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Evidence for Calcium Mediated Conformational Changes in Calbindin-D_{28K} (the Vitamin D-Induced Calcium Binding Protein) Interactions With Chick Intestinal Brush Border Membrane Alkaline Phosphatase as Studied Via Photoaffinity Labeling Techniques

Valerie L. Leathers and Anthony W. Norman

Department of Biochemistry and Division of Biomedical Sciences, University of California, Riverside, California 92521

Abstract The role of the vitamin D-induced calcium binding protein termed calbindin-D (CaBP) in the biological response to 1,25-dihydroxyvitamin D_3 was assessed by photoaffinity labeling techniques. The heterobifunctional cross-linking reagent methyl-4-azidobenzoimidate was employed for studies with the 28 KD chick intestinal calbindin-D_{28K}. Calcium-dependent interactions were evident with purified chick intestinal CaBP-immunoglobulins and bovine intestinal alkaline phosphatase; in the absence of Ca^{2+} there was a greatly diminished crosslinking process. There were also at least two membrane components of chick intestinal brush border membranes, with M_R = 60,000 and 130,000, which were photoaffinity cross-linked with CaBP in a calcium-dependent manner. Similar interactions were demonstrated following incubations of CaBP with phosphatidylinositol-specific phospholipase C (PI-PLC)-treated supernatant fractions from chick intestinal brush borders. PI-PLC was shown to release 14% of the alkaline phosphatase from chick intestinal brush borders compared to greater than 80% for rabbit and chick kidney BBM preparations. Specific interactions between CaBP and brush border membrane proteins could also be demonstrated in the absence of photoaffinity labeling by Sephadex G-150 chromatography of Triton X-100 solubilized incubations between calbindin-D_{28K} and chick intestinal BBMS, with 17% of the radiolabelled CaBP comigrating with alkaline phosphatase activity. These studies collectively demonstrate that calbindin-D_{28K} undergoes calcium-dependent conformational changes which alter its subsequent interactions with cellular proteins in a way consistent with other calcium-binding proteins such as calmodulin or troponin C.

Key words: calbindin, $1,25(OH)_2D_3$, intestinal Ca^{2+} transport, alkaline phosphatase, brush border

Calbindin-D_{28K} (CaBP) (Calbindin-D_{28K} corresponds to the M_R = 28,000 vitamin D-dependent calcium binding protein.) is the protein principally induced by 1,25-dihydroxyvitamin D_3 in the chick intestine [Leathers et al., 1990, Hinghetti et al., 1988]; CaBP can constitute up to 2–3% of the total soluble protein [Wasserman and Taylor, 1968] with intestinal CaBP levels closely correlated to vitamin D stimulated intestinal calcium absorption [Wasserman and Taylor, 1968; Theofan et al., 1986] as well as occupancy of the intestinal nuclear receptor for $1,25(OH)_2D_3$ [Theofan et al., 1986]. As yet a precise physiological function of CaBP in vitamin D mediated cellular actions remains to be demonstrated, although we have shown that the subcellular distribution of calbindin-D in the intestinal epithelial cell is altered in response to the onset of calcium transport [Nemere et al., 1991]. Nuclear magnetic resonance [Dalgarno et al., 1983], fluorescence, circular dichroism [O'Neil et al., 1982], and X-ray crystallographic studies [Szebenyi et al., 1981] of the smaller mammalian calbindin-D_{10K} (mw = 10,000) have

The abbreviations used are CaBP, calbindin-D; MABI, methyl-4-azidobenzoimidate; PI-PLC, phosphatidylinositol specific phospholipase C; BBM, brush border membrane; ATPase, alkaline phosphatase.

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Address reprint requests to Professor Anthony W. Norman, Department of Biochemistry, University of California, Riverside, CA 92521.

suggested that significant conformational changes occur as a consequence of the protein binding calcium. This effect is reminiscent of that seen with the class of calcium-binding proteins of which calbindin-D is a member, which includes calmodulin, troponin C, parvalbumin, and brain S-100 [Norman et al., 1987]. In the case of calmodulin, the protein undergoes tertiary and secondary conformational changes exposing hydrophobic surfaces which allows the modulation of enzymes such as phosphodiesterase and adenylate cyclase [Means and Dedman, 1980].

The proposal [Norman and Leathers, 1982; Leathers and Norman, 1985] that calbindin-D may also interact with cellular proteins following calcium induced conformational changes was explored by photoaffinity labeling experiments performed on the 28 KD chick intestinal CaBP. Following a preliminary study by Freund and Borzemsky [1977] of CaBP activation of rat intestinal membrane-bound alkaline phosphatase, we reported evidence for a calcium-dependent interaction between the CaBP photoaffinity label and bovine intestinal alkaline phosphatase [Norman and Leathers, 1982]. Brush border membrane bound alkaline phosphatase [Leathers and Norman, 1985; Freund and Borzemsky, 1977; Norman et al., 1970; Putkey et al., 1982; Goodman et al., 1972] as well as Ca^{2+}-stimulated ATPase [Melancon and DeLuca, 1970] have previously been found to be vitamin D dependent. In the present study we have further characterized the calcium dependency of the chick intestinal CaBP photoaffinity labeling in addition to an identification of homologous interactions between CaBP and chick intestinal brush border membrane proteins.

MATERIALS AND METHODS

Radioisotopes were purchased from ICN (Irvine, CA), and methyl-4-azido benzoimidate was purchased from Pierce Chemical. Bovine intestinal alkaline phosphatase was obtained from Sigma Chemical Co. (St. Louis, MO). Polyacrylamide gel electrophoresis supplies were from Biorad.

