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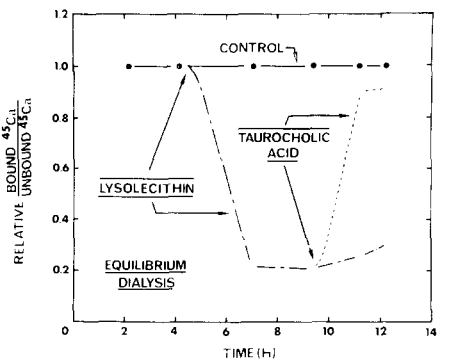
Interaction of vitamin D-dependent calcium binding protein with lysolecithin: Possible relevance to calcium transport

The vitamin D-induced calcium binding protein, which occurs in the intestine[1,2], kidney[3] and shell gland of the laying hen[4], has been localized in the brush border and goblet cells of the intestinal epithelium by fluorescein-labeled antibody techniques[5]. Considerable correlative data suggest that calcium binding protein is intimately involved in calcium translocation[6,7] but its exact role in this process is unknown. This also applies to other binding proteins isolated from bacteria, as reviewed recently by PARDEE[8]. Reported herein are observations showing that calcium binding protein can form a complex with lysolecithin and, as a consequence of this interaction, the binding affinity of the protein for calcium is considerably reduced. This type of interaction might possibly bear on the mechanism by which this and other binding proteins enhance substrate translocation.

Lysolecithin (egg), from Applied Science Laboratories, University Park, Pa.,



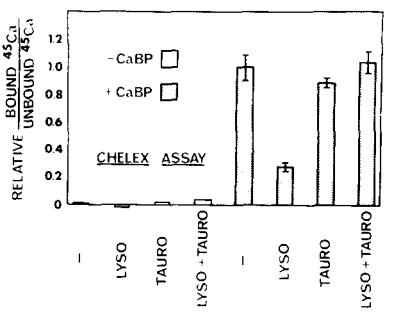


Fig. 1. Effect of lysolecithin on calcium binding by calcium binding protein, and the reversal of this effect by taurocholate, as shown by equilibrium dialysis. The results are expressed as the ratio of protein-bound ^{45}Ca to unbound ^{45}Ca relative to that of the untreated calcium binding protein controls, with the latter arbitrarily set at unity. See text for details.

Fig. 2. Effect of lysolecithin and taurocholate on calcium binding by calcium binding protein, as shown by an ion-exchange resin assay. The assay procedure is essentially as described previously[2], except that the volumes were scaled down by a factor of 10. Components of the *minus* calcium binding protein (−CaBP) control tubes were: buffer mixture (0.15 M KCl, 1 mM PIPES, 5 μM $CaCl_2$, pH 6.8), 0.12 ml; Chelex resin suspension in 0.15 M KCl, 1 mM PIPES, pH 6.8, 0.02 ml; ^{45}Ca solution in the latter buffer mixture, 0.01 ml. The components of the *plus* calcium binding protein (+CaBP) tubes were identical except that the first buffer mixture contained 500 μg calcium binding protein per ml. Lysolecithin and taurocholate were added in a volume of 0.01 ml each, replacing equal volumes of buffer mixture alone. The final lysolecithin and taurocholate concentrations, where applicable, were 0.91 mg/ml and 3.74 mg/ml, respectively. The order of addition was: protein, lysolecithin, taurocholate, Chelex resin, ^{45}Ca, with 5-min incubation periods allowed between lysolecithin and taurocholate addition, and taurocholate and Chelex addition. After mixing and centrifuging, 0.02-ml duplicate aliquots were taken from each tube, added to BRAY's[9] solution, and counted with a liquid scintillation detector. The results are expressed as the ratio of bound ^{45}Ca to unbound ^{45}Ca relative to the calcium binding protein control value. The values are depicted as the mean ± the range.

Abbreviation: PIPES, piperazine-*N*,*N*^{1}-bis-(2-ethanesulfonic acid) monosodium monohydrate.

was dissolved in the following buffer mixture: 0.15 M KCl, 1 mM piperazine-N,N^1-bis-(2-ethanesulfonic acid) monosodium monohydrate (PIPES), 5 μM $CaCl_2$, pH 6.8. Calcium binding protein was isolated from chick intestinal mucosa by the procedure of WASSERMAN *et al.*[2], with only slight modification.

