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Detection and Distribution of Intestinal Calcium-Binding Protein in the Young Adult Male Guinea Pig[1] (39715)

M. W. CHAPMAN,[2, 3] A. N. TAYLOR,[4] R. H. WASSERMAN, AND W. G. POND

Department of Animal Science, Cornell University, Ithaca, New York and Department of Physical Biology, New York State Veterinary College, Ithaca, New York 14853

Intestinal calcium-binding protein (CaBP) has been detected in a wide variety of animals including frog, carp, and pike (1, 2), chick (1, 3–5), rat (5–9), hamster (10), guinea pig[2] (13), dog (14), pig (1, 5, 13), bovine (15), horse (13), squirrel and cebus monkeys (16), and human (17–20). In at least the chick (3, 4), rat (6–8), dog (14), and monkey (16) CaBP is vitamin D-dependent, i.e., in the absence of vitamin D, synthesis of CaBP is reduced or absent.

Previously we attempted to produce rickets in the guinea pig using a Ca/P ratio of 1.1 to 1 and depriving the animals of exogenous vitamin D and light of wavelengths < 350 m, thereby excluding those wavelengths found to be antirachitic in rats (11, 12, 21). No evidence of rickets was found based on changes in plasma calcium concentration or by histological examination of the parathyroids and of the growth plate of the humerus. Since this attempt was unsuccessful and it was not yet known whether the guinea pig had intestinal CaBP, it was of interest to resolve the question.

Methods. Four male English short-hair guinea pigs,[5] raised on commercial pellets prior to sacrifice and weighing 300 to 400 g, were killed by exsanguination. Removal and extraction of duodenal musoca were essentially the same as described by Wasserman and Taylor (3) with slight modification. Mucosae from the four animals were pooled.

Five additional guinea pigs from the same colony were used to compare the calcium-binding activity in different segments of the intestine. The mucosae from the animals were pooled as follows: for the duodenum, the first 18 cm of the small intestine; for the jejunum, the middle 18–25 cm of the small intestine; for the ileum, the last 18 cm of the small intestine; for the cecum, the tip 1/3 to 1/2; for the colon, all. The pooled samples were processed as described above and the supernatant was used for analysis of calcium-binding activity for each segment.

The mucosal supernatant was fractionated on a 2.5 × 85-cm Sephadex G-100 column which was eluted with pH 7.4 Tris buffer at 4°. The flow rate was 0.3 to 0.6 ml/min, and 2-ml fractions were collected. The fractions were analyzed for protein (22) and for calcium-binding activity by the Chelex-100 method (3). The Chelex-100 method depends on the competition between a cation-exchange resin and a calcium-binding substance for added radiocalcium; in the presence of a chelator or soluble complexing factor, less ^{45}Ca is sequestered by the resin.

To see whether the calcium-binding factor was a charged particle and to visualize it, the proteins in the mucosal supernatant were separated by gel electrophoresis (7% gel) (23). Samples of 100 μl from fractions 20, 32, 46, 64, and 72 from the Sephadex gel filtration experiment (see Fig. 1) and the crude supernatant were run at pH 8.6 for 1.5 hr and then stained with buffalo black.

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[2] The work reported here was initially presented at ASAS/ADSA Meeting, University of New Hampshire, July 16–18, 1972 (11, 12). It represents a portion of a thesis submitted by M. W. Chapman to the Graduate School of Cornell University in partial fulfillment of the requirements for the M.S. degree.

[3] Send all correspondence to M. W. Chapman, 254 Morrison Hall, Cornell University, Ithaca, New York 14853.

[4] Present address: Department of Microscopic Anatomy, Baylor College of Dentistry, Dallas, Texas 75226.

[5] From a breeding colony which had been closed to the introduction of animals for 25 years; originally from a cross between animals of strain 13 from NIH and animals from Carworth Farms, New City, N. Y.

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To check further whether the calcium-binding factor was indeed a protein, it was subjected to enzymatic digestion by trypsin and by Pronase in a concentration of 100 μg of enzyme/ml of supernatant. The samples were incubated at 37° with shaking and aliquots of 0.1 ml were removed at 0, 15, 30, 60, 120, and 180 min and analyzed for calcium-binding activity as above.

Results and discussion. The elution diagram of Fig. 1 shows that the calcium-binding activity was associated primarily with the third major protein peak in the region of relatively low molecular weight substances and was associated with only a single major peak in the region of fraction 48. A much lower binding activity was associated with the column void volume and probably could be attributed to high molecular weight proteins and aggregates bearing negatively charged groups. The major calcium-binding peak was well-defined, and rose 60-fold above background. A similarly prominent peak in the elution pattern was reported for the chick (23), rat (6), bovine (24), and horse (13). If this peak is a single protein, the fact would be consistent with data for those CaBPs.

