Different Conformation Changes Induced by Calcium and Terbium of the Porcine Intestinal Calcium-Binding Protein

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Received July 23, 1986; Revised Manuscript Received October 10, 1986

ABSTRACT: The fluorescence of 1-anilino-8-naphthalenesulfonate (ANS) is progressively enhanced with increasing concentration of it, showing a proportionate blue shift of the emission maximum, by the interaction with the porcine intestinal Ca^{2+}-binding protein (CaBP) in the absence of Ca^{2+}. The apo-CaBP has a single binding site for ANS as determined by the fluorescence change, the apparent dissociation constant (K_d) estimated at 49.1 μM. Addition of Ca^{2+} or Tb^{3+} to the ANS–apo-CaBP system is capable of enhancing its fluorescence up to about 2- or 5-fold, respectively, causing further blue shift of the emission maximum. These metal ions do not affect the capacity of ANS binding, but Ca^{2+} slightly increases the K_d value. Increase of the fluorescence of the ANS–CaBP complex by increasing binding of Ca^{2+} to it was monophasic, while that with Tb^{3+} was biphasic, both saturated at the same molar ratio, 2, of added cations to the complex. Biphasic change of response has also been observed in UV absorption of the CaBP with increasing concentration of Tb^{3+}. With a half-saturating concentration of Tb^{3+}, Ca^{2+} can induce a much higher enhancement of the ANS fluorescence than excess Ca^{2+} alone. All these results indicate that the CaBP molecule contains a single ANS binding site and the conformation and/or microenvironment surrounding bound ANS of the protein is altered reversibly with binding of Ca^{2+} or Tb^{3+} to it and that there are differences between Ca^{2+}- and Tb^{3+}-induced conformation changes around the ANS-binding site and the tyrosine residue of it.

The intestinal calcium-binding proteins (CaBP's)[1] found in several mammalian species have individually at least two high-affinity Ca^{2+}-binding sites (Wasserman & Fullmer, 1982; Wasserman et al., 1983). They are located separately in the C- and N-terminal parts of the molecules in the porcine and bovine intestinal CaBP's (Szebenyi, 1981), forming a normal EF hand and a pseudo EF hand, respectively, as defined by Kretsinger (1976). We previously demonstrated by a quantitative determination of Ca^{2+} binding and measurements of Ca^{2+}-induced changes in the intrinsic tyrosine fluorescence, CD, and UV absorption that the porcine CaBP molecule has two noncooperative Ca^{2+}-binding sites with essentially the same affinity to Ca^{2+} (Chiba et al., 1983) and that a slight but conspicuous change in the secondary structure was induced upon binding of Ca^{2+} to the CaBP. It has been also indicated that the two calcium-binding sites of the porcine CaBP bind a pair of Tb^{3+} ions with different affinities (Chiba et al., 1984). This latter finding is consistent with the experimental results of O'Neil et al. (1984) and Shelling et al. (1983) using lanthanides. Vogel et al. (1985) have recently analyzed the cooperative interaction between the two calcium-binding sites of the porcine CaBP using $^{112}Cd^{2+}$ NMR.

Lanthanide ions have certainly been recognized as useful Ca^{2+} analogues in the studies on the binding sites of Ca^{2+}-binding proteins including membrane proteins [cf. Mikkelson (1976)], but at the same time, they are reported to inhibit several biological functions of Ca^{2+}-binding proteins (Oikawa et al., 1980; Chantler, 1983). Yazawa et al. (1984) pointed out that the orders of the binding sites in binding to Ca^{2+} in troponin C and calmodulin, estimated from the Tb^{3+} binding to these proteins, should be reevaluated.

