, and cholate on brush border and basal lateral alkaline phosphatase, and the results are presented in Figure 7. Neither alkaline phosphatase shows inhibition due to detergent solubilization of the membrane; this lack of detergent inhibition, particularly the lack of inhibition by SDS, is remarkable for a membrane-bound enzyme.

It is clear from Figure 7 that the alkaline phosphatases of the two membranes differ in their response to detergents. The basal lateral enzyme shows a marked stimulation with detergents, although cholate is not as effective as SDS or Triton X-100 at lower detergent concentrations. In contrast, the brush border alkaline phosphatase shows little change in activity with detergent treatment. The detergent treatment has therefore shown a major difference between the two membrane-bound alkaline phosphatases.

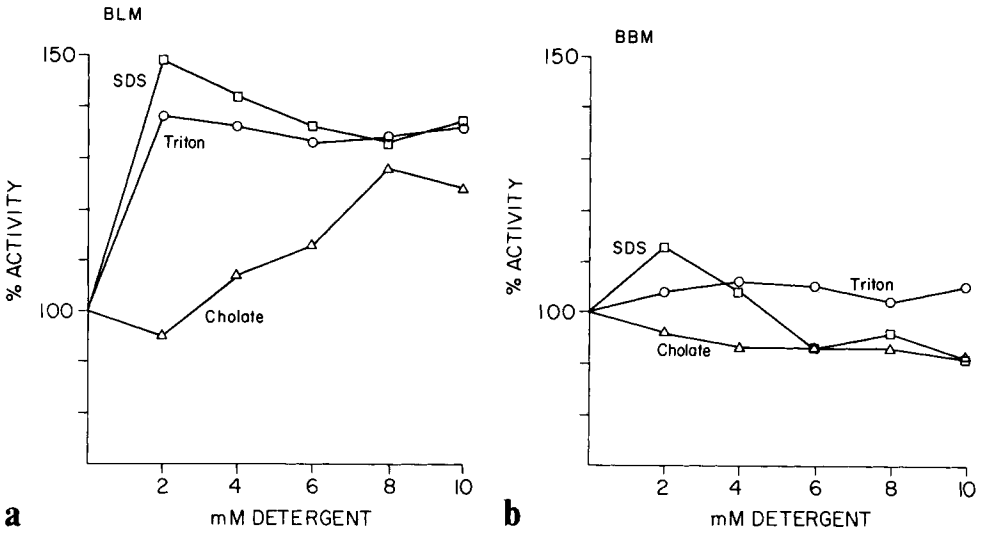

Fig. 7. Effect of detergents on basal lateral (a) and on brush border (b) alkaline phosphatase. Alkaline phosphatase activity was measured in the presence of SDS, Triton X-100, or cholate, as described in Materials and Methods.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gels have been used for the comparison of various alkaline phosphatases using Triton X-100 as a solubilizing agent [33]. We have taken advantage of the stability of alkaline phosphatase in detergents by running polyacrylamide gels in the presence of either Triton X-100 or SDS, followed by histochemical staining of the gels for alkaline phosphatase localization. The use of Triton X-100 or SDS in the gel system allows us to compare brush border and basal lateral alkaline phosphatase on the basis of intrinsic charge, using Triton X-100, or molecular weight, using SDS. On Triton X-100 gels, the brush border and basal lateral enzymes show identical migration distances, and each alkaline phosphatase shows a major band and a more diffuse, faster-migrating band (Fig. 8). Calf intestine alkaline phosphatase migrates significantly faster than the rat alkaline phosphatases, indicating that Triton X-100 gels are sensitive enough to detect species differences in alkaline phosphatases. The similarity between brush border and basal lateral enzymes in terms of migration distances on Triton X-100 gels indicates little or no difference in intrinsic charge.

