Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 656-660

CALMODULIN-LIKE ACTIVITY IN THE SOLUBLE FRACTION OF *ESCHERICHIA COLI*

Yasushi Iwasa, Kosei Yonemitsu*, Kazuo Matsui#
Kohji Fukunaga and Eishichi Miyamoto

From the Department of Pharmacology
Kumamoto University Medical School, Kumamoto 860, Japan

Received December 23,1980

SUMMARY: A heat-stable factor with properties similar to those of calmodulin was found in the fraction containing Ca^{2+}-dependent cyclic AMP phosphodiesterase of *Escherichia coli*. The factor activated such enzymes as cyclic nucleotide phosphodiesterase of bovine brain, (Ca^{2+},Mg^{2+})ATPase of human erythrocyte menbrane and myosin light chain kinase of rabbit myometrium in a Ca^{2+}-dependent fashion with an apparent K_a of 5×10^{-5} M. The factor and brain calmodulin had no effect on the phosphodiesterase of *E. coli*. It may be concluded that calmodulin or a calmodulin-like protein occurs in prokaryotes.

A heat-stable, acidic Ca^{2+}-binding protein referred to as calmodulin has been shown to have multiple Ca^{2+}-dependent regulatory activities, affecting erythrocyte membrane (Ca^{2+},Mg^{2+})ATPase, myosin light chain kinase and other Ca^{2+}-dependent reactions (1-3). Although calmodulin has been reported to be ubiquitous in eukaryotes, it has not yet been reported in a prokaryote. In this communication we report that a heat-stable factor with similar properties to that of calmodulin is associated with the Ca^{2+}-dependent phosphodiesterase in the soluble fraction of *E. coli*. Studies on the Ca^{2+}-dependent phosphodiesterase of *E. coli* will be described elsewhere.

---

* Present address: The Department of Legal Medicine, Kumamoto University Medical School, Kumamoto 860, Japan

On leave from the Department of Obstetricus and Gynecology, Kumamoto University Medical School, Kumamoto 860, Japan

0006-291X/81/030656-05$01.00/0

EXPERIMENTAL PROCEDURES

E. coli (O-143, K-X1), outdated red blood cells from local blood bank, domestic rabbit (2,6 kg) and chicken gizzard from local slaughter hause were employed for the present studies.

Ca^{2+}-dependent phosphodiesterase of E. coli was purified as follows. All manipulations were performed at 0 to 4°C. 16 g of wet cells, which were grown aerobically at 37°C in a nutrient broth and harvested at late logarithmic phase, were washed three times with Tris-buffered saline at pH 7.5 and were sonicated for 5 min with 2 volumes of Buffer A (20 mM Tris-HCl, pH 7.5, 1 mM imidazole, 1 mM magnesium acetate, 10 mM 2-mercaptoethanol, 0.1 mM EGTA[1/] and 0.43 mM phenylmethylsulfonylfluoride). The homogenate was centrifuged for 20 min at 35,000 x g. To the supernatant was added a saturated ammonium sulfate solution (pH 7.5) to a final saturation of 55%. The pellet was collected, dissolved in Buffer A containing 0.1 M NaCl and dialysed against 100 volumes of the same buffer for 18 h. The dialysate was applied to a DEAE-cellulose (DE-52) column (2.5 x 20 cm) equilibrated with the buffer. After the column was washed with 200 ml of the buffer, phosphodiesterase was eluted by a 600 ml of linear concentration gradient of NaCl (0.1 to 0.5 M) in Buffer A. Fractions 6 ml each were collected. Erythrocyte membranes were prepared by the method of Gopinath and Vincenzi (4). Myosin light chain kinase was purified from rabbit myometrium at the stage of DEAE-cellulose column chromatography as described previously (5). Chicken gizzard myosin light chain free of calmodulin was prepared by the method of Perrie and Perry (6) and Matsuda et al. (7). Calmodulin and calmodulin-deficient phosphodiesterase of bovine brain were purified by the methods of Teo et al. (8) and Klee and Krinks (9), respectively. [γ-^{32}P]ATP was prepared by the method of Post and Sen (10). Cyclic AMP, Crotalus atrox venom and ATP were products of Sigma, and other chemicals were obtained from commercial sources.