It has been reported that the family of calcium binding proteins, calmodulin, troponin C, and S-100, is induced commonly to express the hydrophobic domain in their structure by Ca^{2+} binding to the proteins and has Ca^{2+}-dependent interaction with phenothiazine (LaPorte et al., 1980; Marshak et al., 1981; Baudier & Gérard, 1983). The hydrophobic property of these proteins is thought to be important for their biological activities (Tanaka & Hidaka, 1980; Tanaka et al., 1984; Marshak et al., 1985). However, Marshak et al. (1981) have reported that parvalbumin and the chicken intestinal CaBP do not bind phenothiazine. The structure of the hydrophobic region of the mammalian intestinal CaBP and its relation to ligand binding are important problems to be elucidated.

In this work we have studied the interaction between ANS and the porcine CaBP with special reference to the fluorescence parameters of the complex and the UV absorption of the metal-bound forms and revealed that bindings of Ca^{2+} and Tb^{3+} to the protein induce notably different conformation changes around the ANS-binding site of it.

Materials and Methods

Preparation of CaBP. The porcine CaBP was prepared from the small intestine as previously described (Chiba et al., 1983). The calcium contents of the CaBP preparations (apo form) were always less than 10% of the Ca^{2+}-binding capacity of the protein (Chiba et al., 1983).

Fluorescence Measurements. Fluorescence of ANS was measured at 25 °C for 2.5-mL samples in 10 mM Tris-HCl buffer, pH 7.4, with a Hitachi MPF-4 fluorescence spectrophotometer. The wavelengths 390 and 450 nm were used as excitation and emission, respectively, for measurement of the

[1] Abbreviations: CaBP, calcium-binding protein; ANS, 1-anilino-8-naphthalenesulfonate; NPN, *N*-phenyl-1-naphthylamine; K_d, dissociation constant of the ANS–CaBP complex; CD, circular dichroism; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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fluorescence of ANS bound to the CaBP in order to eliminate the contribution of free dye fluorescence, unless otherwise specified in the results. Fluorescence intensities and spectra were corrected for base-line fluorescence. The CaBP and ANS concentrations used were in the ranges 2.5–62.7 and 10–67.6 μM, respectively, as specified under Results. Mg^{2+} in ANS stock solution did not interfere with Ca^{2+} and Tb^{3+} binding to the CaBP, and the fluorescence of ANS was not affected by Ca^{2+} or Tb^{3+} addition in the absence of the protein. The maximal fluorescence intensity (F_{max}) at the infinite dye concentration and the apparent dissociation constant (K_d) for the ANS–CaBP complex were obtained by plotting the ratio of the fluorescence intensity (f) to a given ANS concentration (D) against f and calculating according to

$$f/D = F_{max}/K_d - f/K_d \quad (1)$$

The number of ANS binding sites on the CaBP was determined according to the equation by Christian and Janetzko (1971):

$$\frac{P}{XD} = \frac{1}{n} - \frac{K_d}{n(1-X)D} \quad (2)$$

where n is the number of binding site per protein molecule, K_d is the dissociation constant, D is the total ANS concentration, P is the protein concentration, and X is the fraction of ANS bound. XD/P and $(1 - X)D$ are equivalent to the number of moles of bound ANS per mole of CaBP and the free ANS concentration, respectively. The above calculation assumes that the dye–protein interaction is not cooperative and that all binding sites are characteristically identical. Daniel and Weber (1966) have previously suggested that the fluorescence efficiency is variable with the change of the molar ratio of ANS to protein in solution. It was confirmed that the fluorescence intensity is linearly changed with various concentrations of ANS within a range from 2.49 to 55.6 μM in the used CaBP concentration (110 μM), irrespective of its forms, free or metal bound. Furthermore, relative enhancements of the fluorescence intensity due to binding of the metal ions to the ANS–CaBP complex were much the same in the range of the ratio, [ANS]/[CaBP], mentioned above. Therefore, the fraction of ANS bound in a given protein concentration can be written as $X = (f/F)D$, where F is the fluorescence intensity in the presence of excess CaBP (110 μM) at the various concentration of ANS in the range of 2.49–55.6 μM.

