Conformations of Polypeptide Hormones by Optically Detected Magnetic Resonance and a Zimm–Bragg Analysis of Helical Folding in Glucagon[1]

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Abstract: The zero-field splitting (zfs) parameter $|E|$ determined from optically detected magnetic resonance (ODMR) studies of several polypeptide hormones or shorter peptide segments of hormones has been utilized to investigate conformation in the vicinity of the single phosphorescent tryptophan residue present in each case. ODMR line widths and the position of the phosphorescence maxima were also investigated. Adrenocorticotropin-(1–24) was found to be essentially a random coil in the vicinity of the tryptophan residue, in agreement with conclusions reached by others using different methodology. Glucagon and tryptophan-containing glucagon peptides having C-terminal Thr-29 and eight or more residues behave as if they were in an α-helical conformation, and the Zimm–Bragg parameters deduced from approximate fitting of the glucagon data indicate a high potential for helix formation. Evidence was also obtained pointing to the existence of nonrandom structure in several peptides which are too short to form a stable helix, including glucagon peptides with five or fewer residues and human parathyroid hormone peptides with four, five, and seven residues. We suggest that in glucagon, and glucagon peptides with eight or more residues terminating in Thr-29, the perturbation of the optical and magnetic parameters arises from the nonuniform electric field of the α helix presumed to be present in each case (Stark effect). The optical and magnetic resonance data for somatostatin are consistent with the view that this hormone has a β-pleated sheet structure, with tryptophan occupying the position of the hairpin turn.

The triplet states of biomolecules have long held the interest of chemists and biologists alike because of the wealth of structural and functional information potentially available from the study of such excited states. In the 25 years which have elapsed since it was first realized that the luminescence of proteins originated from ultraviolet excitation of the aromatic amino acids,[3-5] the study of the excited states of these systems has progressed to the level of examination of the magnetic interactions within the triplet state by electron paramagnetic resonance (EPR)[6] spectroscopy[7-10] and, more recently, by optically detected magnetic resonance spectroscopy (ODMR).[11-13]

In a brief note,[14] we reported that ODMR measurements on glucagon, a polypeptide hormone consisting of a single chain of 29 amino acids containing a tryptophan residue at position 25 in the chain, showed evidence of secondary structure in the region of the tryptophan residue in dilute solid solution. In essence, we have been able to show[15] that the phosphorescence maxima, the ODMR zero-field splitting parameters $|D - E|$ and $|2E|$, and the ODMR line widths of glucagon and glucagon peptides all depend on (a) the chain length of the tryptophan-containing peptide and/or (b) the presence or absence of the C-terminal tripeptide sequence Met[27]-Asn[28]-Thr[29]. CO_2^-. The observations are consistent with the existence of an α helix in the region of Trp-25 for peptides with chain lengths longer than 8–10 amino acid residues terminating in Thr-29. For shorter peptides or peptides terminating in Leu-26, the evidence suggests that a random structure is a more appropriate description of the chain conformation in the region of Trp-25.

In the meantime, we have extended our study to include three sequences from human parathyroid hormone (PTH), two additional hormones, ACTH-(1–24) and somatostatin, and several model peptides. The present paper details our work on these systems, and in addition gives an analysis of glucagon folding in terms of the Zimm–Bragg parameters for α-helix formation. The luminescence and magnetic resonance properties of the triplet state of the single tryptophan residue present in each of the molecules under consideration are used to investigate the conformational state of the polypeptide chain in the vicinity of the tryptophan residue.

