Comparative Study of Human and Salmon Calcitonin Secondary Structure in Solutions with Low Dielectric Constants*

(Received for publication, October 12, 1992)

Tudor Arvintes§ and Alex F. Drake†

From ‡Ciba-Geigy Pharmaceuticals, Horsham RH12 4AB, United Kingdom and the †Department of Chemistry, Birkbeck College, University of London, London WC1H 0AJ, United Kingdom

Molecular conformations of salmon (sCT) and human (hCT) calcitonin in media with different concentrations of methanol/water and trifluoroethanol/water have been investigated by fluorescence, circular dichroism (CD) and infrared spectroscopy techniques. In these media, sCT and hCT adopt an α-helical structure comprising up to 40–48% of the amino acids. CD experiments reveal that for both peptides, the ordering of the Cys1-Cys7 disulfide link and the α-helix formation can be distinguished. Disulfide bond ordering is similar in both calcitonins. sCT adopts the α-helical structure more readily than hCT, respectively. Tyrosine fluorescence measurements correlate with the far ultraviolet CD changes associated with the peptide backbone. hCT is seen to adopt a left-handed, extended conformation in aqueous media below −50 °C.

Calcitonin (CT)1 is a peptide hormone produced by the parafollicular cells of the thyroid gland in mammals and by the ultimobranchial gland of birds and fish. Secreted in response to increased serum calcium levels, it has a regulatory function in calcium-phosphorus metabolism (Austin and Heath, 1981). CT receptors are widely spread in the body (i.e. brain, Guidabono et al. (1987); lung, Foucheur-Peron et al. (1981); testis, Chausmer et al. (1980); placenta, Nicholson et al. (1988); cancer cells, Moran et al. (1978); lymphocytes, Body et al. (1990)), with the highest densities of CT receptors being present in bone cells (Warshawsky et al., 1980; Nicholson et al., 1986) and in the kidney (Sexton et al., 1987).

Osteoclast-mediated bone resorption is inhibited by CT (Austin and Heath, 1981) through the regulation of both the number and activity of osteoclasts (Macintyre, 1986). Therefore, CT is a potent drug for various bone metabolism diseases such as Paget’s disease (Avramides et al., 1974), hypercalceemia (Hosking and Heller, 1986), and osteoporosis (Wallach et al., 1977). Calcitonins of different origins, mainly salmon, pig, eel, and human, are currently used therapeutically.

CT consists of 32 amino acids in length, having an amino-terminal 1–7 disulfide bridge and a prolineamide at the carboxyl terminus, at position 32 (Scheme I) (Macintyre, 1989; Azria, 1989).

Fish calcitonins are more potent than mammalian calcitonins in reducing calcium concentration in the blood stream of mammals, i.e. salmon calcitonin (sCT) is 1 order of magnitude more potent than human calcitonin (hCT) (Findlay et al., 1983). Structure-activity studies suggested that high hypocalceemic activity of CT can be associated with the ability of the molecule to solubilize lipids and to adopt an amphipatic α-helix (Epand, 1983; Epand et al., 1983; Moe et al., 1983; Moe and Kaiser, 1985) and with conformational flexibility in aqueous media (Epand et al., 1985, 1986). Circular dichroism (CD) studies have shown that sCT and hCT have little ordered secondary structure in water at room temperature, whereas only sCT exhibits significant α-helical structure in the presence of dimyristoylphosphatidylglycerol or sodium dodecyl sulfate (Epand et al., 1983). To gain information on CT conformation in the presence of membrane-bound receptors, studies have been reported characterizing calcitonins in different structure-promoting solvents. sCT has been shown to adopt an α-helix in the presence of methanol (MeOH) (Orlowski et al., 1987; Meadows, et al., 1991), trifluoroethanol (TFE) (Moe et al., 1983), and sodium dodecyl sulfate (Motta et al., 1991a). In a 14 mol % (v/v) TFE/H2O mixture, the 9–22 residue region of hCT is also seen to adopt a helical conformation (Doi et al., 1989). In 90% dimethyl sulfoxide, 10% H2O, short, intramolecular, antiparallel, double-stranded β-sheets over the residues 16–21 in hCT and 12–18 in sCT are formed, with an isolated β-turn at 3–6 and 28–31 for hCT and 6–9 and 25–28 for sCT (Motta et al., 1989, 1991b).

