INTERACTION OF METAL IONS WITH GASTROINTESTINAL HORMONES

Binding of Ca^{2+} to Nle^{11}-minigastrin I*

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1. Introduction

The biological action of the gastrin hormones is confined to the C-terminal tetrapeptide amide Trp–Met–Asp–Phe–NH_2 [2–5]. The physiological role of the remaining peptide sequences is, however, still obscure, as no clear evidence for a specific function has been found. Among the various possibilities the –$(Glu)_5$– sequence common to big gastrin, little gastrin and minigastrin could be suited for the binding of metal ions such as calcium or magnesium.

To test this hypothesis we studied the interaction of Nle^{11}-minigastrin I (Nle^{11}-HG-13) with Ca^{2+}. The investigation was carried out in trifluoroethanol as the solvent, which mimics the lipophilic environment of a membrane [6], where the binding process could occur.

2. Materials and methods

The synthesis and characterization of the peptides used here will be reported in [7]. Reagent grade $CaCl_2 \cdot 2\ H_2O$ and $CaClO_4 \cdot 4\ H_2O$ were purchased from Fluka AG, Buchs; Uvasol 2,2,2-Trifluoroethanol (TFE) was obtained from Merck AG, Darmstadt.

Solutions of Ca^{2+} were prepared by dissolving known amounts of $CaCl_2$ or perchlorate in TFE. The metal ion concentration was precisely determined by atomic absorption measurements. The hormone concentrations, ranging from 2×10^{-5}–7.6×10^{-7} M, were determined by the weight and peptide content of the samples and/or by absorption measurements in the near UV. CD measurements were performed at 25°C using a Mark III Jobin-Yvon Dichrograph. In the spectra reported here, $[\theta]_R$ represents the ellipticity value/mol peptide residues. UV measurements were made with a Carry 118C recording spectrometer. Atomic absorption data were obtained using a Perkin Elmer 360 AA spectrometer.

Of the experimental methods reported for the characterization of metal ion interactions with peptides, only CD measurements were suitable here. In fact the fluorescent probes Tyr and Trp present in Nle^{11}-HG-13 in the positions 8 and 10, respectively, were not sensitive enough to the binding process; NMR investigations were prevented by precipitation of the Ca-adducts at high ($\geqslant 10^{-2}$ M) hormone levels; equilibrium dialysis and ion-sensitive electrodes could not be employed due to the low M_r of Nle^{11}-HG-13 (1628) and/or to the nature of the solvent.

3. Results and discussion

Upon addition of Ca^{2+} to a dilute (2×10^{-5} M) solution of Nle^{11}-HG-13 in TFE a dramatic change in the CD pattern of the hormone is observed (fig.1). The amount of metal ion required to fully perform the conformational transition is rather low and related to the hormone concentration. Therefore, Ca^{2+} strongly interact with the Nle^{11}-HG-13 molecule. The CD pattern of the Ca-bound peptide is characterized by a minimum absorption at 195 nm and by a negative

* According to the revised structure [1] for the natural hormone, the correct name for the tridecapeptide amide H–Leu–$(Glu)_5$–Ala–Tyr–Gly–Trp–Nle–Asp–Phe–NH_2 is Des-Trp^1–Nle^{12}-minigastrin I

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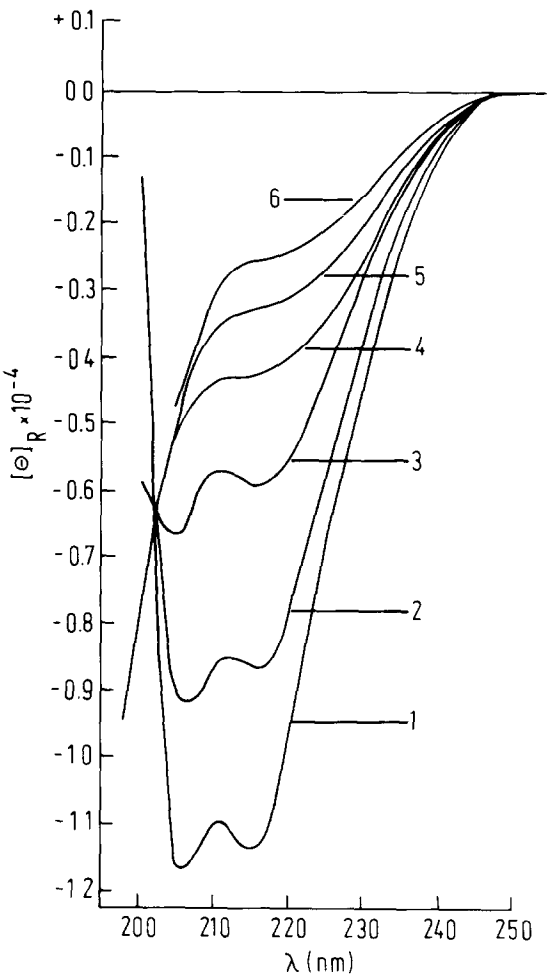