UV Difference Absorption. The difference absorption was measured with a UNION SM-401 spectrophotometer at 25 °C on an expanded scale between 240 and 320 nm. Each (initially 2.5 mL) of the reference and sample contained concentrations of the CaBP, 138 or 392 μM for Tb^{3+} titration and 518 μM for Ca^{2+} titration. In titration, small volumes of concentrated $TbCl_3$ solution were successively added to the sample cell and the same volumes of solvent (10 mM Tris-HCl, pH 7.4) only to the reference. Appropriate corrections were made for the dilution effect on absorption.

Reagents. $TbCl_3 \cdot 6H_2O$ was obtained from Mitsuwa Pure Chemical Co., Japan. 1-Anilino-8-naphthalenesulfonate was purchased from Nakarai Chemical Co., Japan, as the magnesium salt. All other chemicals used were of the purest grade commercially available.

Results

Fluorescence of ANS–CaBP Complex and Enhancement by Ca^{2+}. A large enhancement and blue shift of the emission maximum of ANS fluorescence are shown on addition of a solution of the porcine CaBP without addition of Ca^{2+} (inset of Figure 1). Addition of Ca^{2+} (up to a molar ratio, Ca^{2+}/CaBP, of 2) further enhanced the fluorescence intensity and shifted the emission maximum maximally by about 4 nm.

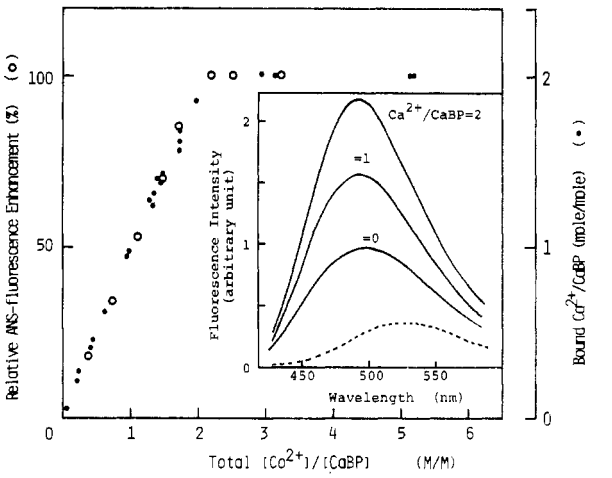

FIGURE 1: Correspondence between ANS fluorescence change and Ca^{2+} binding. Protein and ANS concentrations added were 52.8 and 59.1 μM, respectively. Other conditions in the assay are described under Materials and Methods. (○) Fluorescence change expressed as percent of the maximum; (●) Ca^{2+} binding expressed as the number of molecules of bound Ca^{2+} per mole of protein against the molar ratio of total Ca^{2+} per CaBP concentration, on the data previously reported (Chiba et al., 1983). (Inset) Emission spectra of ANS fluorescence in the presence and absence of the CaBP. Protein and ANS concentrations added were 62.7 and 67.6 μM, respectively. (--) ANS alone; (—) molar ratios of added Ca^{2+}/CaBP = 0, 1, and 2, as indicated in the figure.

Table I: Effects of Ca^{2+} and Tb^{3+} on Binding Parameters of ANS–CaBP Complex[a]

addition	K_d (μM)	increase of F_{max} (%)	n (mol/mol of protein)
	53.6 ± 4.6 (6)	(100) (6)	0.74 (1)
Ca^{2+}	80.7 ± 4.1 (3)	240.0 ± 18.5 (3)	0.92 (1)
Tb^{3+}	32.6 ± 3.1 (3)	398.9 ± 29.9 (3)	0.86 (1)

[a] K_d, dissociation constant of the complex; F_{max}, maximal fluorescence intensity at 450 nm in infinite ANS concentration in metal-bound form or apo form; n, maximum of ANS bound. K_d and n values were calculated from the data shown in Figure 2 (A and B) according to eq 1 and 2 described under Materials and Methods, respectively. Values are means ± SE of the numbers of experiments shown in parentheses.

Figure 1 shows that the fluorescence intensity of the ANS–CaBP complex increased linearly with increasing total Ca^{2+} concentration up to the molar ratio of 2, in a good correspondence with a Ca^{2+} binding curve previously reported (Chiba et al., 1983).