Phosphodiesterase was assayed by the method of Kakiuchi et al. (11) employing 0.8 mM cyclic AMP as a substrate except that instead of 0.1 mM $CaCl_2$, 1 mM $CaCl_2$ or 5 mM EGTA was used. (Ca^{2+},Mg^{2+})ATPase was assayed by the method of Kobayashi et al. (12). Myosin light chain kinase was assayed as described previously (5). Protein was determined by the method of Lowry et al. (13) with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Fig. 1 shows the elution profile on DEAE-cellulose column chromatography of E. coli phosphodiesterase. The enzyme emerged as two active fractions. The first and major peak of phosphodiesterase activity was absolutely Ca^{2+}-dependent, and the second was independent. The first peak, fractions 45 through 65 , were pooled and concentrated by an Amicon ultrafiltration cell equipped with a PM-10 filter. This preparation was employed for the following studies. The preparation was heated for 5 min

1/ The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethylether)N,N,N',N'-tetraacetic acid.

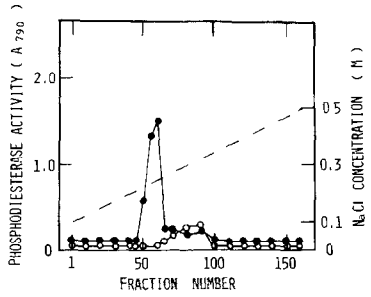

Fig. 1. Resolution of a Ca^{2+}-dependent phosphodiesterase on DEAE-cellulose column chromatography employing linear concentration gradient of NaCl (---). Detailed experimental conditions are described under "EXPERIMENTAL PROCEDURES". Phosphodiesterase activity was assayed in the presence of 1 mM $CaCl_2$ (●—●) and in the presence of 5 mM EGTA (o—o).

in a boiling water bath to abolish the endogenous activity of phosphodiesterase, and then assayed for its ability to activate Ca^{2+}-dependent enzymes. As shown in Table 1, the boiled sample (E. coli factor) increased the activities of brain phosphodiesterase, erythrocyt membrane (Ca^{2+},Mg^{2+})ATPase and myometrium myosin light chain kinase

Table 1

Effects of E. coli factor and brain calmodulin on various enzyme activities

Additions	Enzyme activity			
	Brain PDE*	Membrane (Ca^{2+},Mg^{2+})ATPase	MLCK**	E. coli PDE*
	nmol	nmol	pmol	nmol
None	12	18	10	34
E. coli factor	88	68	167	50
Brain calmodulin	120	87	197	34

* PDE, phosphodiesterase. ** MLCK, myosin light chain kinase. The enzyme activities were assayed under the respective standard conditions described under "EXPERIMENTAL PROCEDURES" and expressed as total one. Brain phosphodiesterase (13 μg protein), erythrocyte membrane preparation (15.6 mg wet weight), myometrium myosin light chain kinase (14.5 μg protein), E. coli factor (62 μg protein) and brain calmodulin (1 μg protein) were employed for this experiment.