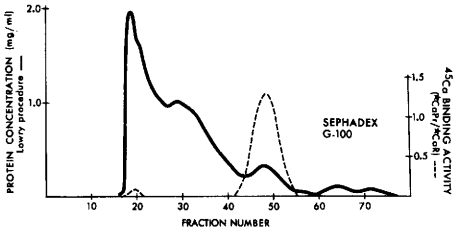

FIG. 1. Distribution of proteins and calcium-binding activity in fractions of supernatant after separation by dextran gel filtration (Sephadex G-100). Elution was performed with pH 7.4 Tris buffer at 4°, and 2-ml fractions were collected. Protein by the Lowry *et al.* procedure (22) and calcium-binding activity by the Chelex method (see text) were determined in aliquots of alternate fractions. (- - -) ^{45}Ca-binding activity expressed as the ratio of ^{45}Ca associated with the protein to that associated with resin (*CaPr/*CaR) (26); (——) protein concentration.

Figure 2 shows acrylamide gel electrophoretic patterns of several fractions of the Sephadex gel filtration and of the crude supernatant. The arrow indicates a prominent protein band in fraction 46 which may be the calcium-binding factor since this fraction corresponds to the calcium-binding peak seen in Fig. 1. Figure 3 shows the changes in calcium-binding activity with time for the incubation of 100 μg of trypsin or Pronase/ml of guinea pig intestinal supernatant. Pronase acted very quickly to destroy almost completely the calcium-binding activity, but trypsin had practically no effect. At the end of the incubation period (180 min) 80% of the original calcium-binding activity remained in the trypsin digest whereas only 6% remained in the Pronase digest. This is similar to what was seen in the bovine (15, 24) and in the dog (14). As previously pointed out (24), potential reasons for lack

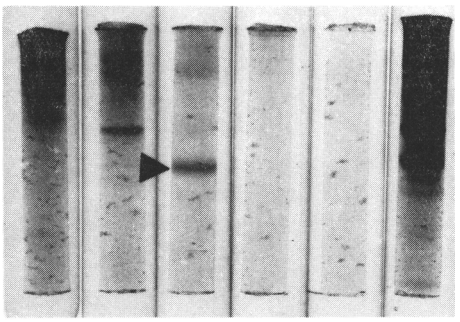

FIG. 2. Acrylamide gel electrophoresis pattern of protein in selected fractions of mucosal supernatant before and after gel filtration. Mucosae were pooled from normal pellet-fed guinea pigs. Arrow indicates probable CaBP band. Migration was toward the anode (bottom).

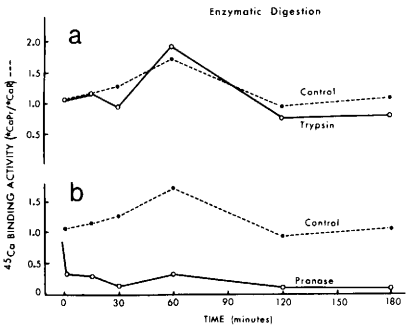

FIG. 3. Effect of trypsin and Pronase digestion on the calcium-binding activity of guinea pig CaBP compared to control. Incubation was at 37° for designated times and at enzyme concentration of 100 μg/ml of mucosal supernatant. (·- - -·) intestinal supernatant with no enzyme added; (O——O) CaBP incubated with either trypsin or Pronase.

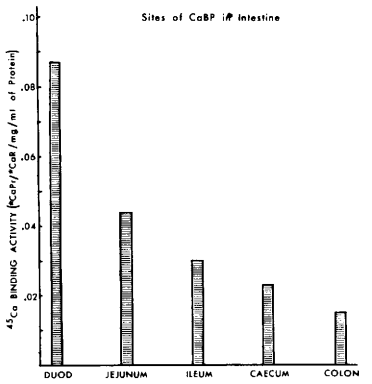

FIG. 4. Comparison of guinea pig CaBP activity as a function of intestinal site.

of digestion by trypsin are several including (a) lack of cleavage, since trypsin is quite specific and (b) cleavage at points too far removed from the active binding site(s) of the molecule to impair calcium-binding activity. However, it is now evident that trypsin-resistance is a function of the calcium content of the protein and the conformational masking of trypsin-susceptible groups on CaBP which might take place when the protein binds to calcium (25). The fact that Pronase so thoroughly digested the binding factor strongly suggests that it is a protein; in addition, what may have been the same factor in a fraction near peak ^{45}Ca-binding activity was charged and moved in an electric field.

Figure 4 shows that the guinea pig has calcium-binding activity throughout the GI tract with the activity decreasing with increasing distance from the stomach. This distribution is similar to that of the chick (23), squirrel and cebus monkeys (16), and the golden hamster (10). In the chick and hamster the rate of calcium absorption per unit segment correlates well with the relative amount of CaBP in a given segment (3, 10).

Summary. A calcium-binding factor was detected in guinea pig intestinal mucosa. It was negatively charged and moved in an electrical field. Pronase quickly destroyed almost completely the calcium-binding activity although trypsin had practically no effect. The major calcium-binding peak was well defined. It was concluded that the factor is probably a protein. Calcium-binding protein was found throughout the gastrointestinal tract, with the calcium-binding activity decreasing with increasing distance from the stomach.

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