Effects of Ca^{2+} and Tb^{3+} on ANS Binding Parameters. As shown in Figure 2A, a plots of $f/[ANS]$ vs. f (f = fluorescence intensity of the ANS–CaBP complex) were linear both in the presence and in the absence of a saturating level of Ca^{2+}. The plots of $P/(XD)$ vs. $1/[(1 - X)D]$ also showed linear relationships with and without Ca^{2+} (Figure 2B). These relationships were also investigated in the presence of Tb^{3+} (Figure 2). ANS binding parameters calculated on the basis of these data and the effects of Ca^{2+} and Tb^{3+} are listed in Table I. The porcine CaBP is assumed to have a single binding site for ANS in the molecule, and the apparent K_d and n values for the ANS–CaBP complex were not greatly altered with either Ca^{2+} or Tb^{3+} ion.

Fluorometric Titration of ANS–CaBP Complex with Ca^{2+} and Tb^{3+}. Figure 3A shows Ca^{2+} and Tb^{3+} titration profiles of the ANS fluorescence of the ANS–CaBP complex. The enhancement of the fluorescence with Ca^{2+} was linear up to

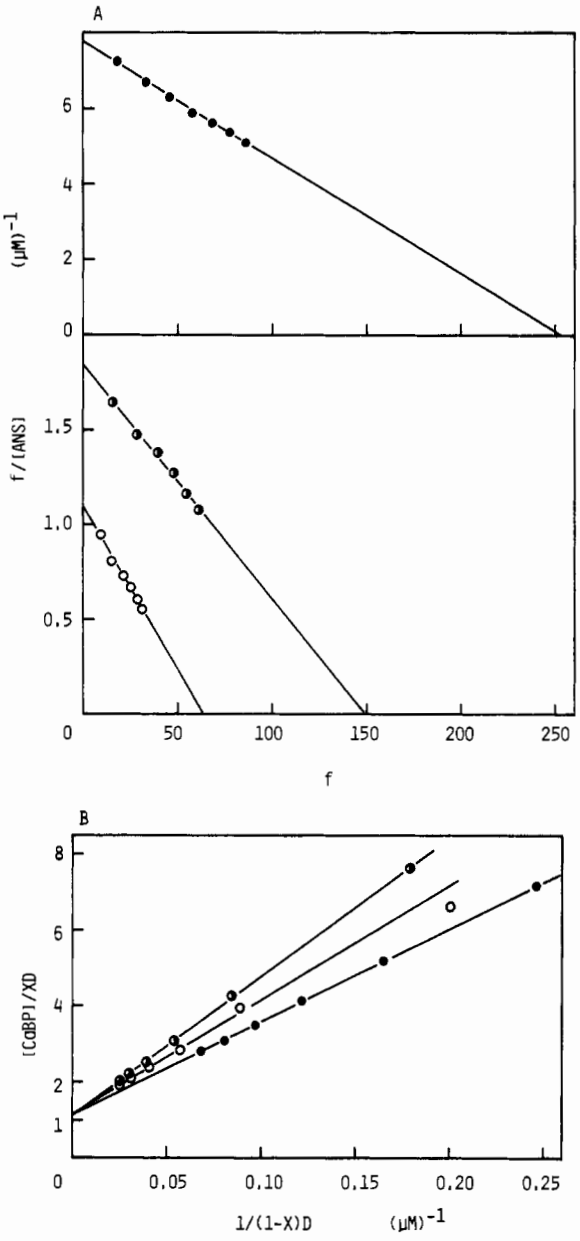

FIGURE 2: ANS binding parameters to CaBP in the presence and absence of Ca^{2+} or Tb^{3+}. (A) Plots of $f/[ANS]$ vs. f. Protein concentration was 29.8 μM. ANS was added in a concentration range from 9.48 to 55.56 μM. (○) No addition of $CaCl_2$; (◑) $CaCl_2$, molar ratio of added $Ca^{2+}/CaBP = 2$; (●) $TbCl_3$, molar ratio of added $Tb^{3+}/CaBP = 2$. Fluorescence intensities were expressed as arbitrary units. Other conditions in the assay are described under Materials and Methods. (B) Plots of $P/(XD)$ vs. $1/[(1-X)D]$: P = [CaBP]; D = [ANS]. (○) No addition of metals; (◑) addition of $CaCl_2$, $Ca^{2+}/CaBP = 2$; (●) addition of $TbCl_3$, $Tb^{3+}/CaBP = 2$. Other conditions in the assay are described under Materials and Methods.