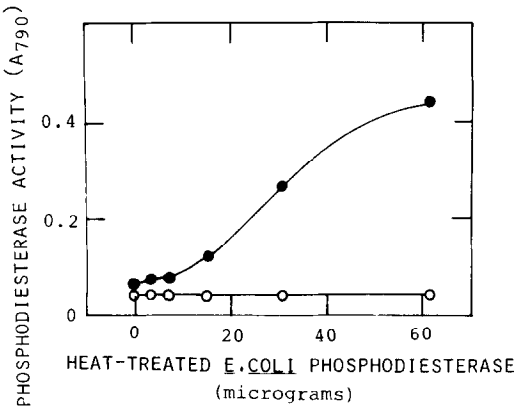

Fig. 2. Effect of various amounts of E. coli factor on activation of brain phosphodiesterase. The enzyme activity was assayed in the presence of 1 mM $CaCl_2$ (●—●) and in the presence of 5 mM EGTA (o—o). The amount of brain phosphodiesterase employed was 25.5 µg of protein in the reaction mixture.

by about 7-, 4- and 17-fold, respectively. These were comparable to the 10-, 5- and 20-fold increases obtained with brain calmodulin. On the other hand, E. coli factor and brain calmodulin had little effects on the activity of E. coli phosphodiesterase. When E. coli factor was treated with trypsin prior to assay, the activation of the enzymes was no longer observed. Fig. 2 shows the effect of various amounts of E. coli factor on a fixed amount of brain phosphodiesterase in the presence or absence of Ca^{2+}. The enzyme activity increased progressively with increase in the amount of E. coli factor, and a sufficient amount of the factor was found to result in maximal increase in the enzyme activity. In addition, the reaction was absolutely dependent on Ca^{2+}. When the effect of various concentration of Ca^{2+} on the activity of brain phosphodiesterase in the presence of E. coli factor was studied, the maximum activity was obtained at 1 mM Ca^{2+} with an apparent K_a value of 5×10^{-5} M. The K_a value was higher than that with brain calmodulin. This is probably due to the presence of an inhibitor protein[2/] which is associated with the phos-

2/ This inhibitor was heat-stable and showed a molecular weight of 20,000. Properties of this protein will be described elsewhere.

phodiesterase of E. coli, as reported for the enzyme of rat brain (14). Similar results were obtained also for (Ca^{2+},Mg^{2+})ATPase and myosin light chain kinase.

These results shows that a component of E. coli phosphodiesterase has many of the properties associated with calmodulin, namely, heat-stability, sensitivity to trypsin digestion, ability to activate three Ca^{2+} and calmodulin-dependent enzymes.

ACKNOWLEDGMENT —— We are grateful to Dr. T. Katsuki of the Department of Bacteriology, Kumamoto University Medical School for valuable discussion and advice for E. coli.

REFERENCES

1. Klee, C.B., Crouch, T.H., and Richman, P.G. (1980) Ann. Rev. Biochem. 49, 489-515.
2. Means, A.R., and Dedman, J.R. (1980) Nature 285, 73-77.
3. Cheung, W.Y. (1980) Science 207, 19-27.
4. Gopinath, R.M., and Vincenzi, F.F. (1977) Biochem. Biophys. Res. Commun. 77, 1203-1209.
5. Miyamoto, E., Hirose, R., and Setoyama, C. (1980) Biomed. Res. 1, 158-163.
6. Perrie, W.T., and Perry, S.V. (1970) Biochem. J. 119, 31-38.
7. Matsuda, G., Suzuyama, Y., Maita, T., and Umegane, T. (1977) FEBS Lett. 84, 53-66.
8. Teo, T.S., Wang, T.H., and Wang, J.H. (1973) J. Biol. Chem. 248, 588-595.
9. Klee, C.B., and Krinks, M.H. (1978) Biochemistry 17, 120-126.
10. Post, R.L., and Sen, A.K. (1967) Methods Enzymol. 10, 773-776.
11. Kakiuchi, S., Yamazaki, R., and Nakajima, H. (1970) Proc. Jap. Acad. 46, 587-592.
12. Kobayashi, R., Tawata, M., and Hidaka, H. (1979) Biochem. Biophys. Res. Commun. 88, 1037-1045.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
14. Wallace, R.W., Lynch, T.J., Tallant, E.A., and Cheung, W.Y. (1978) Arch. Biochem. Biophys. 187, 328-334.