Preparation of ^{125}I-CaBP-MABI

The purification of chick intestinal CaBP was accomplished by the method of Friedlander and Norman [1980]. Iodination of the protein was achieved by the lactoperoxidase-glucose oxidase procedure [LaPorte and Storm, 1978] in which lactoperoxidase, glucose oxidase, and glucose at 0.2 units/ml, 0.25 μg/ml, and 5 mM final reagent concentrations, respectively, were added to 50–100 μg of purified protein and 1.0 μCi of carrier-free Na ^{125}I in a buffer consisting of 125 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM Hepes (pH 7.5). The reaction was allowed to proceed for 30 min at 21°C and was terminated by the addition of 10 μg/ml Na metabisulfite (final reagent concentration 2.5 μg/ml). The sample was immediately applied to a 0.5 × 10 cm Sephadex G-100 column equilibrated with 50 mM sodium borate and 100 mM NaCl (pH 9.4). Fractions containing the ^{125}I-CaBP were collected. A final concentration of approximately 1.0 nM CaBP with a specific radioactivity of 60 μCi/nmole was obtained. The ^{125}I-CaBP (10 μg) was then incubated with methyl-4-azidobenzoimidate (MABI) at a concentration of 0.6 μg/ml and nonradioactive CaBP (90 μg) in 50 mM sodium borate and 100 mM NaCl pH 9.4, for 2 h at 4°C in the dark. This allows the imidoester functionality of the photoaffinity reagent to covalently bind to primary amines on the CaBP. The sample was chromatographed on a 0.5 × 10 cm Sephadex G-25 column and eluted with 20 mM Hepes (pH 7.2), thereby separating the ^{125}I-CaBP-MABI from unreacted MABI. The number of MABI molecules conjugated to one molecule of CaBP was calculated to be approximately 3.0 following spectrophotometric measurement of the incorporation of azido groups [Ji, 1977].

Photoaffinity Labeling and SDS-PAGE

Incubations of ^{125}I-CaBP-MABI (50–1,000 nM) and purified anti-CaBP immunoglobulins (see below) were 4 h at 4°C, while bovine intestinal alkaline phosphatase (1,140 units/mg) and membranes were incubated with the photoaffinity label for 30 min to an hour at 21°C. A 2 min irradiation with a shortwave UVG-11 mineralight at a 1 cm distance was followed by sample solubilization as described by Laemmli [1970]. The samples were heated at 100°C for 2 min and applied to SDS slab gels (1.5 mm spacers) composed of a 7–20% polyacrylamide gradient. The methods of O'Farrell [1975] and Laemmli [1970] for preparation of gel buffers and running buffers were followed. The gels were run at constant power with an initial current of 40 mA per gel, followed by staining overnight by the procedure of Fairbanks [Fairbanks et al., 1971] and destaining in 10% acetic acid. The dried gels were

autoradiographed at −70°C on Kodak XR-5 X-ray film. A Schoeffel densitometer was used to densitometrically scan the autoradiographs.

Purified Anti-CaBP-Immunoglobulins and ELISA

Rabbit antisera against purified chick intestinal CaBP was applied to a 1 × 5 cm Protein A column and washed with 0.90 M NaCl, 5 mM KCl, and 50 mM Na_2HPO_4. Following elution with 0.5 M acetic acid and 0.15 M NaCl, the immunoglubulin fractions were immediately neutralized with 10 M ammonium hydroxide. The peak fractions were dialyzed in 1 liter of 0.90 M NaCl, 5 mM KCl, and 50 mM Na_2HPO_4 to yield purified anti-CaBP immunoglobulins.

The method of Miller and Norman [1983] was used for the enzyme-linked immunosorbent assay (ELISA).

Isolation of Membrane Fractions

Chick intestinal brush border membranes and brush borders were prepared by the method of Putkey et al. [1982]. Rabbit kidney brush border membrane sheets were prepared by a modification of Thuneberg and Rostgaard [1968] as described by Yusufi et al. [1983]. The same method was utilized for chick kidney brush border membranes.

Digestion of BBM With Phosphatidylinositol-Specific Phospholipase C

Phosphatidylinositol-specific phospholipase C (PI-PLC) prepared from Staphylococcus aureus culture medium [Low and Zilversmit, 1980] was generously provided by Dr. Martin G. Low. Digestion of the membranes with the PI-PLC was effected by a modified procedure of Yusufi et al. [1983]. Brush border preparations (50–200 μg) were preincubated with PI-PLC (0.15–10 μg/tube) in 60 mM sucrose and 50 mM Hepes (pH 7.4) for 60 min at 4°C to ensure equilibration of the enzyme with the membrane vesicles. The samples were incubated for 30 min at 37°C, followed by a rapid cooling on ice and centrifugation at 100,000*g* for 60 min at 4°C. Control membranes, which were not digested with PI-PLC, were treated in the same manner. Alkaline phosphatase activity was measured as the rate of hydrolysis of p-nitrophenylphosphate by the procedure of Nemere et al. [1983].

RESULTS

The ^{125}I-CaBP-MABI interaction with bovine intestinal alkaline phosphatase (subunit mw = 65,000), which results in a complex at 95,000 daltons, has previously been described [Norman and Leathers, 1982; Leathers and Norman, 1985] in terms of its calcium dependency and specificity as evidenced by the lack of interaction between *E. coli* alkaline phosphatase and ^{125}I-CaBP-MABI. Photoaffinity labeling experiments with chick intestinal ^{125}I-CaBP-MABI and bovine intestinal alkaline phosphatase shown in Figure 1A further demonstrate specific photoaffinity labeling as seen by the lack of interaction when excess nonradioactive CaBP is added to the sample. Additionally in the absence of ultraviolet irradiation there is no photoaffinity complex present. Panel B illustrates the effect of excess nonradioactive CaBP on ^{125}I-CaBP-MABI incubations with the heavy and light chains of purified anti-CaBP immunoglobulins. Complexes of 82,000 and 56,000 daltons are effectively diminished by the addition of excess CaBP.