a molar ratio of about 2, of added Ca^{2+} to CaBP, while the enhancement with Tb^{3+} was prominent and sigmoidal. The level of the fluorescence at the ratio, 1.0, of Tb^{3+} corresponds to that at the ratio, 2.0, of Ca^{2+}. The ANS fluorescence enhancement with Tb^{3+} is saturated at a ratio of about 2. Biphasic enhancement of the fluorescence was also observed with other lanthanide ions (La^{3+}, Nd^{3+}) (data not presented).

As shown in Figure 3B, in the presence of a saturating level of Ca^{2+} a linear enhancement of the ANS fluorescence intensity was found with an increase of Tb^{3+} up to a molar ratio of Tb^{3+} to CaBP of about 1, while in the presence of saturating Tb^{3+} the fluorescence was progressively quenched with an increase of Ca^{2+} up to a molar ratio of 1. It is noted that, in

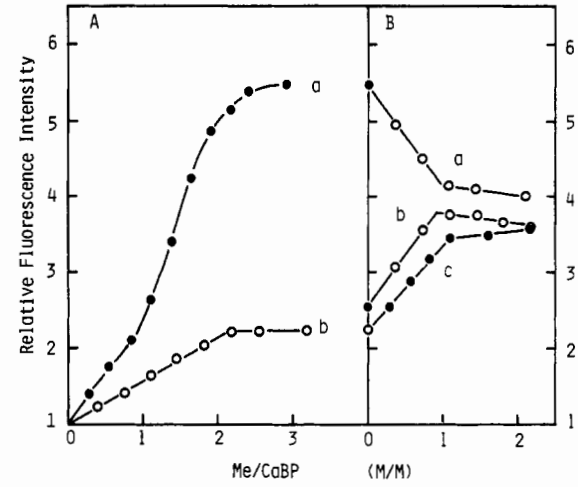

FIGURE 3: Fluorescent titration of ANS–CaBP complex with Ca^{2+} and Tb^{3+}. Protein and ANS concentrations added were 52.8 and 59.1 μM, respectively. Fluorescence intensity is presented as relative maximum fluorescence of metal-bound form to that of unbound form: metal is Ca^{2+} or Tb^{3+}. (A) Tb^{3+} (a) and Ca^{2+} (b) titration profiles. Added Ca^{2+} and Tb^{3+} were varied from 0.37 and 0.28 to 3.22 and 2.92, respectively, as Me/CaBP molar ratios. (B) Titration profiles with mixed metal ions, Ca^{2+} and Tb^{3+}. Curves a and b show the Ca^{2+} titration in the presence of fixed molar ratios of Tb^{3+}, 2 and 1, to CaBP, respectively; curve c shows the Tb^{3+} titration in the presence of Ca^{2+} at a ratio of 2.

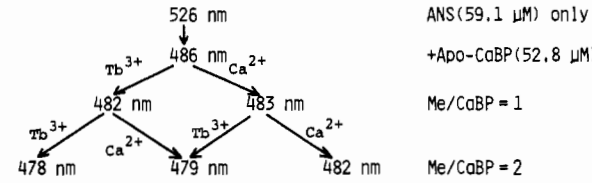

FIGURE 4: Effects of Ca^{2+} and Tb^{3+} on the emission maximum wavelength of the fluorescence of the ANS–CaBP complex. The experimental conditions are the same as in Figure 3. Each metal ion was added sequentially as indicated with arrows in the figure. Metal is Ca^{2+} or Tb^{3+}. All spectra were corrected for base-line fluorescence.

the presence of a half-saturating level of Tb^{3+}, Ca^{2+} could induce much higher enhancement of the fluorescence than excess Ca^{2+} alone (cf. Figure 3A, curve b).