Experimental Section

Materials. Crystalline glucagon was obtained from Elanco Products, Indianapolis, Ind., and was purified by ion-exchange chromatography.[16a] Glucagon peptides were kindly provided by Dr. W. Bromer, Eli Lilly and Co., Indianapolis, Ind.; Dr. H. Edelhoch, National Institutes of Health, Bethesda, Md.; and Dr. A. Fontana, Institute for Organic Chemistry, Padova, Italy. Synthetic adrenocorticotropin-(1–24) (ACTH) was a gift from Dr. W. Rittel of CIBA-Geigy, Ltd., Basel, Switzerland, and somatostatin was donated by Dr. J. Shields, Eli Lilly and Co., Indianapolis, Ind. Three short fragments of human parathyroid hormone (PTH), including the 21–24 peptide which has the same sequence as the 23–26 peptide in glucagon (Val-Gln-Trp-Leu), were gifts from Professor K. Wüthrich, Swiss Federal Institute of Technology, Zürich, Switzerland. Additional peptides and amino acids were purchased from various U.S. suppliers and were the highest quality obtainable. Chromatoquality ethylene glycol was obtained from Matheson Coleman & Bell Co.

Methods. All samples were made up in ethylene glycol–H_2O (EGW), 1:1 (v/v), buffered at pH 7.0 with 0.1 M K_xPO_4, at concentrations of ca. 10^{-5} M or less. ODMR measurements were carried out at 1.2–1.3 K as previously described,[15,16b] sweeping microwaves toward both higher and lower frequencies and averaging the resulting line positions. For readers unfamiliar with the ODMR experiment, a brief synopsis follows.

The sample, immersed in liquid He at temperatures below the λ point (obtained by evacuating the space above the liquid), is excited by monochromatic radiation at or near the long-wavelength ultraviolet absorption band of the chromophore under study. Excess electronic energy is lost through various radiative (fluorescence) and radiationless processes including vibrational relaxation and intersystem crossing to the metastable triplet state. From the triplet state the molecule can likewise return to the ground state by spontaneous emission of a photon (phosphorescence), or via radiationless processes. Intersystem crossing to the triplet state takes place with spin inversion, and the mutual interaction of the unpaired electrons gives rise to a small but significant magnetic interaction. This small interaction typically splits the degenerate energy level of the triplet state into three distinct sublevels, separated from each other by energies that fall in the microwave region of the electromagnetic spectrum. Usually the magnetic sublevels are unequally populated. Inducing transitions between sublevels by microwave irradiation may temporarily increase (or decrease) the population of the sublevel(s) primarily responsible for optical emission, leading to an increase (or decrease) in the observed phosphorescence. Experimentally, microwaves are delivered

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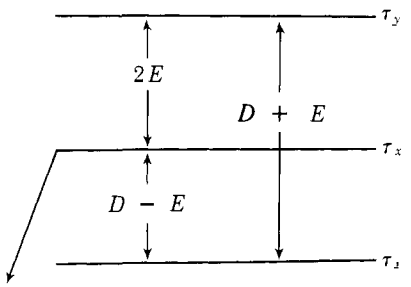
Table I. Maxima in the Phosphorescence Spectra and zfs Parameters and ODMR Line Widths for Tryptophan-Containing Hormones and Peptides[a]

	Phosphorescence		zfs parameters		ODMR line widths	
Substance (n)	$\lambda_{0\text{-}0}$, nm	λ_m, nm	$\|D\|$, cm^{-1}	$\|E\|$, cm^{-1}	$\gamma_{\|D-E\|}$, MHz	$\gamma_{\|2E\|}$, MHz
Glucagon (29)	411.6	438.9	0.0994	0.0432	102	206
Glucagon peptides:						
18-29 (12)	411.2	437.2	0.0992	0.0432	116	217
19-29 (11)	411.7	438.6	0.0990	0.0431	116	231
20-29 (10)	410.4	436.4	0.0994	0.0430	135	220
21-29 (9)	410.3	436.4	0.0992	0.0430	144	246
22-29 (8)	410.4	436.7	0.0995	0.0430	144	279
22-26 (5)	409.5	435.6	0.0994	0.0420	135	282
23-26 (4)[b]	408.6	435.2	0.0992	0.0418	140	263
1-26 (26)	409.2	435.0	0.0991	0.0417	139	293
PTH peptides:						
18-24 (7)	408.9	434.9	0.0989	0.0419	134	291
20-24 (5)	408.9	436.8	0.0994	0.0420	133	278
21-24 (4)[b]	408.6	435.2	0.0992	0.0418	140	283
ACTH (24)	408.2	434.5	0.0993	0.0413	152	296
Somatostatin (14)	408.9	435.0	0.0993	0.0415	147	296
Estimated precision of measurement:						
All samples	±0.5 nm	±0.5 nm	±0.0003	±0.0002	±10	±13

