MOLECULAR PHARMACOLOGY, 22:105–108

Cation-Induced Conformational Change in Glucagon

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Received January 11, 1982; Accepted March 1, 1982

SUMMARY

Glucagon can bind to terbium, resulting in a 1000-fold enhancement of terbium fluorescence. This process is critically dependent on pH and apparently requires that the NH_2-terminal histidine residue of glucagon be in an unprotonated form. The terbium ion can be displaced by zinc but not readily by calcium. The binding of zinc or terbium to glucagon induces a large conformational change in the peptide resulting in a shift in the fluorescence emission maximum of glucagon from 352 to 339 mm and in a marked increase in the helix content of the peptide. Similar conformational changes occur with this peptide upon self-association or upon binding to lipids.

INTRODUCTION

Cations are known to bind to many peptides and proteins. Glucagon, a 29-amino acid polypeptide hormone, has several functional groups which could play a role in cation binding, including four carboxyl groups, an imidazole group, and an α-amino group. In addition, several biological effects of cations could be related to their ability to bind directly to glucagon. For example, $CoCl_2$ has been shown to have a relatively specific cytotoxic effect on the glucagon-producing α-cells of the pancreas (1). Both Co(II) and Ni(II) have been shown to give rise to acute hyperglycemic responses which are thought to result from hyperglucagonemia (2, 3), again making an association between metals and glucagon storage. Insulin is known to bind Zn(II) (4), and a role for this interaction in the storage of insulin has also been suggested (5). The zinc content of the α-cells of the pancreas is similar to that of the β-cells (6). It is thus possible that zinc could also play a role in glucagon storage. We have therefore investigated the possibility that glucagon, like insulin, can also form stable complexes with certain cations. We have found that such interactions can occur and that they initiate a large conformational change in the glucagon molecule.

EXPERIMENTAL PROCEDURES

Materials

$TbCl_3 \cdot 6\ H_2O$, gold label grade, was obtained from Aldrich Chemical Company (Milwaukee, Wisc.), and crystalline glucagon was purchased from Elanco Products Company (Indianapolis, Ind.). Both materials were used without further purification. The water was deionized and distilled. All other chemicals were reagent-grade. Unless otherwise indicated, the buffer used in this study was 0.01 M Hepes[1] (Ultrol grade; Calbiochem, San Diego, Calif.), which had been adjusted to pH 6.90 with KOH.

Methods

Fluorescence spectroscopy. Fluorescence emission spectra were measured using a Perkin-Elmer MPF-44 spectrofluorimeter in the ratio mode with slit widths of 4 nm. With measurements of terbium fluorescence a 390-nm cutoff filter was used. The temperature of the sample was maintained at 25° with a thermostated cell holder.

Circular dichroism. CD spectra were measured using a Cary 61 spectrometer with the slit width programmed to give a constant spectral bandwidth of 2 nm. The temperature of the sample was maintained at 25° with a thermostated cell holder. A 2-mm pathlength cell was used with solutions containing about 0.1 mg of peptide per milliliter. The concentration of glucagon was determined spectrophotometrically using 2.38 as the absorbance of solution containing 1 mg/ml. The CD data were expressed as the mean residue ellipticity $[\theta]$ using 120.2 as the calculated mean residue weight of glucagon.

RESULTS

The addition of aliquots of a solution of $TbCl_3$ to a solution of glucagon in 10^{-2} M Hepes buffer (pH 6.90) resulted in the appearance of a typical terbium fluorescence emission spectrum with emission maxima at 490, 545, 588, and 622 nm using an excitation wavelength of 280 nm. The emission band at 545 nm was the most intense by a factor of about 2. The intensity of the fluorescence emission was saturable, reaching a half-maximal value at about 0.3 mM terbium. The observed terbium titration curves (Fig. 1) could not be simply analyzed in terms of a single equilibrium binding process. It is possible that several terbium glucagon complexes were

This research was supported by Grant A9848 from the National Scientific and Engineering Council of Canada.

[1] The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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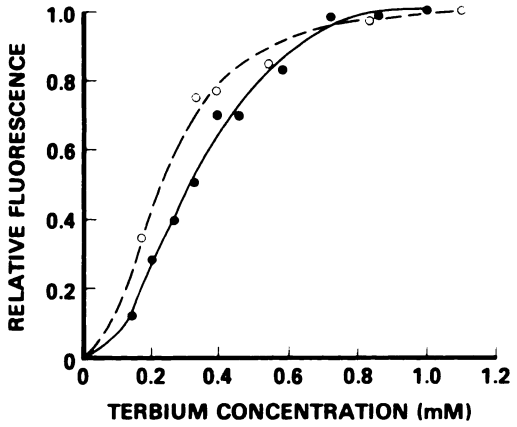

FIG. 1. *Effect of terbium concentration on the intensity of fluorescence emission at 545 nm from a solution of 6 μM (●) or 40 μM (○) glucagon in 0.01 M Hepes buffer (pH 6.90)*

Excitation wavelength was 280 nm; temperature was 25°.

formed. In the absence of glucagon, the intensity of the fluorescence emission from terbium was very weak. Assuming that one terbium ion was bound per glucagon monomer, the increase in the intensity of terbium emission caused by glucagon was about 1000-fold. When the excitation wavelength was raised from 280 nm to 295 or 300 nm, the emission intensity from the terbium-glucagon complex at 545 nm decreased to 64% or 40% of its initial value (uncorrected), respectively, indicating that at least a portion of the energy transferred to terbium came from tryptophan as a donor, since this was the only chromaphore in this system that absorbed light above 295 nm.