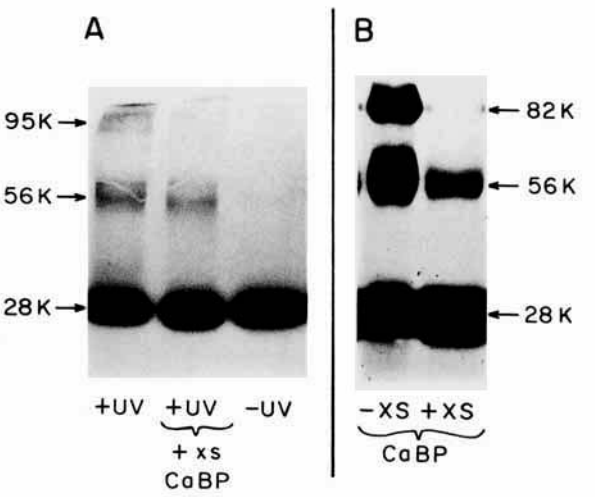

Fig. 1. Autoradiographs of SDS-PAGE gels of ^{125}I-CaBP-MABI ± excess nonradioactive CaBP which had been incubated with bovine intestinal alkaline phosphatase or purified anti-CaBP immunoglobulins. **A:** ^{125}I-CaBP-MABI (80 nM) and commercially obtained bovine intestinal alkaline phosphatase (2.0 μM) were incubated 45 min at 21°C. Lane 1 and 2 received UV irradiation, while lane 3 did not. Lane 2 is with the addition of excess unlabeled CaBP (6.0 μM). **B:** ^{125}I-CaBP-MABI (100 nM) and anti-CaBP immunoglobulin (0.22 μg) were incubated 4 h at 4°C. Both samples received irradiation. Lane 2 is in the presence of 4.2 μM CaBP. Samples were evaluated by SDS-PAGE as described in Materials and Methods.

The photoaffinity labeling of purified anti-CaBP immunoglobulins might be anticipated to exhibit calcium-dependent interactions considering the calcium-dependent conformations of other calcium-binding proteins such as calmodulin, parvalbumin, and troponin C. As seen in Figure 2, the ^{125}I-CaBP-MABI conjugates with

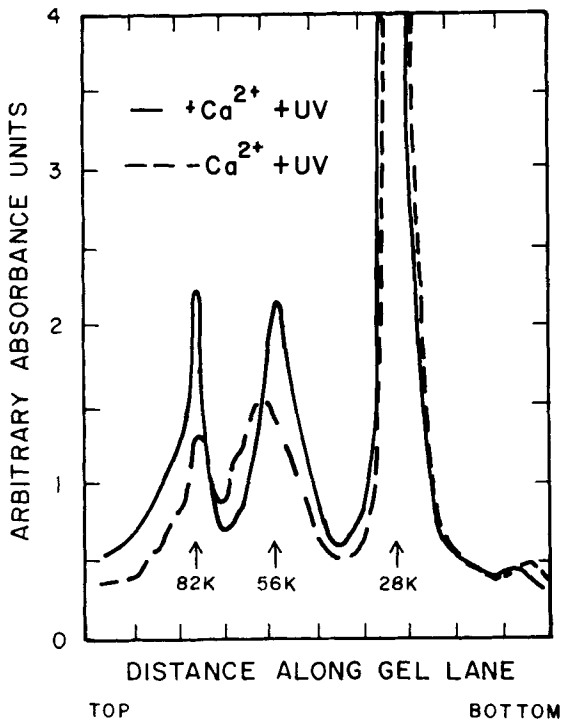

Fig. 2. Densitometric trace demonstrating calcium dependency of ^{125}I-CaBP-MABI conjugates with purified anti-CaBP immunoglobulins. ^{125}I-CaBP-MABI (300 nM) and anti-CaBP immunoglobulins (0.2 μg) were incubated 4 h at 4°C prior to irradiation for 2 min. Incubations were in the presence of 1 mM $CaCl_2$ (—) or 1 mM EGTA (---). Complexes were assessed via SDS-PAGE and autoradiography.

the light and heavy chains of immunoglobulins are shown to be calcium dependent; however, due to the polyclonal nature of the antisera photoaffinity labeling continues to some degree in the absence of calcium.

Another approach for evaluating calcium-dependent chick intestinal CaBP interactions with anti-CaBP immunoglobulins is by the ELISA (Fig. 3). In this assay, calcium-deplete CaBP interacts with immunoglobulins to a lesser extent than calcium replete CaBP resulting in decreased p-nitrophenol production.

Our previous experiments [Norman and Leathers, 1982] had indicated a specific photoaffinity labeling by ^{125}I-CaBP-MABI of bovine intestinal alkaline phosphatase. Since it is known that intestinal alkaline phosphatase is localized in the brush border membrane [Norman et al., 1970], we chose to pursue putative interactions between ^{125}I-CaBP-MABI and chick intestinal brush border membrane (BBM) proteins. Incubations of ^{125}I-CaBP-MABI with +D BBM proteins in the presence (1 mM $CaCl_2$) and absence (no added calcium) of calcium followed by SDS-PAGE and autoradiography resulted in complexes visible at 180,000 and 90,000 daltons which are clearly calcium dependent (Fig. 4). The molecular weight of chick intestinal alkaline phosphatase, as determined by alkaline phosphatase assays on 1 mm SDS-PAGE gel slices, was found to be 130,000–150,000 daltons.