Fig.1. CD spectra of Nle[11]-HG-13 in TFE at different Ca/peptide ratios (R): (1) $R = 0$; (2) $R = 0.5$; (3) $R = 1.0$; (4) $R = 1.5$; (5) $R = 2.2$; (6) $R = 4/6$. Peptide was 2.1×10^{-5} M.

shoulder at 215 nm with molar ellipticities/residue of −10 900 and −2300, respectively. These data indicate that coordination to the metal ion forces Nle[11]-HG-13 into an essentially disordered conformation. The results obtained at 3 different hormone levels (over 2×10^{-5}–8×10^{-7} M) are summarized in fig.2, where the relative change in circular dichroism (θ_R/θ_∞) is reported as a function of the Ca/peptide ratio (R). It clearly appears that 2 Ca^{2+} can bind to Nle[11]-HG-13 under our experimental conditions. If further binding occurs after the structural change, it cannot obviously be detected by CD measurements.

Due to the low hormone concentration we used, it is reasonable to assume that no intermolecular gastrin–Ca–gastrin interactions occur, so that 1 Nle[11]-HG-13

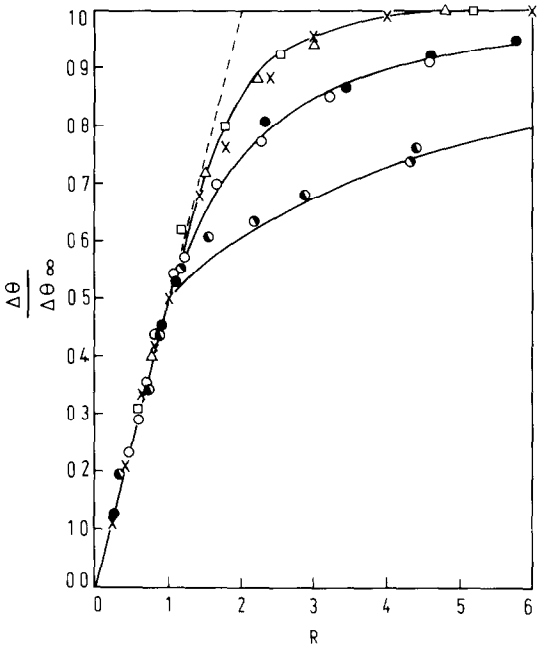

Fig.2. Binding curves for the system Ca–Nle[11]-HG-13 in TFE at different hormone levels: (□,△,×) 2.1×10^{-5} M; (○,●) 4.2×10^{-6} M; (◐,◑) 7.6×10^{-7} M. Solid lines are the theoretical binding curves with $K_1 = \infty$ and $K_2 = 4.3 \times 10^5$ at the above hormone levels.

molecule/complex unit can be considered.

From fig.2 it also appears that the conformational change is directly related to the binding process, as the relative CD modification at a given wavelength is proportional to the amount of added calcium at sufficiently high gastrin concentrations.

About 50% of the spectral change therefore can be attributed to the binding of the first metal ion, and the remaining 50% to the binding of the second Ca^{2+}. While no complex dissociation takes place by lowering the hormone concentration by a factor of 25 when $R = 1$ (all experimental points fall on the same straight line), different curves are obtained as a function of the peptide concentration at $R > 1$. As a consequence the affinity constant for the first binding defined as:

$$K_1 = \frac{[\text{Nle}^{11}\text{-HG-13–Ca}]}{[\text{Nle}^{11}\text{-HG-13}] \cdot [\text{Ca}^{2+}]}$$

will be substantially higher than the corresponding constant for the second binding defined as:

$$K_2 = \frac{[\text{Nle}^{11}\text{-HG-13–Ca}_2]}{[\text{Nle}^{11}\text{-HG-13–Ca}] \cdot [\text{Ca}^{2+}]}$$

A precise value for K_1 cannot be inferred from our data, but it should exceed 5×10^8, as no detectable dissociation occurred at $\sim 8 \times 10^{-7}$ M complex.