In the present study, we compare the conformational changes induced in the secondary structures of hCT and sCT by media with low dielectric constants. CD and fluorescence measurements showed that in MeOH and TFE, the folded forms of hCT and sCT are similar. The data indicate that the conformational changes consist of the ordering of the cysteine disulfide bridge and the formation of the α-helix. Although the disulfide ordering is similar, sCT adopts the α-helix more readily than hCT. In TFE, the α-helix formation involves a two-step folding process for both calcitonins.

EXPERIMENTAL PROCEDURES

Materials—Synthetic hCT was obtained from Ciba-Geigy Pharmaceuticals, Basel, Switzerland. Synthetic sCT was purchased from NovaBiochem, Switzerland. TFE (puriss., >99%) was from Fluka Chemie AG, Switzerland. All other chemicals were Analar grade.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Ciba-Geigy Pharmaceuticals, Wimblehurst Rd., Horsham RH12 4AB, UK. Tel.: 44-403-272827 or 44-403-323711; Fax: 44-403-323253.

1 The abbreviations used are: CT, calcitonin; CD, circular dichroism; hCT, human calcitonin; sCT, salmon calcitonin; MCD, magnetic circular dichroism; MeOH, methanol; TFE, 2,2,2-trifluoroethanol.
Scheme 1. Amino acid sequence of hCT and sCT.

Peptide Concentration—CT concentration was determined from its weight concentration or from its ultraviolet absorption spectrum. Absorption coefficients at 275 nm of 1531 and 1515 cm⁻¹ M⁻¹ were used for hCT and sCT, respectively (Epand et al., 1983).

Titration Experiments—Samples for the titration experiments were prepared by mixing two calcitonin solutions, one in water and one in a low dielectric constant solvent (MeOH or TFE). The conversion from volume % to mole % of a mixture of two solvents 1 and 2 was calculated by using the following equation: (mol %)₁ = (k₁/(k₁ - 1 + 100% vol %₁)) × 100, where k is the constant (d₁ × (mol %₁))/(d₂ × (mol %₂)). In this equation, (mol %), and (vol %), are the mole % and volume % of solution 1, i.e. ethanol or TFE, in mixtures with solution 2, in our case water; d₁, d₂ are the densities at the temperature where the experiment was performed, and (mw)₁ and (mw)₂ are the molecular weights of solvents 1 and 2, respectively. For the MeOH/water and TFE/water mixtures, k(Me0H) = 0.445 and k(TFE) = 0.25, at 20°C.

Absorption Measurements—All absorption spectra were recorded using a dual-beam Perkin-Elmer Lambda 5 spectrometer at 20°C.

Fluorescence Measurements—Fluorescence emission spectra were obtained with a Perkin-Elmer LS 5 spectrophotometer at 20°C. The tyrosine fluorescence of CT was excited at 270 nm, and the emission was monitored between 280 and 400 nm.

Circular Dichroism—CD spectra were obtained using a JASCO J810 spectropolarimeter equipped with a thermostatted cell holder. Peptide concentrations (per residue) of 0.1–0.2 and 1–2 mM were used for the wavelength regions of 190–250 and 250–350 nm, respectively. Estimates of peptide secondary structure from CD were performed using the standard CONTIN program (190–240 nm) (Provencher and Glöckner, 1981; Provencher, 1982).

Magnetic circular dichroism (MCD) was measured using a JASCO J440 CD spectropolarimeter equipped with a JASCO MCD-1 electromagnet operated at 1.0 tesla. MCD spectra are reported in terms of ΔA₉₀₀/tesla, corrected for the corresponding natural CD observed with zero magnetic field strength.

Infrared Spectroscopy—Fourier transform infrared spectra were obtained with a Perkin-Elmer 1750 infrared Fourier transform spectrometer at 20°C. Typically, the spectra were averaged over 20 scans. Peptide concentration in the Fourier transform infrared measurements was 20 mg/ml; no aggregation was observed in the solvent conditions used during the measurements.

In all spectroscopic investigations, reference spectra were recorded under identical conditions with only the media in which the peptides were dissolved in the cells; these spectra were subtracted from the corresponding peptide spectra.