Attempts to purify chick intestinal BBM bound alkaline phosphatase were uniformly unsuccessful. Greater than 80% of the alkaline phosphatase enzyme remained membrane bound following butanol extraction unlike most mammalian BBM-bound alkaline phosphatases. In an effort to partially purify alkaline phosphatase from chick intestinal BBM for further photoaffinity labeling experiments, a bacterial phosphatidylinositol-specific phospholipase C was utilized for specific release of the ATPase. Treatment of alkaline phosphatase from purified chick intestinal BBM and brush borders with the phospholipase C resulted in only a 22% and 14.3% release of enzyme respectively, as compared to greater than 80% for chick kidney BBM and rabbit kidney BBMs (Fig. 5). The proteins released from chick intestinal brush borders following PI-PLC treatment were collected in the 100,000g supernatant and subsequently incubated with ^{125}I-CaBP-MABI resulting in complexes at 160,000 and 90,000 daltons. Both conjugates are dependent on the previous digestion of the membranes with the PI-PLC (Fig. 6). As seen in Figure 5 the control untreated membranes for chick intestinal brush borders, as well as BBM, released a low level of alkaline phosphatase into the supernatant, possibly explaining the apparent interactions occurring between ^{125}I-CaBP-MABI and the untreated membranes.

In addition to the experiments described utilizing SDS-PAGE followed by autoradiography and densitometric scanning to identify interactions between ^{125}I-CaBP-MABI and chick intestinal BBM proteins, we have also evaluated these interactions chromatographically on Sephadex G-150 columns under nondenaturing conditions. Figure 7 demonstrates Triton X-100 solubilization of chick intestinal BBM previously incubated with ^{125}I-CaBP, resulting in 17% of the radioactive ^{125}I-CaBP comigrating in the region of alkaline phosphatase activity. The addition of excess nonradioactive CaBP resulted in decreased elution of ^{125}I-CaBP with the alkaline phosphatase activity.

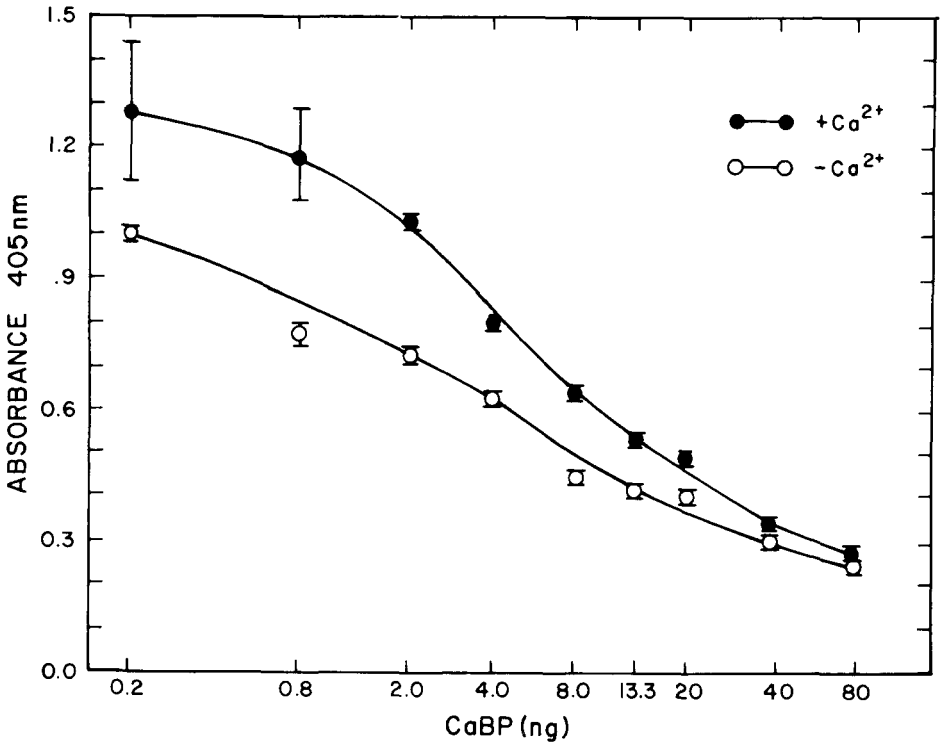

Fig. 3. Calcium dependency of ELISA for the calbindin-D_{28K}. Standard curves were obtained in a 20 mM Hepes, 0.15 M NaCl tween 20 buffer with either 5 mM $CaCl_2$ (●—●) or 5 mM EDTA (○—○). Values are mean ± SD of triplicate determinations.

DISCUSSION

Photoaffinity labeling studies have been used in a wide variety of systems predominantly for evaluating hormone-receptor interactions [Yip et al., 1978] such as the insulin receptor [Andreasen et al., 1981] and its ligand. Calmodulin modulation of calcium-sensitive phosphodiesterase has also been assessed via photoaffinity techniques [Thorens et al., 1982].

Photoaffinity studies with chick intestinal CaBP have had a two-fold purpose. The first has been to identify potential CaBP-protein interactions which may contribute to the vitamin D_3 mediated response. In this respect ^{125}I-CaBP-MABI has been found to interact specifically with bovine intestinal alkaline phosphatase. In addition there is photoaffinity labeling of chick intestinal BBM proteins of mw 150,000 and 60,000.

The second purpose of these studies has been to illustrate the calcium dependence of CaBP interactions such as those seen with anti-CaBP immunoglobulins, bovine intestinal alkaline phosphatase, and chick intestinal BBM proteins. Although considerable data has been presented for the smaller calcium-binding proteins such as calmodulin and calbindin-D_{10K} outlining the conformational effects of calcium, there is little data on the larger calbindin-D_{28K}. The results presented here clearly demonstrate the calcium dependency of calbindin-D_{28K} interactions confirming that the large 28 KD CaBP does have a calcium-dependent conformation.