Figure 4 summarizes the changes of the emission maximum of the fluorescence of the ANS–CaBP complex with Ca^{2+} and/or Tb^{3+} titration. The half-saturating level of Ca^{2+} and Tb^{3+} induced a blue shift of the emission maximum by about 3 and 4 nm, respectively, from that of the ANS–apo-CaBP system. When 1 mol each of Ca^{2+} and Tb^{3+} was simultaneously added to 1 mol of the ANS–apo-CaBP system, the blue shift of the wavelength was greater by about 3 nm than that induced with 2 mol of Ca^{2+} in the system, irrespective of the order of addition of the metal ions, comparable to that induced with 2 mol of Tb^{3+}. La^{3+} and Nd^{3+} showed a similar effect on the ANS fluorescence changes with Ca^{2+} as Tb^{3+} (data not shown).

UV Difference Spectra of CaBP with and without Ca^{2+} or Tb^{3+}. As shown in Figure 5A, the UV absorption of the CaBP decreased on binding of Tb^{3+} up to a half-saturation, a decrease corresponding to the change of a single tyrosine residue with 1 mol of Tb^{3+}/mol of the protein. With a 1.5 or greater molar ratio of Tb^{3+} to the protein, the absorption was progressively increased depending on Tb^{3+} concentration, even larger than that without Tb^{3+} in two regions of wavelength, shorter (below 250 nm) and longer (270–290 nm). On the other hand, the UV difference spectra corresponding to the change of phenylalanine residue(s) were negative over all the Tb^{3+} concentrations tested.

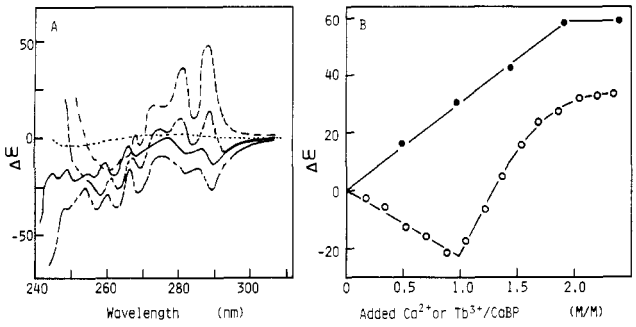

FIGURE 5: UV difference spectra of the CaBP before and after addition of Tb^{3+} or Ca^{2+}. (A) UV difference spectra with Tb^{3+}. Protein concentration was 392 μM. Tb^{3+} was added in the following molar ratios to the CaBP: 0.5 (—), 1 (- - -), 1.5 (- · - · -), or 2 (— —); (···) base line. (B) Titration profiles of the UV differences upon Ca^{2+} and Tb^{3+} bindings at 287 and 288 nm, respectively. Protein concentrations were 138 and 518 μM for Tb^{3+} and Ca^{2+} titrations, respectively. (●) Changes upon Ca^{2+} binding; (○) changes upon Tb^{3+} binding. Other conditions in the assay are described under Materials and Methods. Added Ca^{2+} and Tb^{3+} concentrations were varied from 0.48 and 0.19 to 2.38 and 2.34, respectively, molar ratio to the CaBP.

As previously reported by us (Chiba et al., 1983), the UV difference spectra before and after binding of Ca^{2+} to the CaBP are negative as for phenylalanine and positive as for tyrosine bands. Both the increase in extinction in the tyrosine band (Figure 5B) and the decrease around 240 nm (data not shown) were linear with increase of Ca^{2+} up to a saturating level.