SDS gels stained for protein and alkaline phosphatase activity are presented in Figure 9A and $9A_1$. Gels stained for enzyme activity show a relatively broad band, in the molecular weight range of 124,000–150,000 daltons (Fig. 9D and $9D_1$). The brush border and basal lateral enzymes have similar molecular weights, and the components of the broad band appear to be a major band at 133,000 daltons and a minor band at 124,000 daltons. This observed heterogenity is in agreement with previous results obtained using purified rat intestinal alkaline phosphatase [18, 19]. The molecular weight of the major band, 133,000, differs with that of 160,000 found by Malik and Butterworth [19], yet is close to the molecular weight of 140,000 for intestinal alkaline phosphatase found by other investigators [34].

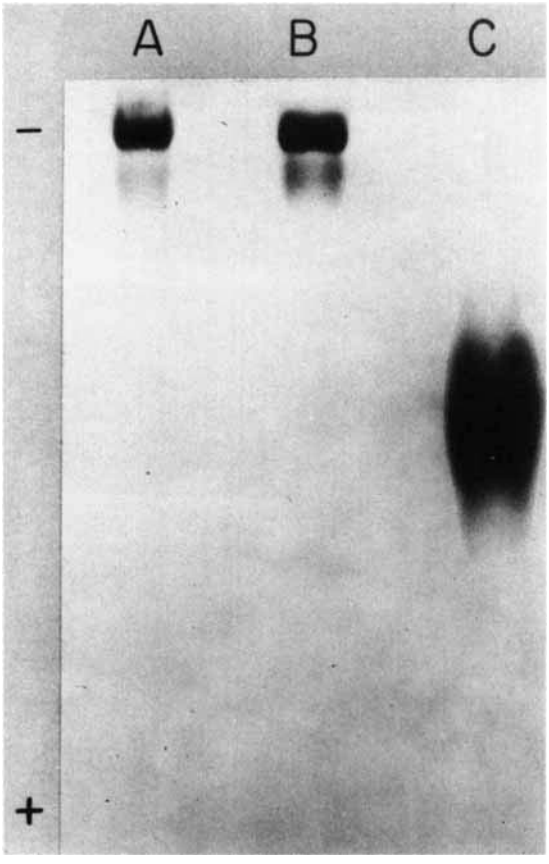

Fig. 8. Polyacrylamide gels of alkaline phosphatases in the presence of Triton X-100. Samples were solubilized in 5% Triton X-100, run on 5% polyacrylamide gels, and stained for alkaline phosphatase activity as described in Materials and Methods. A: basal lateral alkaline phosphatase; B: brush border alkaline phosphatase; C: calf intestine alkaline phosphatase (Sigma).

A proteolytic digestion method has recently been shown to be very sensitive when closely related proteins are being compared [22], and this method has been successfully used for the comparison of membrane proteins as well [35]. Initially, we hoped to use this method to compare the structures of brush border and basal lateral alkaline phosphatases, by comparing the molecular weights of active subfragments on polyacrylamide gels. This approach seemed feasible, as Malik and Butterworth [19] had converted purified intestinal alkaline phosphatase to an active lower-molecular-weight form by proteolytic digestion. However, we found no appreciable digestion of brush border or basal lateral alkaline phosphatase, even though we used 20 times more proteolytic enzyme per unit membrane protein for 60 times as long as was necessary with sarcoplasmic reticulum [35]. The remarkable insensitivity to proteolytic digestion is illustrated in Figure 9D–F and $9D_1$–F_1; no observable molecular weight alteration is found upon extensive digestion of the membranes. The degree of digestion of membrane protein is seen in Figure 9A–C and $9A_1$–C_1. After digestion, very little protein is present on SDS-polyacrylamide gels,