The effect of lysolecithin on calcium binding by calcium binding protein was examined by two techniques, equilibrium dialysis across Visking dialyzing membrane and the Chelex 100 (Bio-Rad) ion-exchange assay[1]. For the former, calcium binding protein (380 μg/ml), in 0.4 ml of the above buffer mixture, was placed in one chamber of a dialysis cell (1-ml chambers; Chemical Rubber Co.) and the same volume of buffer mixture *plus* tracer ^{45}Ca (5 μC) placed in the other. In one experiment, six cells were shaken at room temperature, and 0.02-ml aliquots were removed at about 2 h and 4 h with a micro-pipette (Eppendorf) for ^{45}Ca analysis. After the 4-h sampling, 0.05 ml lysolecithin solution (in 0.15 M KCl, 1 mM PIPES, pH 6.8) was added to both sides of four of the cells, and 0.05 ml of buffer mixture to the others. At 7 h and 9.5 h, all cells were resampled. After the 9.5-h sampling, 0.01 ml sodium taurocholate (in 0.15 M KCl, 1 mM PIPES, pH 6.8) was added to both chambers of two cells containing lysolecithin. The same volume of the buffer mixture was added to the other cells. All cells were sampled again at about 11.3 h and 13 h. The final lysolecithin concentration was 1.52 mg/ml, and that of sodium taurocholate, 1.40 mg/ml. The ^{45}Ca was counted by placing the sampled volume (0.02 ml) into 5 ml BRAY's[9] solution, and the count rate assessed with a liquid scintillation detector (Nuclear-Chicago, Mark I). The data are expressed as the ratio of protein-bound ^{45}Ca to that of free ^{45}Ca, relative to the untreated controls at each time period.

The Chelex-100 assay is essentially as published previously[2], except that it was scaled down such that the binding capacity of small volumes (in the range of 0.10 ml) could be determined. This miniaturization of the procedure was developed and evaluated by Dr. R. A. Corradino of this laboratory. The protocol is detailed in the legend and main body of Fig. 2. The ion-exchange data are expressed in similar units as that of the equilibrium dialysis experiment, relative to that of the untreated calcium binding protein control.

Complex formation between calcium binding protein and lysolecithin was further explored by acrylamide gel electrophoresis, using the slab gel technique described previously[3]. The protein and lysolecithin were pre-incubated for 10–15 min prior to placing the sample in the gel slot. Other details of this experiment are given in the legend of Fig. 3.

Fig. 1 shows that the addition of lysolecithin to the equilibrium dialysis cell causes an appreciable decrease in the ratio of bound to unbound ^{45}Ca in the calcium binding protein-containing compartment. This, by itself, suggests that lysolecithin interacts with calcium binding protein. Taurocholate, when added to the lysolecithin-loaded equilibrium cells (Fig. 1) reversed, at least in large part, the inhibitory effect of the phospholipid, undoubtedly by removing the lipid from the protein. An estimate of ^{45}Ca binding by the ion-exchange procedure showed that lysolecithin and taurocholate, either alone or in combination, bound ^{45}Ca only minimally or not at all under the conditions of this experiment (Fig. 2). With this technique, the depression of the calcium binding activity of calcium binding protein by lysolecithin and its reversal by taurocholate were again demonstrated (Fig. 2). Taurocholate itself did not alter the binding activity of the protein.

The acrylamide gel pattern (Fig. 3) clearly shows that lysolecithin and calcium binding protein form a complex which is not readily dissociated by an electrical field (compare A and B). Such an association is not unexpected after the fact, since the occurrence of lipid–protein interactions has been well documented[10–12]. Taurocholate alone did not detectably alter the migration of the protein (Fig. 3, C) but did restore its mobility when lysolecithin was present (Fig. 3, D). The specificity of the calcium binding protein interaction with various lipoid substances is currently under investigation.

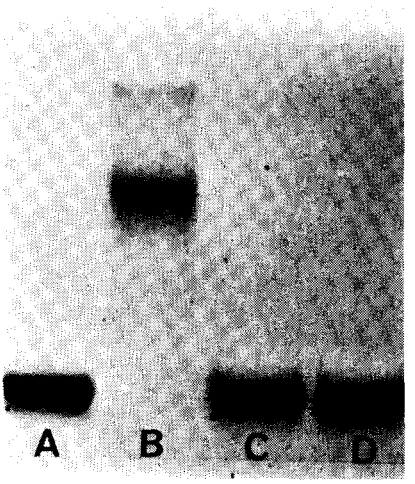


Fig. 3. Interaction of lysolecithin with calcium binding protein, as shown by acrylamide gel electrophoresis. The procedure is identical to that described previously[3]. The gel slots were loaded with 0.2 ml of the following preparations: (A) purified calcium binding protein (320 μg/ml); (B) purified calcium binding protein + lysolecithin (0.57 mg/ml); (C) purified calcium binding protein + lysolecithin + taurocholate (2.34 mg/ml); (D) purified calcium binding protein + taurocholate. The pH of the electrophoresis buffer was 8.2, the potential was 200 V, and the running time, 2 h. Migration was toward the anode (bottom).