The ability of glucagon to enhance the fluorescence emission from terbium was highly dependent on pH (Fig. 2). The titration was not carried above pH 7.1 because of the appearance of a precipitate of terbium hydroxide. It appeared that a group with pK of about 6.3 must be in an unprotonated form for glucagon to enhance the fluorescence of terbium. However, the pH dependence of the fluorescence seemed somewhat more cooperative than a single titration curve (Fig. 2). The only ionizable groups on glucagon are the α-NH_2 group and the imidazole group, with pK values of 7.3 and 5.3, respectively (7–9). It is likely that for terbium binding both of these groups must be in an unprotonated form and that the pK of the α-amino group is shifted to a lower value by terbium binding.

Terbium could be displaced from its complex with glucagon by other cations. Zinc was particularly effective and appeared to have affinity comparable to that of terbium (Fig. 3). The addition of Mn(II) also resulted in a decrease in the intensity of terbium fluorescence, comparable to the effects of Zn(II). However, manganese did not induce a shift in the fluorescence emission of glucagon, and part of its quenching ability could result from collisional quenching since Mn(II) is a paramagnetic ion. In contrast, Ca(II) was only moderately effective in displacing terbium whereas Na(I) and K(I) were very weakly active in this regard (Fig. 3).

Cations were also capable of inducing a large conformational change in glucagon. The fluorescence emission spectrum of glucagon, which was dominated by tryptophan emission, shifted its maximum from 352 to 339 nm in the presence of Tb(III) in 0.01 M Hepes buffer (pH 6.90). As with the enhancement of terbium fluorescence (Fig. 2), the shift in tryptophan emission was also pH-dependent and appeared to require that the histidine residue be in unprotonated state (Fig. 2). No significance was placed on the shape of this curve since the shifts in emission maxima from one point to another were relatively small and sometimes within the experimental error of ±1 nm, although the over-all change from pH 4.9 to 6.9 was quite large and significant.

Zinc, which we have shown to be capable of displacing terbium from the glucagon complex (Fig. 3), also induced a shift in the fluorescence emission maximum of glucagon. A solution of 20 μM glucagon in 0.01 M Hepes buffer (pH 6.9) had an emission maximum at 352 nm which was shifted to 340 nm in the presence of 1 mM $ZnCl_2$. No effect on the fluorescence properties of glucagon was observed upon the addition of 1 mM NaCl, KCl, $CaCl_2$, or $MnCl_2$.

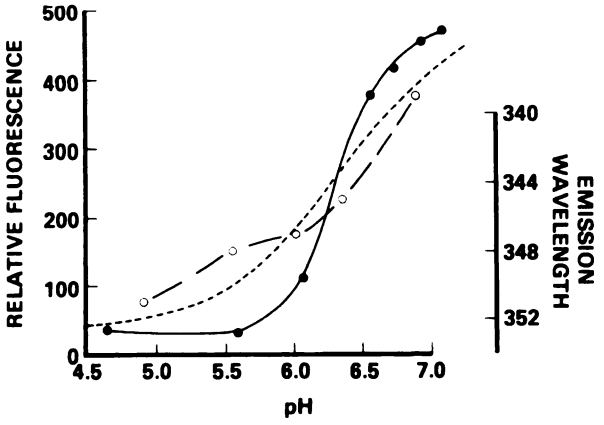

FIG. 2. *Effect of pH on the intensity of fluorescence emission at 545 nm (●) and on the wavelength of maximal emission from glucagon (○) of a solution of 1 mM $TbCl_3$ and 10 μM glucagon in 0.01 M Hepes buffer*

Excitation wavelength was 280 nm; temperature was 25°. - - -, The theoretical titration curve for a single pK of 6.325.