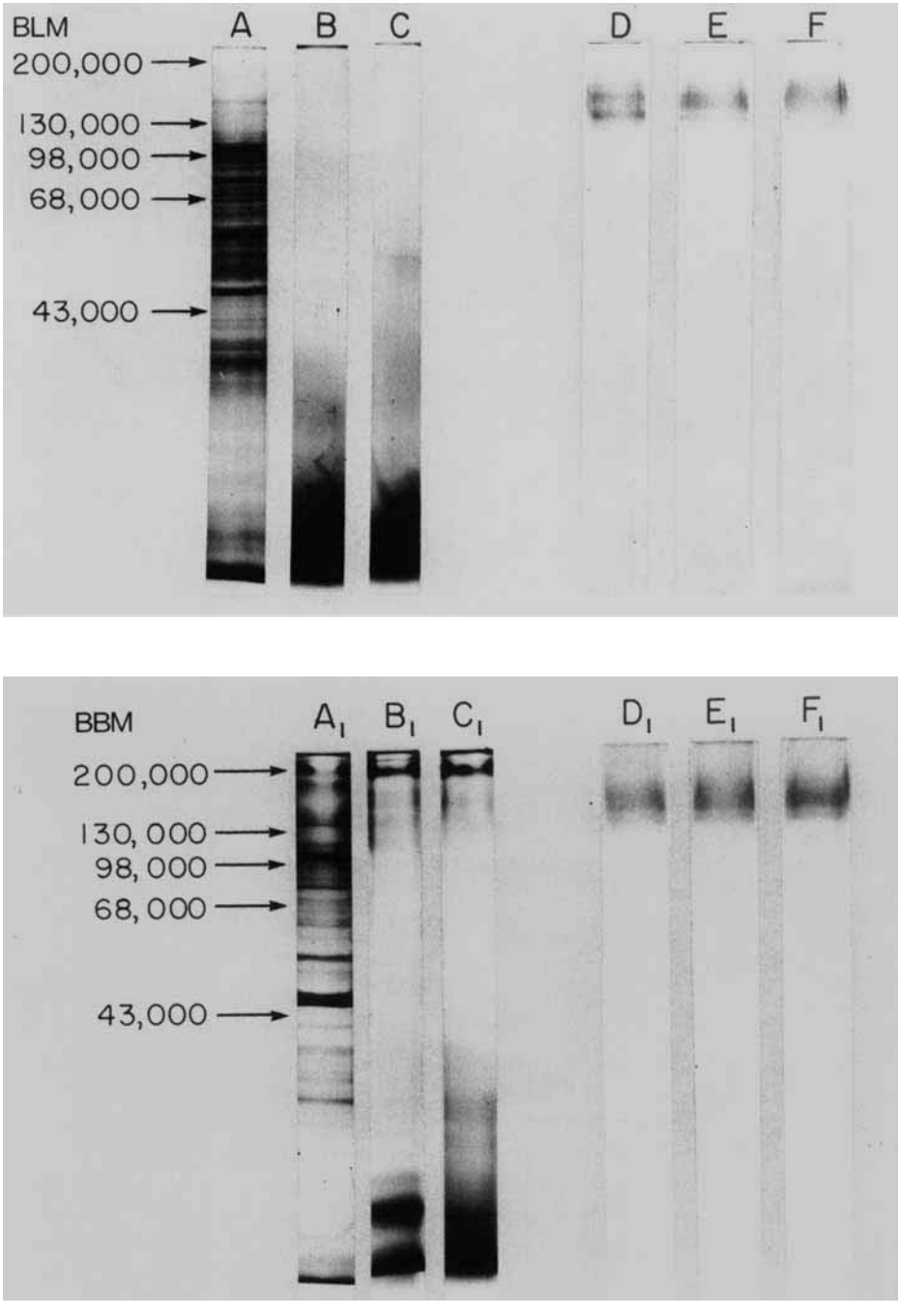

Fig. 9. SDS-polyacrylamide gel electrophoresis. Gels A–F are basal lateral membranes, and gels A_1–F_1 are brush border membranes. Gels A–C and A_1–C_1 were stained for protein, and gels D–F and D_1–F_1 were stained for alkaline phosphatase activity. A,A_1,D,D_1, untreated membranes; B,B_1,E,E_1, membranes digested for 60 min with papain; C,C_1,F,F_1, membranes digested for 60 min with trypsin. Further details are given in Materials and Methods. The molecular weights and arrows correspond to standard proteins. The concentration of the running gel was 10% acrylamide.