[a] Explanation of symbols: $\lambda_{0\text{-}0}$ and λ_m are the wavelengths of the phosphorescence intensity at, respectively, the maximum of the 0–0 band and the maximum of the highest intensity band. Excitation in all cases was at 297 nm with a 3-nm maximum band-pass. Emission spectra were recorded with a 1.5- or 3.0-nm maximum band-pass, and the ODMR transitions were monitored at 432 nm with a 1.5- or 3.0-nm band-pass. γ is the full width of the ODMR line at half-height. All zfs values are averages for at least two independent samples run on different days, except somatostatin (single determination of $|2E|$), and PTH-20-24 (single determination of both $|D-E|$ and $|2E|$). The number n in parentheses following the sample identification is the total number of amino acid residues in the substance. [b] These peptides have the same sequence in both glucagon and in PTH (Val-Gln-Trp-Leu). The double table entry of the same data is for comparative purposes.

to the sample by means of a helical wire coil surrounding the sample inside the liquid helium Dewar.

The zero-field splitting (zfs) transition energies are labeled, by convention, $|D-E|$, $|2E|$, and $|D+E|$, where $|D|$ and $|E|$ are the two parameters used to specify the triplet sublevel energies of a chromophore in a given molecular environment. For tryptophan, the zfs parameters are given in the following diagram ($D > E \geq 0$):



where τ_x, τ_y, and τ_z designate the energy levels associated with the principal magnetic axes x, y, and z, and z is perpendicular to the plane of the ring.[12] The slanted arrow represents optical emission from the primary radiative level τ_x.

Results

Hormones and Peptides. Table I gives the average values of the phosphorescence maxima, the zfs parameters, and the ODMR line widths for the hormones and partial hormone sequences examined in this work. The table values fall into two rather loose categories. Glucagon and the glucagon peptides with C-terminal Thr-29 and 8–10 or more amino acid residues exhibit phosphorescence maxima near 411 and 437 nm, $|E|$ values near 0.0430 cm^{-1}, and ODMR line widths of around 100 ($|D-E|$) and 200 ($|2E|$) MHz. ACTH, somatostatin, the three PTH peptides, and the glucagon peptides ending in Leu-26 have phosphorescence maxima near 409 and 435 nm, $|E|$ values of 0.0420 cm^{-1} or less, and line widths of about 150 ($|D-E|$) and 300 ($|2E|$) MHz.

Although the differences between the two categories suggested by the table are small and exhibit a fair degree of noise, they are clearly significant. The phosphorescence shift of 3–4 nm represents a change in energy of around 150 wavenumbers (cm^{-1}), or roughly 30% of the optical line width of ca. 500 cm^{-1} for the 0–0 phosphorescence band, with an estimated precision of ±15%. The differences occurring in the zfs parameters are, though much smaller in absolute magnitude than the differences in the optical parameters, even more dramatic because of their greater experimental reliability. Comparing the zfs parameters of ACTH and glucagon, for example, we observe differences of nearly 60 MHz in $|D-E|$ and 120 MHz in $|2E|$, with estimated precisions of ±5 MHz (9%) and ±13 MHz (11%), respectively. These shifts in the ODMR line positions amount to 40–60% of the line widths, depending on which peptide is chosen for the calculation.

Model Compounds. The phosphorescence maxima, zfs parameters, and ODMR line widths of several short model peptides are given in Table II. These compounds were studied in an effort to develop reasonable models on which to base interpretations of the ODMR results obtained with longer peptides, hormones, and proteins. It is apparent by comparing Tables I and II that the dipeptides Trp-Leu-amide, Met-Trp, and Gly-Trp and the tripeptide Gly-Trp-Gly exhibit phosphorescence maxima, zfs parameters, and ODMR line widths closely resembling those of ACTH and somatostatin. The ODMR line widths of tryptophan and N^{α}-acetyltryptophanamide are similar to those of the di- and tripeptides, yet as discussed below, the zfs parameters and phosphorescence maxima are somewhat different.