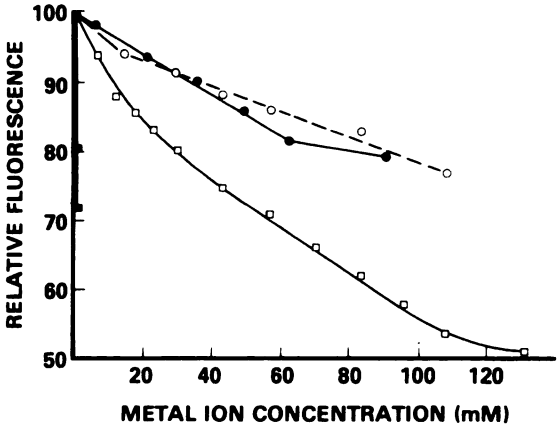

FIG. 3. *Effect of the addition of other salts on the intensity of fluorescence emission at 545 nm from a solution of 1 mM $TbCl_3$ and 30 μM glucagon in 0.01 M Hepes buffer (pH 6.90)*

Excitation wavelength was 280 nm; temperature was 25°. ■, $ZnCl_2$; □, $CaCl_2$; ○, KCl; ●, NaCl.

In addition to the effect of certain cations on the fluorescence properties of glucagon, these cations also affected a large change in the CD spectra of the peptide. In the presence of 1 mM terbium a marked increase in the magnitude of the ellipticity was observed in the far UV spectrum, with a band forming at 222 nm, indicative of the generation of helical structures (Fig. 4). In contrast, Ca(II) had only a weak effect on the observed CD (Fig. 4).

DISCUSSION

Terbium has been used as a fluorescent probe for studying cation binding to proteins and peptides (10–12). A marked enhancement, by as much as a factor of 10^5, of the terbium fluorescence is observed in some systems, probably as a result of energy transfer from tyrosine and tryptophan residues. Terbium has been especially useful as a probe for calcium binding sites in biological systems (13). We have shown that the peptide hormone glucagon can also bind to terbium and enhance its fluorescence emission by 1000-fold.

The pH dependence of the glucagon-promoted terbium fluorescence (Fig. 2) suggests that the NH_2-terminal histidine residue must be unprotonated for terbium to bind to glucagon. Zn(II), which, as with insulin (14), is also likely to be coordinated to a histidine residue, can also displace terbium from glucagon. It is possible that the state of the histidine residue is critical in effecting a conformational change required for terbium binding or that the histidine residue (probably along with carboxyl groups) is directly coordinated to terbium. In addition, there may also be a pH-dependent association of complexes, probably through hydroxide linkages (15). Studies are currently in progress to investigate this question further. The ability of glucagon to acquire an enhanced helical content, similar to what is found in the self-associated form of glucagon (16), suggests that the terbium-glucagon complex may contain several glucagon molecules as well as more than one terbium ion. It is possible that under most conditions a mixture of cation-glucagon complexes is formed, similar to the situation with insulin (4).

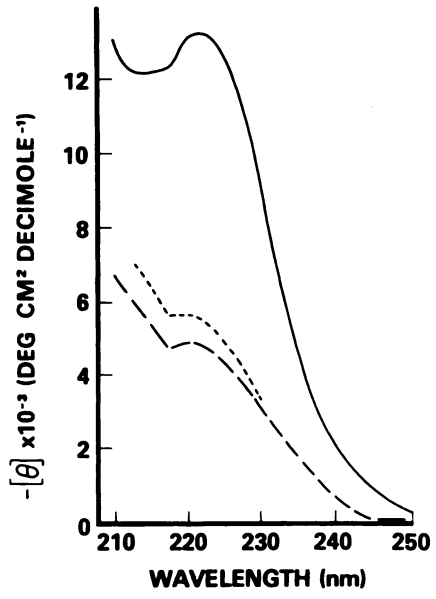

FIG. 4. *CD specta of 30 μM glucagon in 0.01 M Hepes buffer (pH 6.90) (— —) and with the addition of 100 mM $CaCl_2$ (- - -) or 1 mM $TbCl_2$ (— —), 25°*

The conformational change induced by millimolar concentrations of terbium is marked (Fig. 4). Similar increases in the helical content of glucagon are also observed in detergent-bound (17, 18) and lipid-bound (19) forms of the hormone as well as in the self-associated state (16). High concentrations of NaCl, in the range of several molar, can also induce some increase in the helical content of glucagon (18). In the case of the terbium-induced conformational change, the tryptophan fluorescence emission is shifted to lower wavelengths, suggesting that this residue becomes incorporated into a more hydrophobic environment in the presence of the cation. The tryptophan emission maximum for the terbium-glucagon complex is similar to that for glucagon incorporated into a phospholipid (20). Since the glucagon monomer contains only 29 amino acids it is not likely to be able to fold into a conformation in which the interior has reduced contact with the aqueous solvent. Thus, the shift in the tryptophan fluorescence also suggests the formation of glucagon oligomers in the terbium complex.

As with the zinc-insulin complex, the biological role of cation-glucagon interactions is uncertain. In connection with the possibile role of cations in the action of insulin or glucagon, it is interesting to note the observed importance of Cr(III) in the diet in connection with glucagon tolerance (21). The most likely function of the association of cations with insulin and glucagon is in connection with the storage of these hormones.

ACKNOWLEDGMENT

We wish to thank Dr. Evert Nieboer of the Department of Biochemistry, McMaster University, for his interest and helpful discussions.

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