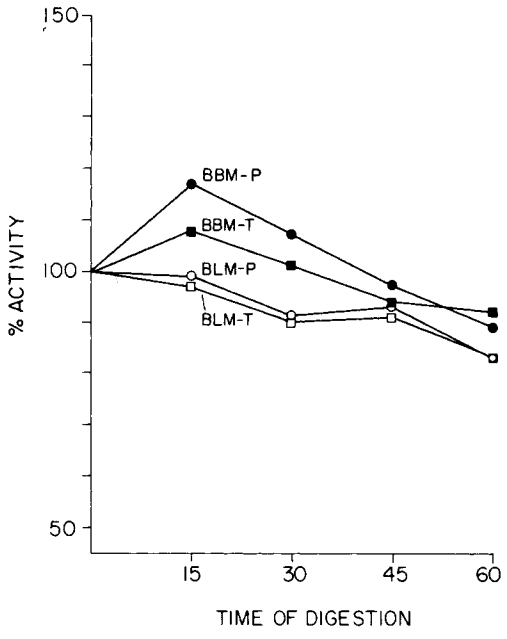

Fig. 10. Effect of proteolytic digestion on brush border and basal lateral alkaline phosphatase activities. Digestions were performed as described in Methods, for up to 60 min. BBM-P, Brush border membranes digested with papain; BBM-T, brush border membranes digested with trypsin; BLM-P, basal lateral membranes digested with papain; BLM-T, basal lateral membranes digested with trypsin.

and most of the staining occurs at low molecular weight in the region of the tracking dye. The activity stains in Figure 9D–F and $9D_1$–F_1 indicate that the alkaline phosphatases are not being inactivated by digestion, and this is shown quantitatively in Figure 10. The maximum inhibition found after 60 min digestion was to 83% of the original activity for basal lateral alkaline phosphatase. The brush border enzyme shows even less inhibition after 60 min digestion, and even shows a slight stimulation at shorter digestion times. The insensitivity of these alkaline phosphatases to proteolytic digestion is probably not due to protection by the membrane environment, as most membrane protein was severely digested by the same treatment. The enzyme may be somehow protected from digestion by its native configuration, as successful tryptic peptide maps have been performed on denatured placental alkaline phosphatase [36].

DISCUSSION

This study represents the first comparison of similar proteins present in both the brush border and basal lateral plasma membranes of intestinal cells. The major points that have been established are the following.

1. The alkaline phosphatase activity measured in basal lateral membranes is a true alkaline phosphatase, and not merely the expression of another phosphatase at alkaline pH. This conclusion is based on measurement of pH optimum, sensitivity to known inhibitors of intestinal alkaline phosphatase, and molecular weight. Recent results from

our laboratory indicate that the known phosphatases of basal lateral membranes, Na,K-ATPase, acid phosphatase, and alkaline phosphatase, differ from one another in terms of molecular weight and response to various inhibitors [37].

2. The brush border and basal lateral alkaline phosphatases are similar to each other. On a physical level, the two enzymes have similar intrinsic charges and molecular weights. On a catalytic level, the two enzymes have similar pH optima, substrate K_m's, responses to various inhibitors, cation sensitivities, and resistances to proteolytic digestion. This information is summarized in Table I. A difference does occur in the response of the two alkaline phosphatases to various detergents, and that will be dealt with below. A major difference also exists in the V_{max}'s of the two enzymes, with a value of 140 μmoles/mg-h for brush border alkaline phosphatase and 14 μmoles/mg-h for basal lateral alkaline phosphatase. This is consistent with the known distribution of alka-

TABLE I. Summary of Properties of Brush Border and Basal Lateral Alkaline Phosphatases

	Basal lateral	Brush border
pH optimum	9.6	9.8
K_m (PNPP)	71 μM	71 μM
V_{max} (PNPP)	14 μmoles/mg-hr	140 μmoles/mg-hr
50% Inhibition by:		
2-Mercaptoethanol	0.18% (v/v)	0.15% (v/v)
Theophylline	6 μM	4 μM
L-Phenylalanine	4 mM	3 mM
Activity after 5 min EDTA treatment	36%	24%
Reactivation of EDTA-treated alkaline phosphatase with $ZnCl_2$:		
Maximum reactivation	58%	76%
$ZnCl_2$ necessary for 50% reactivation	0.17 mM	0.16 mM
Activity in 1 mM:		
$CaCl_2$	144%	136%
$MgCl_2$	155%	136%
$ZnCl_2$	108%	96%
Activity in 100 mM:		
NaCl	144%	149%
KCl	161%	154%
Activity in 10 mM:		
SDS	137%	93%
Triton X-100	136%	106%
Cholate	125%	93%
Activity after 60 minutes digestion with:		
Trypsin	84%	93%
Papain	84%	90%
Molecular weight	124,000–150,000	124,000–150,000
R_f on 5% polyacrylamide Triton X-100 gels	0.092	0.092