The PI-PLC release of membrane-bound alkaline phosphatase was previously demonstrated by Yusufi et al. [1983] in rabbit kidney brush border membrane sheets. In this system digestion of the membranes released greater than 95% of the alkaline phosphatase in a specific manner as evidenced by the lack of release of marker enzymes such as maltase and leucine aminopeptidase. The original goal in treating chick intestinal BB with PI-PLC was to partially purify the enzyme from other membrane proteins subsequent to photoaffinity labeling studies. While alkaline phosphatase is readily released from rabbit and chick kidney membrane preparations, less than 25% of the total enzyme activity was released from the chick intestinal membranes after treatment with PI-PLC. This resistance to PI-PLC digestion, as well as to butanol extraction, suggests a unique attach-

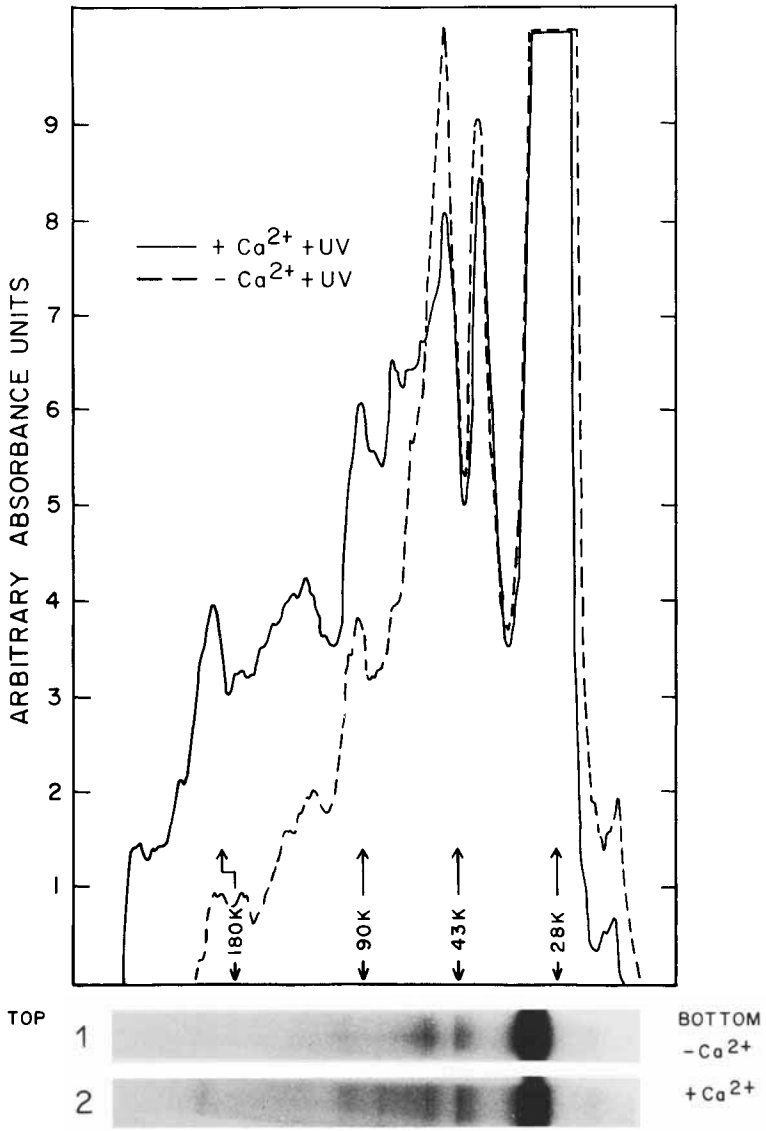

Fig. 4. Calcium-dependent interactions between ^{125}I-CaBP-MABI and chick intestinal brush border membrane proteins. ^{125}I-CaBP-MABI was mixed with 1 mM EGTA followed by chromatography on a 0.5 × 10 cm Sephadex G-25 column. The calcium-deplete ^{125}I-CaBP-MABI (300 nM) was then incubated with chick intestinal brush border membranes (80 μg) for 30 min in a 20 mM Hepes, 1 mM $ZnCl_2$, and 2 mM MgCl (pH 7.2) buffer. The samples were irradiated for 2 min and centrifuged at 2,000*g* for 5 min. The final pellets were subjected to SDS-PAGE as described in Materials and Methods. The autoradiogram lanes are presented in the bottom panel with the corresponding densitometric scans in the top panel. Samples which were incubated in the presence of added calcium (1 mM $CaCl_2$) are in autoradiogram lane 2 and the solid line densitometric tracing. Samples which received no additional calcium appear in autoradiogram lane 1 and the dashed line densitometric tracing.

ment of the chick intestinal brush border membrane-bound alkaline phosphatase unlike that seen in mammalian tissue or chick kidney.

The PI-PLC-treated chick intestinal BB, while yielding only a small fraction of released ATPase, was sufficient for photoaffinity labeling experiments in which complexes of 90,000 and 160,000 mw were detected. This cross-linking occurred despite the fact that there were undetectable levels of protein as assessed by Bradford protein assay and Coomassie blue staining of the slab gels.