RESULTS

Effects of Solvent Polarity on hCT and sCT Fluorescence and CD Spectra—The fluorescence properties of tryptophan and tyrosine are often used to study the conformation, environmental properties, and interaction of proteins containing these amino acids (Longworth, 1971; Cowgill, 1976; Lakowicz, 1983). Both calcitonins contain 1 tyrosine amino acid in their sequence: Tyr² in hCT and Tyr²⁵ in sCT. For both hCT and sCT, an increase in intrinsic Tyr fluorescence was observed as the polarity of the solvent was decreased (Fig. 1). Polarity-induced changes in hCT and sCT fluorescence intensity were not accompanied by changes in emission maxima, except for a blue shift in the Tyr emission maximum from 304 to 298 nm for both hCT and sCT in very high TFE concentrations (data not shown).

Parallel CD experiments with the two calcitonins revealed that a high α-helical content is induced in low dielectric solvents (Figs. 2, 3, and 8).

hCT and sCT Conformational Changes Induced by TFE/Water Mixtures—Increasing TFE concentration induced CD spectral changes in the far ultraviolet, peptide backbone (190–250 nm) region and in the near ultraviolet (250–320 nm) region, as illustrated in Fig. 3. Circular dichroism reveals (Figs. 3, A and C, and 4A) that the peptide backbone folding involves two steps, with sCT folding more readily than hCT. The first backbone folding step is completed by about 6 mol % TFE/H₂O for sCT and 11 mol % TFE/H₂O for hCT (Fig. 4A). The second folding step is completed at 100% TFE and represents 50%, in the case of hCT, and 25%, in the case of sCT, of the whole CD conformational changes at 220 nm in going from water to pure TFE (Fig. 4A).

The assignment of near UV circular dichroism spectra of hCT and sCT required further investigations. The CD in the range 250–320 nm (Fig. 3B) for hCT dissolved in 100% TFE exhibits a negative tail extending from 290 to 320 nm, a negative maximum at 278 nm, fine structure around 290 nm, and a positive peak at 252 nm. Assignment of these features was obtained using MCD, the dichroism induced by a magnetic field superimposed on a natural dichroism. The aromatic function of tyrosine (and tryptophan) are known to present characteristic, relatively large, MCD signals and can therefore be readily assigned (Holmquist and Vallee, 1978). Fig. 5
For hCT, increases in the CD intensity in the 185-260 nm (0.25 mg/ml sCT) region and the 245-320 nm (2.5 mg/ml hCT) region. For sCT, spectra were obtained at 220 nm (U, 1.1, 2, 3, 4, 5, 6.4, 7.7, 10.22% (only 290 nm), 33.4, 42.9, 60, and 100% in the 185-260 nm (0.4 mg/ml hCT) region and the CD in the backbone region. The tyrosine MCD absorption was characterized by a peak at 278 nm and was the same in water and in the cryogenic solvent ethanediol/water (Fig. 9A), leading to similar final states in both cases. The disulfide CD components are methanol-sensitive; Fig. 9B shows no significant differences between the two peptides. Similar ordering of the disulfide bridge is therefore inferred in the folded states for both hCT and sCT.

Fluorescence changes associated with increasing methanol concentrations are shown in Fig. 10; hCT and sCT can be distinguished. As in the case of TFE titration (Fig. 7), there is a linearity between the fluorescence measurements (F/F0) and the CD in the backbone region (∆ε/Δε0) for both hCT and sCT (Fig. 11).

Temperature Dependence CD Studies—The temperature dependence of CD was studied in water between 80 and 10 °C and in the cryogenic solvent ethanediol/water (2:1) (Drake et al., 1988) in the range −85 to +25 °C (Fig. 12). An increase in temperature from 10 to 80 °C induces a monotonic small increase in negative CD at 220 nm for both hCT and sCT (Fig. 12). With decreasing temperature, the CD spectrum of hCT shows the occurrence of a positive dichroism at −85 °C (Fig. 13), indicating a left-handed helical structure of hCT at −85 °C. Spectra similar to that of hCT at −85 °C were shown to correspond to a non-hydrogen-bonded extended helix, known as polyproline II or 3, helix (Drake et al., 1988). The left-handed helix structure could not be seen with sCT, since attempts to go below 0 °C produced the α-helix conformation (spectra not shown) (Fig. 12).