Discussion

It was been found in this work that ANS is bound to the porcine intestinal CaBP (apoprotein) to result in stimulation of the fluorescence that can be further enhanced by Ca^{2+} binding to the complex proportionally to the amount of bound Ca^{2+}, maximal at saturation of binding (Figure 1). The molar ratio of the maximal binding of ANS to the protein is approximately unity (Table I), irrespective of the presence or absence of Ca^{2+}.

A conspicuous blue shift of the fluorescence maximum on binding of ANS to the apo-CaBP (Figures 1 and 4) may be due to either microenvironmental hydrophobicity of the binding site in the protein or just restriction in motion of the dye molecules on electrostatic binding (Johnson et al., 1979). Little effect of increase of ionic strength by addition of 100 mM KCl or NaCl to the CaBP solution on the enhancement of ANS fluorescence (data not shown) suggests a hydrophobic interaction of the dye with the protein. The large enhancement of the fluorescence of the ANS–CaBP complex by Ca^{2+} is not due to a change in the nature of the binding itself (the binding constants are not comparatively changed (Table I), but rather is thought to be due to a conformation change in the microenvironment around bound ANS molecules to restrict their structural flexibility and/or increase hydrophobicity.

It has been reported (LaPorte et al., 1980) that both ANS– and NPN (*N*-phenyl-1-naphthylamine)–calmodulin complexes increase greatly their fluorescence with blue shift on binding Ca^{2+}. But in our experiment the NPN–CaBP complex decreased its fluorescence on binding Ca^{2+} (data not shown). Because NPN lacks an anionic group, while ANS has a free sulfonic group, the ANS-binding site of the CaBP is considered to be specifically accessible to an anionic group in the presence of Ca^{2+}. These findings suggest the effective interaction between the CaBP and the cell membrane components of intestinal epithelium depending on the binding of Ca^{2+} to the protein (Freund & Borzemsky, 1977; Norman & Leathers, 1982; Wasserman et al., 1983) as the consequence of Ca^{2+}-induced conformation change of it, as suggested for the binding of calmodulin to calmodulin-dependent enzymes (LaPorte et al., 1980; Tanaka & Hidaka, 1980; Tanaka et al., 1984).

As shown in Figure 3A, the fluorescence of the ANS–CaBP complex is much more effectively enhanced by Tb^{3+} binding than by Ca^{2+} binding, and it increases cooperatively with increasing ratio of bound Tb^{3+} to protein above 1. This finding suggests a greater conformation change in the vicinity of the ANS-binding site by binding of Tb^{3+} to the N-terminal binding site (with lower affinity for Tb^{3+}) subsequently to the C-terminal site. The increase of the ANS fluorescence by Ca^{2+} binding to the N-terminal site was larger when Tb^{3+} is bound to the C-terminal binding site than when Ca^{2+} is (Figures 3B and 4). Binding of La^{3+} or Nd^{3+} also shows a similar cooperative effect on the fluorescence enhancement induced by subsequent addition of Ca^{2+} (unpublished data).

According to our preliminary experiments with the porcine CaBP, Tb^{3+} was greater in the affinity for the C-terminal binding site and in the effect on the bound ANS fluorescence than any of other cations like Ca^{2+}, Cd^{2+}, Sr^{2+}, and Ba^{2+} (unpublished data). Therefore, fitting the size of the binding site (i.e., approximately 1.1 Å as ionic radius) and charge density (2.75 for Tb^{3+} and 1.90 or less for other ions) of the ligand ion may be important for its potency in binding to the CaBP and resulting cooperative conformation change.

The conformation change induced by Tb^{3+} in the vicinity of a tyrosine residue in the CaBP has also been indicated to be different from that induced by Ca^{2+}. It is proposed that the single tyrosine residue becomes more and more buried in a hydrophobic domain monotonously with increase of bound Ca^{2+}, while the conformation of the microenvironment around that shows a transition at unity of the molar ratio of bound Tb^{3+} to the CaBP, noticeably increasing its hydrophobicity with high extents of Tb^{3+} binding (Figure 5B). A transition of the conformation at a binding ratio of 1 of Tb^{3+} to CaBP was also suggested by reversal at that point of the spectal profile of absorption at around 240 nm (Figure 5A). The absorption change near 240 nm is ascribable to ionization of the phenol group of the tyrosine residue in the protein (Tachibana & Murachi, 1966; Tan & Woodworth, 1969). It has also been reported that absorption increases at 237 nm when Tb^{3+} is bound to a carboxyl group (O'Neil et al., 1984; Charles & Reider, 1966). Therefore, it is inferred that Tb^{3+} binding to the extent of near saturation causes the conformation change of the CaBP that induces exposure of the tyrosine and ionization of the phenol group or exposure of carboxyl group(s) in the side chain of amino acid residues.