The recurring photoaffinity labeling of a brush border protein at 60,000 daltons to yield a complex of 88,000 mw is similar to a report by Shimura and Wasserman [1984] in which they utilized a ^{125}I-CaBP gel overlay technique with chick intestinal BBMs [Thorens et al., 1982]. A

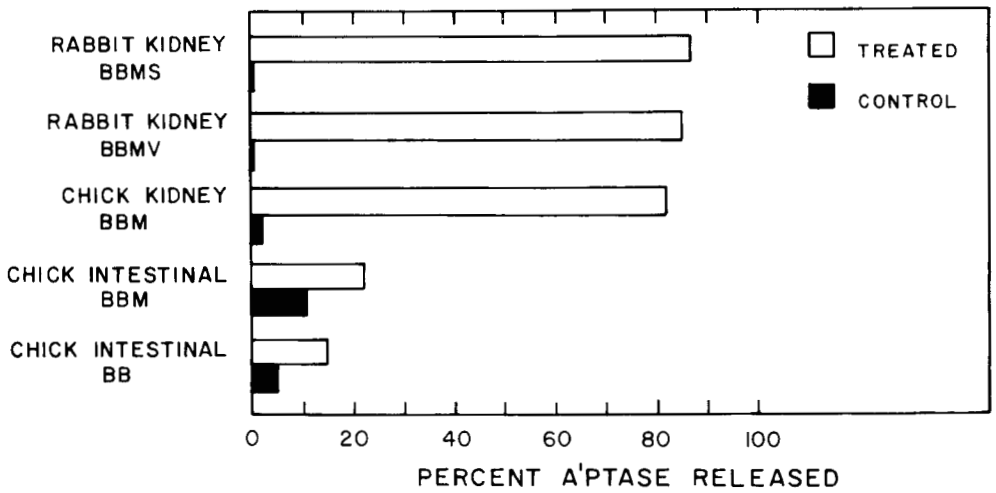

Fig. 5. PI-PLC-mediated release of membrane-bound alkaline phosphatase from rabbit kidney, chick kidney, or chick intestine The indicated brush border membranes (50 μg) were pre-incubated with 4 μg PI-PLC in 60 mM sucrose, 50 mM Hepes pH 7 4 for 60 min at 4°C, with the exception of the chick intestinal membrane preparations which were treated with 10 μg PI-PLC Samples were then incubated at 37°C for 30 min, rapidly cooled on ice, and centrifuged at 100,000*g* for 60 min at 4°C Alkaline phosphatase and protein assays were performed on the pellets and supernatants The percent alkaline phosphatase released is the percent enzyme activity in μmoles product/h released from the treated pellet as compared to the control pellet

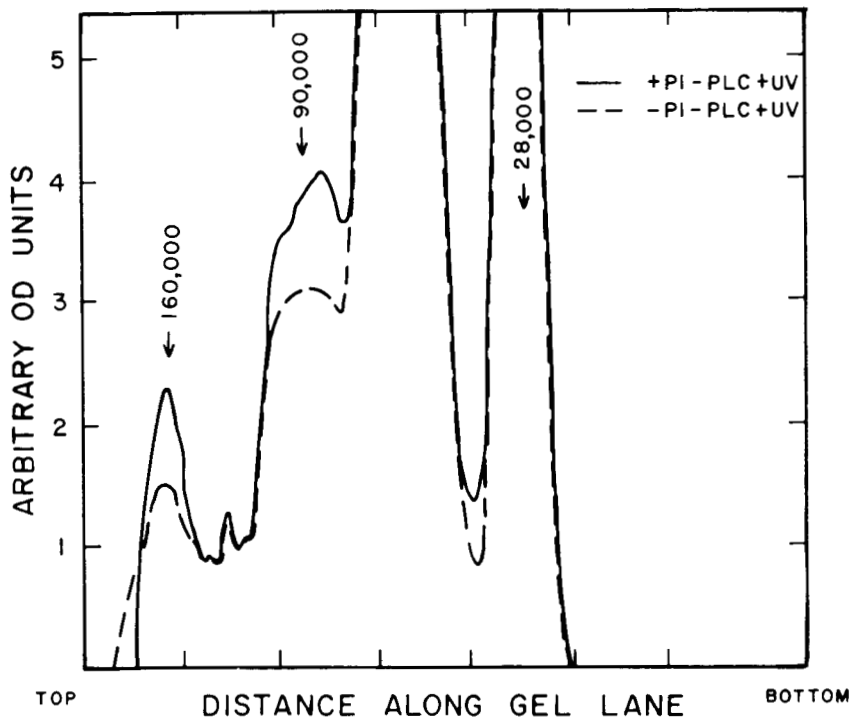

Fig. 6. Densitometric tracing demonstrating PI-PLC-dependent interactions between chick intestinal brush border supernatant components and ^{125}I-CaBP-MABI Chick intestinal brush borders (50 μg) were treated with 10 μg PI-PLC as described in Materials and Methods The 100,000*g* supernatant with released alkaline phosphatase was lyophilized and resuspended in 25 μl The samples, which had less than 2 μg of protein as determined by the Bradford assay, were incubated 60 min at 4°C with ^{125}I-CaBP-MABI (500 nM), irradiated 2 min, and assessed by SDS-PAGE Control (—), treated (---)