Secondary Structure Estimations from CD Data—In water, neither hCT nor sCT has significant detectable ordered structure. The CD data associated with the solvents of low dielectric constants have been run in the CONTIN protein secondary structure analysis program (Provencher and Glöckner, 1981). The calculated α-helix contents (Table I) agree well
Fig. 5. MCD spectra of hCT (dashed line) in water (A) and in 100% TFE (B). The dashed lines represent the CD spectra in the absence of the magnetic field (abscissa units $\Delta A$). hCT concentration was 1.15 mg/ml. MCD spectra have been inverted for clarity.

Fig. 6. Effect of TFE/water concentration on the hCT and sCT intrinsic tyrosine fluorescence. Tyrosine fluorescence intensity was measured at 304 nm, excitation at 275 nm. $F$ is calcitonin fluorescence at a given TFE concentration and $F_0$ is the calcitonin fluorescence in water. For hCT (○) and sCT titration (×), the following procedure was used. From a CT sample in TFE (0.2 mg/ml), small volumes were removed and the same volume of a CT water solution (0.2 mg/ml) was added.

Infrared Spectroscopy—Infrared spectroscopy is an independent method of assessing the components of protein and peptide secondary structure. In Fig. 14 are illustrated the Fourier transform infrared spectra of hCT in water, in 95% MeOH/water, and in TFE. The appearance of 1660 cm$^{-1}$ in the amide region I band in the lower dielectric solvents is consistent with the induction of an $\alpha$-helix component (Erne et al., 1985; Carrier et al., 1990; Rafalski et al., 1990; Dousseau and Pézolet, 1990).

Conformational Reversibility—In both the MeOH and TFE titration experiments, the fluorescence and CD changes were reversible (data not shown). Experiments have been performed to examine the in vivo biological activity following hCT exposure to 99% MeOH, by measuring the decrease in calcium levels after hCT administration to rats (Schwartz et al., 1981). An hCT sample in 99% MeOH was diluted to 0.5% MeOH with water containing 0.9% NaCl to give a solution that had the same biological activity as a reference solution that had not been incubated with MeOH (data not shown). Thus, hCT conformational changes do not result in loss of biological activity.

DISCUSSION

The results presented show that human calcitonin is largely disordered in aqueous solutions at physiological conditions but can adopt an $\alpha$-helical structure in environments of low dielectric constant. The results also show that under similar conditions, hCT adopts the $\alpha$-helical structure less readily than does sCT.

For hCT and sCT in 100% TFE and 100% MeOH, CD data analysis (Table I) indicates that the calcitonins contain 45–50% $\alpha$-helix, in agreement with NMR data (Meyer et al., 1991; Meadows et al., 1991). Upon the addition of TFE to water
**Structure of Human and Salmon Calcitonins**

**Fig. 8.** Effect of MeOH/water concentration on the CD spectra of hCT and sCT. For hCT, increases in the CD intensity correspond to methanol mole % concentrations of 0, 6.3, 12.9, 18.2, 47.1, 57.2, 72.7, and 100% in the 185-260 nm (0.4 mg/ml hCT) region and the 245-320 nm (2.5 mg/ml hCT) region. For sCT, the increased intensity CD signals correspond to methanol mole % concentrations of 0, 1.8, 3.6, 6.9, 18.2, and 30.8% (not on the figure are 47.1, 57.2, 72.7, and 100%) in the 185-260 nm (0.25 mg/ml sCT) region and the 245-320 nm (2.5 mg/ml hCT) region.

**Fig. 9.** Effect of MeOH/water concentration on the hCT and sCT CD absorbance at 220 and 290 nm. For hCT and sCT, spectra were obtained at 220 nm (A) (●, hCT; ▲, sCT) and at 290 nm (B) (○, hCT; ●, sCT) by using the solvent conditions described in the legend to Fig. 8.

**Fig. 10.** Effect of MeOH/water concentration on the hCT and sCT intrinsic tyrosine fluorescence. Tyrosine fluorescence intensity was measured as described in the legend to Fig. 6. From stock samples of hCT in MeOH (●) and hCT in water (○), dilutions were made at a final concentration of 0.2 mg/ml. For sCT (▲), the titration procedure described for sCT in the legend to Fig. 6 was used. $F_0$ is the fluorescence intensity in water. The final concentration of the measured samples was 0.2 mg/ml calcitonin.