Zimm-Bragg Parameters for Helix Formation. Because of strong evidence that the 18–29 segment of glucagon exists in an α-helical configuration in dilute solution,[14,15] we analyzed the glucagon and the glucagon peptide data in terms of the experimental Zimm-Bragg parameters s, σ, and n.[18] No attempt was made to use curve fitting since in the first place the Zimm-Bragg equations are strictly valid only for homopolymers such as polytryptophan, and in the second place the experimental scatter—particularly in the phosphorescence data—does not justify the effort involved in a rigorous analysis.

Table II. Maxima in the Phosphorescence Spectra and zfs Parameters and ODMR Line Widths for Tryptophan Model Compounds[a]

Substance	Phosphorescence		zfs parameters		ODMR line widths	
	$\lambda_{0\text{-}0}$, nm	λ_m, nm	$\|D\|$, cm^{-1}	$\|E\|$, cm^{-1}	$\gamma_{\|D-E\|}$, MHz	$\gamma_{\|2E\|}$, MHz
Monopeptides						
Tryptophan	407.2	433.0	0.1004	0.0423	157	300
N^{α}-Ac-Trp-amide	406.8	433.4	0.0993	0.0403	148	271
Dipeptides						
Trp-Leu-amide	408.1	434.0	0.0995	0.0412	175	283
Met-Trp	408.8	435.0	0.0997	0.0413	172	303
Gly-Trp	408.0	435.0	0.0999	0.0413	148	294
Tripeptide						
Gly-Trp-Gly	407.9	434.1	0.0994	0.0412	154	278

[a] See footnote *a* to Table I for an explanation of symbols and estimates of precision.

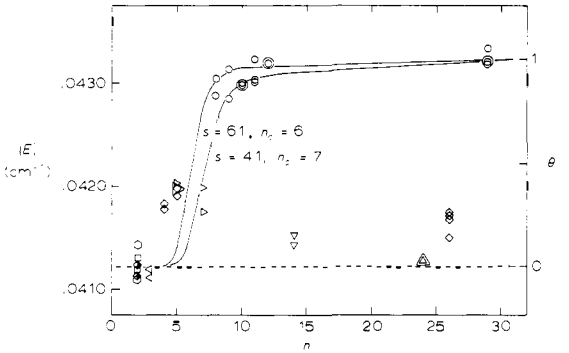


Figure 1. Variation in the zfs parameter $|E|$ as a function of chain length for various peptides and hormones: (○) glucagon and glucagon peptides ending in Thr-29; (◇) glucagon peptides ending in Leu-26; (▷) human parathyroid hormone peptides; (▽) somatostatin; (△) ACTH; (□) Met-Trp; (○) Gly-Trp; (◁) Gly-Trp-Gly; (◆) Trp-Leu-amide. Solid curves are calculated from the Zimm–Bragg equation (eq 2) for $\sigma = 5 \times 10^{-4}$ and the indicated values of s and n_c (critical length).

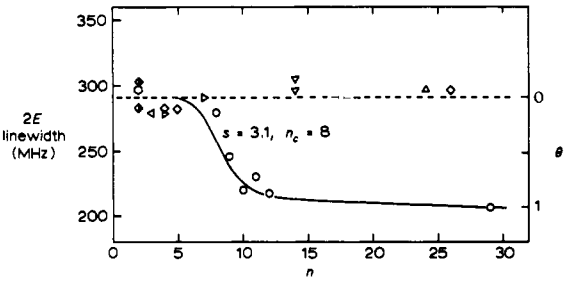


Figure 2. Variation of the ODMR $|2E|$ line width (full line width at half-height) as a function of chain length for various peptides and hormones. Identifying symbols and Zimm–Bragg parameters are the same as for Figure 1.