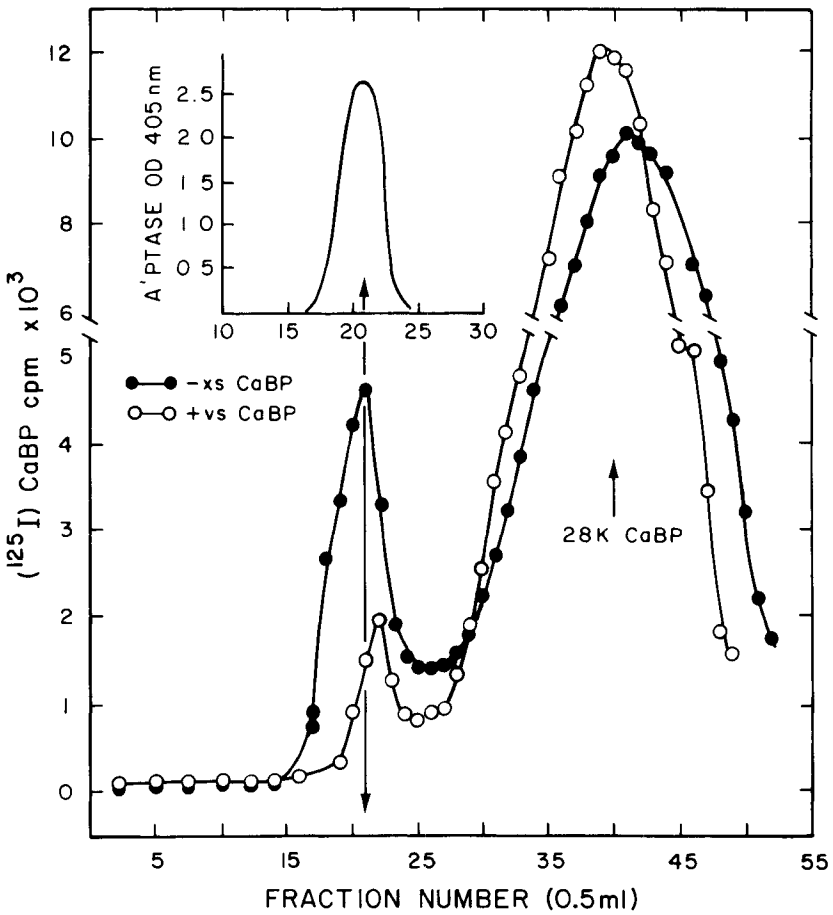

Fig. 7. Interaction of ^{125}I-CaBP with chick intestinal brush border membranes as assessed by Sephadex G-150 filtration. Purified chick intestinal brush border membranes (100 μg) were incubated with ^{125}I-CaBP (500 nM) for 45 min at 21°C in a 20 mM Hepes, 1 mM $CaCl_2$, pH 7.2, buffer. The sample was solubilized in 10% Triton X-100 and chromatographed on a 40 cm Sephadex G-150 column. Fractions (0.5 ml) were collected and alkaline phosphatase activity and ^{125}I-CaBP content determined. The open circles are representative of the sample which had 100-fold excess of nonradioactive CaBP added. The closed circles represent the sample which had no additional CaBP present.

60,000 mw protein was found to be labeled by the ^{125}I-CaBP.

Coelution of ^{125}I-CaBP with alkaline phosphatase activity following Sephadex G-150 gel filtration is further evidence in support of an interaction occurring between chick intestinal CaBP and BBM bound alkaline phosphatase. However, as yet the identity of the 150,000 and 60,000 mw proteins which CaBP associates with remains unknown. The fact that both interactions occur in BB supernatants subsequent to PI-PLC treatment may indicate that the two proteins are closely associated in the membranes.

Calbindin-D is generally considered to be a cytosolic protein [Friedlander and Norman, 1980; Friedlander et al., 1977]; however, recently Shimura and Wasserman [1984] demonstrated that up to 10% of the protein can be found in the BBM. This population of calbindin-D is not normally detected via radioimmunoassay unless the membranes are treated with Triton X-100. Therefore, brush border membrane bound CaBP may in fact be available to interact with brush border proteins. This laboratory has also found a significant amount of calbindin-D associated with lysosomal residues [Nemere et al., 1986]. In addition there is apparent, at the light microscope level, a redistribution of calbindin-D in the intestinal epithelial cell coincident with the onset of calcium transport [Nemere et al., 1991], which may well be dependent upon the calbindin-D membrane protein interactions described in this report.

Collectively these results demonstrate the calcium-dependent nature of calbindin-D interac-

tions with cellular proteins. In particular, brush border membrane proteins of M_R = 60,000 and 130,000 as well as bovine intestinal alkaline phosphatase. This apparent affinity of CaBP for specific brush border membrane proteins was consistently seen in membranes analyzed by SDS-PAGE and Sephadex G-150 chromatography as well as PI-PLC treated membranes. The significance of an interaction between CaBP and alkaline phosphatase remains to be elucidated; however one could propose that CaBP may modulate the activity of the enzyme in a calcium dependent manner. Studies are currently underway to address this issue.

ACKNOWLEDGMENTS

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REFERENCES

Andreasen TJ, Keller CH, LaPorte DC, Edelman AM, Storm DR (1981) Preparation of Azidocalmodulin A photoaffinity label for calmodulin-binding protein Proc Natl Acad Sci USA 78 2782–2785

Dalgarno DC, Levine BA, Williams RJP, Fullmer CS, Wasserman RH (1983) Proton-NMR studies of the solution conformations of vitamin-D induced bovine intestinal calcium-binding protein Eur J Biochem 137 523–529

Fairbanks G, Steck TL, Wallach DFH (1971) Electrophoretic analysis of major polypeptides of human-erythrocyte-membrane Biochemistry 10 2606–2617

Friedlander EJ, Norman AW (1980) Purification of chick intestinal calcium binding protein Methods in Enzymology Vitamins and co-enzymes 67 504–508

Friedlander EJ, Henry H, Norman AW (1977) Studies on the mode of action of calciferol XII Effects of dietary calcium and phosphorus on the relationship between the 25-hydroxyvitamin D_3-1α-hydroxylase and production of chick intestinal calcium binding protein J Biol Chem 252 8677–8683

Freund TS, Borzemsky G (1977) In Wasserman RH, et al (eds) "International Symposium on Calcium-Binding Proteins and Calcium Function in Health and Disease " New York North Holland, pp 353–356

Goodman DBP, Haussler MR, Rasmussen H (1972) Vitamin D_3 induced alteration of microvillar membrane lipid composition Biochim Biophys Res Commun 46 80–86

Ji TH (1977) Novel approach to identification of surface receptors Use of photosensitive heterobifunctional cross-linking reagent J Biol Chem 252 1566–1570

Laemmli UK (1970) Cleavage of structural proteins during assembly of head of bacteriophage-T_4 Nature 227 680–685

LaPorte DC, Storm DR (1978) Detection of calcium-dependent regulatory protein-binding components using 1,25-labeled calcium-dependent regulatory protein J Biol Chem 253 3374–3377