**Fig. 11.** Proportionality of the fluorescence and CD techniques for hCT (●) and sCT (▲) MeOH/water titration experiments. Changes in fluorescence ($F/F_0$) and in the CD signal ($\Delta \varepsilon/\Delta \varepsilon_0$) are plotted for a given MeOH concentration. $F_0$ and $\Delta \varepsilon_0$ are the fluorescence intensity and CD absorption of calcitonin in water; $F$ and $\Delta \varepsilon$ are the corresponding values at a given MeOH concentration. The values for $F/F_0$ and $\Delta \varepsilon/\Delta \varepsilon_0$ were calculated from the data in Figs. 9 and 10. The wavelength at which the CD signals was measured is indicated.

There is a sharp increase in sCT $\alpha$-helix content up to a TFE mole % of 6% (20% by volume) (Fig. 4A). This first $\alpha$-helix formation step is likely to be associated with the main $\alpha$-helix chain (residues 8-22, comprising 47% of the peptide). The situation is similar for hCT, where the first step of $\alpha$-helix formation is completed at 11 mol % TFE (Fig. 4A). Doi et al. (1990) report that in an 11 mol % TFE/H$_2$O solution, the residue 9-22 fragment of hCT is in a helical conformation with only the Thr$^{11}$-Lys$^{18}$ segment being $\alpha$-helical; the bordering residues 9-10 and 19-22 deviate from normality. This may correspond to the first peptide backbone folded intermediate formed at 11 mol % TFE (Fig. 4A). Together with the first step of $\alpha$-helix folding, there is also the ordering of the 1-7 disulfide (Fig. 4B), which is completed for both hCT
Table I  
Secondary structure estimates of hCT and sCT in 100% TFE and 100% MeOH

| Sample  | α-Helix content | Method/reference |
|---------|-----------------|------------------|
| hCT (TFE) | 57 | CD (Fig. 3) |
| sCT (TFE) | 42 | CD (Fig. 3) |
| sCT (TFE) | 60 (40) | NMR (CD) (Meyer et al., 1991) |
| hCT (MeOH) | 48 | CD (Fig. 8) |
| sCT (MeOH) | 44 | CD (Fig. 8) |
| sCT (MeOH) | 44 | NMR (Meadows et al., 1991) |

and sCT at 11 mol % TFE (33 vol %). At higher TFE concentrations, a further ordering of the backbone occurs for both hCT and sCT (Fig. 4A), which may be associated with the extension of the α-helix to residue 4 and the folding back of the carboxyl terminus tail to give the His17-Gly30 connectivity (Meyer et al., 1991; Doi et al., 1990).

The two α-helix folding steps were not detected in the sCT and hCT MeOH/water titration experiments (Fig. 9A). As in the case of TFE, sCT α-helix formation was completed at lower MeOH concentrations, as compared with hCT. Similar to TFE, in the MeOH/water titration experiments, no differences in the ordering of the 1–7 disulfide ring were observed between sCT and hCT (Fig. 9B). This indicates that the conformational change in the S–S bond region in hydrophobic environments is the same in both hCT and sCT.

CD measurements at low temperatures using as solvent ethanediol/water (2:1) showed that sCT adopted the α-helical structure, whereas hCT adopted an extended left-handed helix at −85 °C (Fig. 13) (Drake et al., 1988).

The MeOH and TFE titration experiments indicate the following steps for the folding of hCT and sCT in media with low dielectric constants: (i) the disulfide bond arrangement and (ii) a two-step α-helix formation.

(i) The CD data suggest that at low TFE and MeOH concentrations, the dihedral angle of the S–S bond adopts a preferred conformation that correlates with the initiation of the α-helix. It is likely that in a hydrophilic environment, the hydrophobic S–S bonds with the Cys1 near the central part of the molecule will be thermodynamically favored. Support for these considerations comes from recent NMR studies that showed that in TFE, sCT has the Cys1-Cys7 ring in close association with the beginning of the α-helix (Meyer et al., 1991). The biological significance of the disulfide bond ar-
rangement, which is similar for both hCT and sCT, remains to be determined. Since this S–S bond rearrangement occurs in environments with high dielectric constants (low MeOH/H₂O and TFE/H₂O concentrations) it may play a role in the first steps of CT interactions with receptors.