line phosphatase activity in rat duodenum, most of the activity being present on the brush border membrane [4, 5]. The differences in V_{max}'s could be due to different amounts of enzyme present per milligram of membrane protein and/or differences in membrane environment. All other differences between the two enzymes appear to be minor, with the exception of the reactivation of the alkaline phosphatases with $ZnCl_2$. The maximum reactivation of the basal lateral enzyme is 58%, while that of the brush border enzyme is 76%. However, the $ZnCl_2$ concentrations necessary for 50% of maximum reactivation are similar for both enzymes (0.17 mM for basal lateral and 0.16 mM for brush border), indicating that the two enzymes are responding to the reactivation process in essentially the same manner.

3. Aside from the observed differences in V_{max}'s, one major difference between the two enzymes has been shown – the stimulation of basal lateral alkaline phosphatase and the lack of stimulation of brush border alkaline phosphatase by detergents. This discrepancy does not necessarily reflect a difference between the two enzymes, as the detergent effect may merely reflect differences in the respective membrane environments of the two enzymes. Two possible differences that could cause the observed effect are 1) a more restrictive membrane environment in the basal lateral membrane, which is made less restrictive upon detergent disruption, or 2) the location of some of the basal lateral alkaline phosphatase hydrolytic sites on the inside of the membrane vesicles, where they are not accessible to substrate. The latter possibility could indicate a major difference in the enzyme distribution in the two membranes, or could merely be due to the presence of a certain percentage of inside-out vesicles.

4. Both of the alkaline phosphatases are very insensitive to proteolytic digestion and detergent denaturation. Most membrane proteins are rapidly hydrolyzed by proteolytic enzymes, but the intestinal alkaline phosphatases show little inhibition of activity and no apparent change in molecular weight upon extensive hydrolysis of membrane protein. It must be concluded that the enzyme is very resistant to proteolytic cleavage. The lack of inhibition by detergents was indicated previously by Ey and Ferber [32], and it indicates that solubilizing levels of detergent have no inhibitory effect on rat intestinal alkaline phosphatase.

The similarity between the two alkaline phosphatases implies similar functions for the two enzymes; any differences in function would have to be imposed on the enzymes by their respective membrane environments. Unfortunately, the major functions of alkaline phosphatase are not currently known. Some evidence does exist of a correlation between intestinal alkaline phosphatase, calcium ATPase, and calcium absorption in vitamin-D-replete and rachitic animals [30, 31]. However, claims have also been made for the separation of alkaline phosphatase and calcium ATPase activities, implying that they are separate enzymes [38]. Final resolution of this question may not be complete until the calcium transport system is isolated, purified, and reconstituted. In any event, any explanation of calcium ATPase and calcium transport involving alkaline phosphatase in rat intestine must now account for the presence of very similar membranes. In a similar manner, any other hypothesis of intestinal alkaline phosphatase function must explain the presence of similar enzymes on both plasma membranes.

The mechanisms by which the epithelial cells maintains the functional polarity of the brush border and basal lateral membranes have yet to be elucidated. Essential to the solution of this question is the method of selective insertion of enzymes exclusively into one membrane surface. The present study has indicated that at least

one enzyme is incorporated into both membranes, implying that the epithelial cell also exerts a quantitative level of control over the selective insertion of enzymes into the brush border and basal lateral membrane surfaces.

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NOTE ADDED IN PROOF

Recent work from this laboratory shows that when purified calf alkaline phosphatase is reconstituted into phospholipid vesicles there is a specific increase in Ca^{++} permeability (Gunther, R.D., Mircheff, A.K. & Wright, E.M. J Cell Biol 83:287a 1979).