Leathers VL, Norman AW (1985) Vitamin D-dependent chick intestinal CaBP Calcium dependent interactions as assessed by photoaffinity labeling In Norman AW, Schaefer K, Grigoleit HG, Herrath Dv (eds) "Vitamin D Chemical, Biochemical and Clinical Update " Walter de Gruyter, Berlin, pp 343–344

Leathers VL, Linse S, Forsen S, Norman AW (1990) Calbindin-D_{28K}, a 1α,25-dihydroxyvitamin D_3-induced calcium-binding protein, binds five or six Ca^{2+} ions with high affinity J Biol Chem 265 9838–9841

Means AR, Dedman JR (1980) Calmodulin An intracellular calcium receptor Nature 285 73–77

Melancon MJ, DeLuca HF (1970) Vitamin D stimulation of calcium-dependent adenosine triphosphatase in chick intestinal brush border Biochem 9 1658–1664

Miller BE, Norman AW (1983) Enzyme-linked immunoabsorbent assay (ELISA) and radioimmunoassay (RIA) for the vitamin D-dependent 28,000 dalton calcium-binding protein In Means AR, O'Malley BW (eds) "Methods in Enzymology Hormone Action " Academic Press, New York, Part G, vol 102, pp 291–296

Minghetti PP, Cancela L, Fujisawa Y, Theofan G, Norman AW (1988) Molecular structure of the chicken vitamin D-induced calbindin-D_{28K} gene reveals eleven exons, six Ca^{2+}-binding domains, and numerous promoter regulatory elements Mol Endocrinol 2 355–367

Nemere I, Putkey JA, Norman AW (1983) Studies on the mode of action of calciferol XLIII Vitamin D-mediated alterations in the topography of intestinal brush border proteins effect of papain on hydrolase release and calcium uptake Arch Biochem Biophys 222 610–620

Nemere I, Leathers V, Norman AW (1986) 1,25-Dihydroxyvitamin D_3-mediated intestinal calcium transport Biochemical identification of lysosomes containing calcium and calcium binding protein (calbindin-D_{28K}) J Biol Chem 261 16106–16114

Nemere I, Norman AW (1990) Transcaltachia, vesicular calcium transport, and microtubule-associated calbindin-D_{28K} Emerging views of 1,25-dihydroxyvitamin D_3-mediated intestinal calcium absorption Min Elec Metab 16 109–114

Norman AW, Mircheff AK, Adams TH, Spielvogel AM (1970) Studies on the mechanism of action of calciferol III Vitamin D mediated increase of intestinal brush border alkaline phosphatase activity Biochim Biophys Acta 215 348–359

Norman AW, Leathers V (1982) Preparation of a photoaffinity probe for the vitamin D-dependent intestinal calcium binding protein Evidence for a calcium dependent, specific interaction with intestinal alkaline phosphatase Biochim Biophys Res Commun 108 220–226

Norman AW, Vanaman TC, Means AR (eds) (1987) Calcium-Binding Proteins in Health and Disease, Proceedings of the Fifth International Symposium on Calcium-Binding Proteins in Health and Disease, Academic Press, San Diego

O'Farrell PH (1975) High resolution 2-dimensional electrophoresis of proteins J Biol Chem 250 4007–4021

O'Neil JDJ, Dorrington KJ, Kells DIC, Hofmann T (1982) Fluorescence and circular-dichroism properties of pig intestinal calcium-binding protein (M_r = 9000), a protein with a single tyrosine residue Biochem J 207 389–396

Putkey JA, Spielvogel AM, Sauerheber RD, Dunlap CS, Norman AW (1982a) Studies on the mode of action of calciferol XXXIX Vitamin D-mediated intestinal calcium transport Effect of essential fatty acid deficiency and spin label studies of enterocyte membrane lipid fluidity Biochim Biophys Acta 688 177–190

Putkey JA, Spielvogel AM, Sauerheber RD, Dunlap CS, Norman AW (1982b): Studies on the mode of action of calciferol XXXIX. Vitamin D-mediated intestinal calcium transport: effect of essential fatty acid deficiency and spin label studies of enterocyte membrane lipid fluidity. Biochim Biophys Acta 688:177–190.

Shimura F, Wasserman RH (1984): Membrane-associated vitamin D-induced calcium-binding protein (CaBP): Quantification by a radioimmunoassay and evidence for a specific CaBP in purified intestinal brush borders. Endocrinology 115:1964–1972.

Szebenyi DME, Obendorf SK, Moffat K (1981): Structure of vitamin D-dependent calcium-binding protein from bovine intestine. Nature 294:327–332.

Theofan G, Nguyen AP, Norman AW (1986): Regulation of calbindin-D_{28K} gene expression by 1,25-dihydroxyvitamin D_3 is correlated to receptor occupancy. J Biol Chem 261: 16943–16947.

Thorens B, Roth J, Norman AW, Perrelet A, Orci L (1982): Immunocytochemical localization of the vitamin D-dependent calcium-binding protein in chick duodenum. J Biol Chem 94:115–122.

Thuneberg L, Rostgaard J (1968): Isolation of brush border fragments from homogenates of rat and rabbit kidney cortex. Exp. Cell. Res. 51:123–140.

Wasserman RH, Taylor AN (1968): Vitamin D-dependent calcium-binding protein: Response to some physiological and nutritional variables. J Biol Chem 243:3987–3993.

Yip CC, Yeung CWT, Moule ML (1978): Photoaffinity labeling of insulin receptor of rat-adipocyte plasma-membrane. J Biol Chem 253:1743–1745.

Yusufi ANK, Low MG, Turner ST, Dousa TP (1983): Selective removal of alkaline-phosphatase from renal brush-border membrane and sodium-dependent brush-border membrane transport. J Biol Chem 258:5695–5701.