(ii) The two α-helix formation steps could be defined only in TFE and not in MeOH. This may be related to the smaller dielectric constant of TFE (26.67 debyes at 25 °C; Llinás and Klein (1975)), as compared with MeOH (33 debyes) or to other factors such as TFE stabilization of peptide hydrogen bonds, TFE-induced peptide dehydration, effects of TFE on water structure, or binding of TFE to the peptide. These factors have been suggested as possible explanations for the indication that the low dielectric constant of TFE is not important in the stabilization of the ribonuclease S-peptide α-helix (Nelson and Kallenbach, 1986). The first α-helix step is completed at 11 mol % (TFE/water) for hCT and 6 mol % (TFE/water) for sCT; the second α-helix step comprises 20 and 23% of the whole conformational change in going from water to pure TFE for hCT and sCT, respectively.

Recently, evidence was presented for the existence of CT receptors that distinguish between conformational features of CT molecules (Nakamuta et al., 1990). CT receptors on bones in mammals were suggested to selectively recognize CT molecules that do not easily adopt an α-helical structure, i.e., hCT, whereas helical CT, like sCT, seems to bind specifically to kidney (Nakamuta et al., 1990). In this context, our comparative study of salmon and human calcitonin secondary structure may contribute to a better understanding of the calcitonin structure-function relation and calcitonin receptor recognition.

Acknowledgments—We are grateful to Jeff T. Hoadley and Beat Kaufmann for expert technical assistance and to Dr. Judith Phillips for the rat hypocalcemic experiments.