For the second binding constant we obtained $K_2 = 4.3 \pm 0.3 \times 10^5$, using the graphical methods in [8]. The high values of K_1 and K_2 suggest a specific interaction between Ca^{2+} and the hormone analogue. The remarkable differences in the two constants either reflect the existence of intrinsically different binding sites or a negative cooperativity between identical sites.

The question now arises on what is the nature of the ligand groups in Nle[11]-HG-13. Since Ca^{2+} generally binds to oxygen atoms, the possibilities are restricted to two candidates: the carboxylate groups of the Glu and/or Asp residues and the carbonyl moieties of the peptide group.

To shed light on this problem we investigated the binding properties of Ca^{2+} to a sample of Nle[11]-HG-13 in which all Glu and Asp residues are protected by *t*-butyl groups (Boc-Leu–[Glu(OBu^t)]$_5$–Ala–Tyr(Bu^t)–Gly–Trp–Nle–Asp(OBu^t)–Phe-NH_2). The CD spectrum of this peptide in TFE closely resembles that of the corresponding deblocked hormone (fig.3(1)), so that the presence of the *t*-butyl groups does not appear to substantially affect the conformational properties of Nle[11]-HG-13. The metal ion clearly interacts with the peptide, in spite of the absence of free carboxylate groups (fig.3). However, in this case we obtain evidence of specific binding for only 1 metal ion. The conclusion can be drawn that the presence of negatively charged Glu and/or Asp residues is essential for the binding of at least 1 Ca^{2+} to deprotected Nle[11]-HG-13. On the other hand involvement of peptide oxygens in the binding process is suggested by the dramatic conformational change to a disordered structure caused by Ca^{2+} addition to the hormone (which would not be expected if only sidechain groups were bound to the metal ion), and by the data on the crystal structure of several Ca–proteins in which at least one carbonyl moiety from the peptide group is found in the coordination sphere of calcium [9].

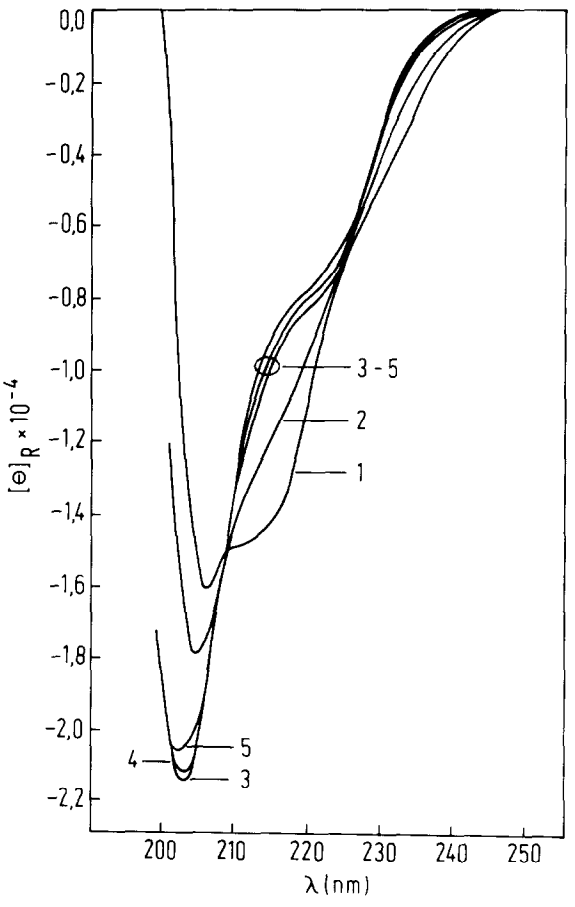

Fig.3. CD spectra of *t*-butylated BOC-Nle[11]-HG-13 in TFE at different Ca/peptide ratios (R): (1) $R = 0$; (2) $R = 0.5$; (3) $R = 1.0$; (4) $R = 1.5$; (5) $R = 2.0$. Peptide was 2.46×10^{-5} M.

Preliminary results indicate that also Mg^{2+} strongly interact with Nle[11]-HG-13. Further studies on the binding properties of our peptide hormones with alkaline earth and other metal ions are in progress.

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