The relevance of these results to the function of calcium binding protein is difficult to surmise at present. However, these observations assume some significance when it is noted that: (a) calcium binding protein is localized in a region of the cell at which membrane lipid interactions could occur[5], (b) a mechanism for releasing the calcium binding protein-bound calcium in or near the surface membrane is provided, (c) a phospholipase A has been localized in the brush border of the intestine[13] which could make lysolecithin available at the site where calcium binding protein is localized. Further, intestine[13], toad bladder epithelium[14] and other tissues contain enzyme systems for catalyzing the conversion of lecithin to lysolecithin and reconverting lysolecithin to lecithin. Because of the membrane-lytic effect of lysolecithin, it has been speculated that the lecithin–lysolecithin cycle might regulate membrane permeability[14] and turnover[15], and it is also conceivable that the same cycle may be involved in nutrient transfer by reacting with a specific "carrier".

In another vein, the present findings might prove of interest to those studying lipid–protein interactions because a readily discernable property of the protein, *i.e.* calcium binding activity, is altered as a consequence of this interaction.

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1 R. H. WASSERMAN AND A. N. TAYLOR, *Science*, 152 (1966) 791.
2 R. H. WASSERMAN, R. A. CORRADINO AND A. N. TAYLOR, *J. Biol. Chem.*, 243 (1968) 3978.
3 A. N. TAYLOR AND R. H. WASSERMAN, *Arch. Biochem. Biophys.*, 119 (1967) 536.
4 R. A. CORRADINO, R. H. WASSERMAN, M. H. PUBOLS AND S. I. CHANG, *Arch. Biochem. Biophys.*, 125 (1968) 378.
5 A. N. TAYLOR AND R. H. WASSERMAN, *J. Histochem. Cytochem.*, in the press.
6 R. H. WASSERMAN AND A. N. TAYLOR, *J. Biol. Chem.*, 243 (1968) 3987.
7 R. H. WASSERMAN, R. A. CORRADINO AND A. N. TAYLOR, *J. Gen. Physiol.*, 54 (1969) 114.
8 A. B. PARDEE, *Science*, 162 (1968) 632.
9 F. G. BRAY, *Anal. Biochem.*, 1 (1960) 279.
10 A. D. BANGHAM, *Progr. Biophys. Mol. Biol.*, 18 (1968) 29.
11 W. E. KOLPFENSTEIN, *Biochim. Biophys. Acta*, 181 (1969) 323.
12 R. M. C. DAWSON, in D. CHAPMAN, *Biological Membranes, Physical Fact and Function*, Academic Press, New York, 1968, p. 203.
13 P. V. SUBBAIAH, P. S. SASTRY AND J. GANGULY, *Biochem. J.*, 113 (1969) 441.
14 A. A. ROSENBLUM AND P. ELSBACH, *Am. J. Physiol.*, 214 (1968) 161.
15 L. L. VAN DEENEN AND G. H. DE HAAS, *Ann. Rev. Biochem.*, 35 (1966) 157.

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Variation in tissue resistance in rat small intestine: Its relationship to observed potential changes

In a previous paper[1] it was shown that the increase in the potential difference between the mucosal and serosal surfaces of rat small intestine, in the presence of actively transported amino acids in the mucosal bathing fluid, was greatest in the ileum and smallest in the proximal jejunum. Similar observations were made both *in vivo* and *in vitro*. The same study, as well as many others[2], suggests that the change in potential difference (PD) is proportional to the rate of amino acid transfer. Varying patterns of amino acid absorption along the length of the intestine have been reported[3] but the differences do not appear to account for the pattern of PD changes observed. It was suggested[1] that either the stoichiometry between amino acid and ion transport varied along the intestine or the results could be due to differences in tissue resistance.

The present study was designed to choose between these alternatives. We have examined the effects of L-histidine on electrical parameters of the small intestine and also compared histidine transfer in the mid-intestine and ileum. Histidine was chosen because it is very little metabolised during transfer[4] and gives a comparatively large maximum PD[1].

Abbreviation: PD, potential difference.