REFERENCES

Austin, L. A., and Heath, H. (1981) N. Engl. J. Med. 304, 296–297
Averyad, A., Baker, R. K., and Wallach, S. (1974) Metabolism 23, 1037–1046
Azria, M. (1989) The Calcitonins: Physiology and Pharmacology, pp. 4–10, S. Karger AG, Basel, Switzerland
Body, J. J., Gilbert, F., Noji, S., Fernandes, G., Van Langendonck, A., and Borkowski, A. (1990) J. Clin. Endocrinol. Metab. 71, 675–681
Carriere, D., Mantch, H. H., and Wong, P. T. T. (1990) Biochemistry 29, 254–258
Chauessier, S., Stuart, C., Stevens, M. (1990) J. Lab. Clin. Med. 26, 933–938
Cowan, R. W. (1976) in Biochemical Fluorescence Concepts (Chen, R. F., and Edelhoch, H., eds) Vol. 2, pp. 441–486, Marcel Dekker, New York
Dai, M., Kobayashi, Y., Kyoistu, Y., Takimoto, M., and Goda, K. (1990) Peptides: Chemistry, Structure and Biology, Proceedings of the Eleventh American Peptide Symposium, July 9–14, 1989, La Jolla, California (River, J. E., and Marshall, R., eds) pp. 165–167, ESCOM, Leiden, The Netherlands
Deye, A. F., Silgward, G., and Gibbons, W. A. (1986) Biochem. Soc. Chem. 31, 143–146
Epand, R. M. (1983) Mol. Cell. Biochem. 67, 41–47
Epand, R. M., Epand, R. F., Orlowski, R. C., Schilliet, R. J., Boni, L. T., and Hui, S. W. (1983) Biochemistry 22, 5074–5084
Epand, R. M., Epand, R. F., and Orlowski, R. C. (1985) Int. J. Pept. Protein Res. 25, 105–111
Epand, R. M., Epand, R. F., Orlowski, R. C., Seiler, J. K., and Colescott, R. L. (1986) Biochemistry 25, 1984–1988
Erez, A., Sargent, D. F., and Sparer, R. (1986) Biochemistry 24, 4261–4263
Finnlay, D. M., Michelangeli, V. P., Orlowski, R. C., and Martin, T. J. (1988) Endocrinology 112, 2290–2291
Fouchereau-Peron, M., Moukhia, M. S., Benson, A. A., and Milbaur, G. (1981) Prog. Natl. Acad. Sci. U. S. A. 78, 7601–7605
Guidotti, P., Netti, C., Pacile, A., Gittis, I., and Mancia, M. (1987) Neuropeptides 10, 315–322
Hocking, D. J., and Heller, S. R. (1986) Eur. J. Clin. Pharmacol. 31, 27–31
Holmquist, E. D., and Vazquez, L. (1975) Methods Enzymol. 49, 149–176
Lagier, J. J. (1983) Principles of Fluorescence Spectroscopy, Plenum Press, New York
Longworth, W. J. (1971) in Excited States of Proteins and Nucleic Acids (Steiner, R. P., and Weinryb, L., eds) pp. 519–545, Marcel Dekker, New York
Linsen, M., and Klein, M. F. (1975) J. Am. Chem. Soc. 97, 4731–4737
MacIver, T. (1986) Clin. Trais. 23, Suppl. 1, 19–18
MacIver, T. (1989) in Endocrinology (DeGroot L. J., ed) Vol. 2, pp. 892–901, W. B. Saunders & Co., Philadelphia
Meadows, R. P., Nikonorwich, E. F., Jones, C. R., Bastian, J. W., and Gorenstein, D. G. (1981) Biochemistry 20, 1247–1254
Meyer, J., Pelton, J. T., Hofack, J., and Saudek, V. (1991) Biopolymers 31, 233–241
Moe, G. R., and Kaiser, E. T. (1985) Biochemistry 24, 1971–1976
Moe, G. R., Miller, R. J., and Kaiser, E. T. (1983) J. Am. Chem. Soc. 105, 4100–4102
Mote, J., Hunziker, W., and Fischer, J. A. (1978) Prog. Natl. Acad. Sci. U. S. A. 75, 3884–3886
Motta, A., Morelli, M. A. C., Goud, N., and Temussi, P. A. (1989) Biochemistry 28, 7900–7906
Motta, A., Pastore, A., Goud, N. A., and Morelli, M. A. C. (1991a) Biochemistry 30, 10444–10450
Motta, A., Temussi, P. A., Wünsch, E., and Bovermann, G. (1991b) Biopolymers 30, 2364–2371
Nakamuta, H., Orlowski, R. C., and Epand, R. M. (1990) Endocrinology 127, 165–169
Nelson, J. W., and Kallenbach, N. R. (1986) Proteins Structure Function Genet. 1, 211–217
Nicholoson, G., Moseley, J. M., Sexton, P. M., Mendelsohn, F. A., and Martin, T. J. (1986) J. Clin. Invest. 76, 355–369
Nishimura, C., D’Santos, C. S., Evans, T., Moseley, J. M., Kemp, B. E., Morelli, V. P., and Martin, T. J. (1988) Biochem. J. 250, 877–882
Orlowski, R. C., Safford, A. R., and Epand, R. M. (1987) Eur. J. Biochem. 162, 392–402
Provencher, S. W. (1982) Comput. Phys. Commun. 22, 213–227, 229–242
Provencher, S. W., and Gluckman, J. (1981) Biochemistry 20, 33–37
Rafaelli, M., Lean, J. D., and DeGrado, W. F. (1980) Biochemistry 29, 7917–7922
Ribeiro, V., Stankowski, S., and Schwarz, G. (1987) Biochemistry 26, 2751–2759
Schwartz, K. E., Orlowski, R. C., and Marcus, R. (1981) Endocrinology 108, 831–835
Sexton, P. M., Adam, W. R., Moseley, J. M., Martin, T. J., and Mendelsohn, F. A. (1987) Kidney Int. 32, 962–965
Silgward, G., Campbell, M. M., Gibbons, W. A., and Drake, A. F. (1992) Eur. J. Biochem. 206, 23–29
Strickland, E. H. (1974) CRC Crit. Rev. Biochem. 2, 113–175
Wallach, S., Cohn, S. H., Atkins, H. L., Ellis, K. J., Koberberger, R., Aloia, J. F., and ZANNI, I. (1977) Curr. Ther. Res. 22, 556–572
Wandersv, H., Goltman, D., Rouleau, M. F., and Bergeron, J. M. (1980) J. Cell. Biol. 85, 862–874