Vogel et al. (1985) have suggested interactions between the two binding sites of the porcine CaBP on the basis of experiments with ^{113}Cd NMR. Actually, the increase of the intrinsic fluorescence of the porcine CaBP stimulated by increasing concentration of Cd^{2+} has been proved by our experiment to be signoidal (data not shown).

In conclusion, the possible cooperation between the two binding sites has been confirmed in this paper by the two-state enhancing effect on Tb^{3+} binding on the fluorescence of ANS bound to the CaBP and by reversal of the absorption profiles around 240 nm of the protein in lower and higher binding ratios of Tb^{3+} to it.

Registry No. ANS, 82-76-8; calcium, 7440-70-2; terbium, 7440-27-9; tyrosine, 60-18-4.

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Effect of Growth Hormone on Protein Phosphorylation in Isolated Rat Hepatocytes

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Received February 14, 1986; Revised Manuscript Received October 20, 1986

ABSTRACT: Hepatocytes from male rats were incubated with [^{32}P]P_i for 40 min at 37 °C, thereby equilibrating the cellular ATP pool with ^{32}P. Subsequent exposure to bovine growth hormone for 10 additional min did not change the specific activity of cellular [γ-^{32}P]ATP. Two-dimensional gel electrophoresis or chromatofocusing followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used to fractionate phosphoproteins solubilized from control or hormone-stimulated cells. Stimulation of hepatocytes with 5 nM growth hormone for 10 min at 37 °C affected the phosphorylation of a number of proteins including an M_r 46 000 species of pI 4.7 whose phosphorylation was augmented (2.65 ± 0.50)-fold. A significant fraction of the maximal effect of growth hormone on phosphorylation of the M_r 46 000 species was elicited by 1–5% receptor occupancy. Bovine growth hormone, which binds to somatogenic receptors with great specificity, or recombinant human growth hormone, which is not contaminated with other hormones, affected phosphorylation of hepatic proteins similarly. The M_r 46 000 phosphoprotein was isolated in a fraction enriched in cytosol after centrifugation of cellular homogenates. Phosphorylation of the M_r 46 000 phosphoprotein was also increased (1.75 ± 0.35)-fold and (2.15 ± 0.50)-fold by insulin and glucagon, respectively. These observations are consistent with the possibility that selective changes in the phosphorylation state of cellular proteins may mediate growth hormone actions in cells.

The first step leading to growth hormone action is binding to receptors, which have been identified in a number of different cells and membranes (Kelly et al., 1974; Lesniak et al., 1974; Posner, 1976; Fagin et al., 1980), including hepatocytes isolated from rats (Ranke et al., 1976; Donner et al., 1978a–c, 1980; Donner, 1980, 1983; Yamada & Donner, 1984). The growth hormone receptor has been affinity labeled (Donner, 1983; Hughes et al., 1983; Yamada & Donner, 1984; Carter-Su et al., 1984; Gorin & Goodman, 1984) and, in the hepatocyte, is a moiety of 300 000 daltons which contains a binding subunit of M_r 100 000 (Donner, 1983; Yamada &

* Correspondence should be addressed to this author at the Memorial Sloan-Kettering Cancer Center. He is the recipient of Research Career Development Award AM 01045 and Grant AM 30788 from the NIH.
‡ Recipient of National Research Service Award AM 07112 from the NIH.

0006-2960/87/0426-0715$01.50/0