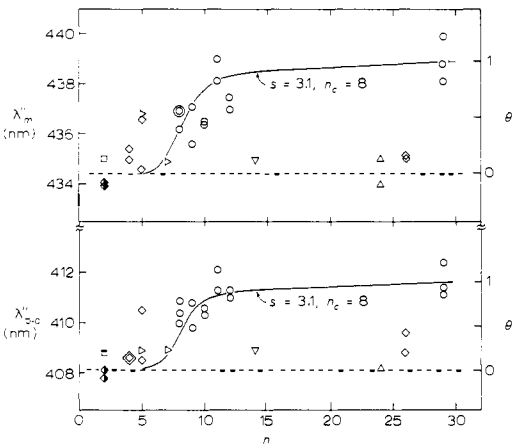


Figure 3. Variation of the position of the phosphorescence maxima as a function of chain length for various peptides and hormones. Identifying symbols and Zimm–Bragg parameters are the same as for Figure 1.

A representative value[19] of $\sigma = 5 \times 10^{-4}$ was selected for the extra free energy lost at the start of a helical section, and the values of s (approximately the equilibrium constant for the incorporation of an amino acid residue which is part of a random coil into an adjacent helical segment) were calculated from the relation:[20]

$$(s-1)^2 s^{-n_c} \simeq \sigma \qquad (1)$$

for various integral values of n_c, the critical number of amino acid residues needed for formation of a substantial amount of helix stabilized by intrachain hydrogen bonds. The fractional helicity θ (fraction of all residues present in helical segments) in a hypothetical polypeptide of n total amino acid residues was then obtained from the Zimm–Bragg equation:[20]

$$\theta = \frac{(n-4)(s-1) - 2 + [(n-4)(s-1) + 2s]s^{-n+1}}{(n-4)(s-1)\{1 + (s-1)^2 s^{-n_c-1} - [(n-4)(s-1) + s]s^{-n+1}\}} \qquad (2)$$

In each of Figures 1–3 the fractional helicity θ and one of the triplet-state parameters are plotted as a function of chain length n for the compounds listed in Tables I and II. The data from the dipeptides Trp-Leu-amide, Met-Trp, and Gly-Trp and the tripeptide Gly-Trp-Gly were averaged and used to set the baseline ($\theta = 0$), and the maximum values ($\theta = 1$, 100% helix) were obtained by averaging the four independent glucagon data sets. Figures 1–3 show more clearly the nature of the two broad classes of data in Tables I and II. A curious feature of the plots is evident in the values of n_c and s which give rise to fractional helicities that follow the zfs parameter $|E|$ on the one hand, and the magnetic line widths and phosphorescence maxima on the other. While the latter two data sets seem reasonably well described by sigmoidal curves with $n_c = 8$ and a corresponding s near 3, the change in $|E|$ is better approximated by theoretical curves with $n_c = 6$ or 7 and s values of 4 or larger.

Discussion

In attempting to develop a method for examining the local conformational environment of a particular amino acid side chain, in this case the indole ring, the question arises as to what model compounds are best suited to describe the "random" arrangement of all groups with respect to each other. By "random", we mean here the ensemble of conformers which are easily interchangeable with each other on the basis of the available thermal energy; that is, structures in which the equilibrium constant is essentially one for the interconversion of any given conformation with any other, different conformation. It was somewhat surprising, therefore, to discover that the simplest models, tryptophan and N^{α}-acetyltryptophana-

mide, showed significantly different zfs parameters than the di- and tripeptides which we finally chose as models for the random state of the indole side chain in a polypeptide. The ODMR line widths of tryptophan and N^{α}-acetyltryptophanamide are comparable with those of the dipeptides and Gly-Trp-Gly, and since the (broad) line widths should be a sensitive measure of the multiplicity of solvent and other interactions with the indole ring,[21] this criterion suggests that the indole ring is freely solvent available in both monopeptide models as well as in the di- and tripeptides.[22]

It is conceivable that the explanation for the altered zfs parameters of the monopeptides lies in a charge effect: N^{α}-acetyltryptophanamide has no charge on either the amide or the carboxyl group, whereas the positive and negative charges of the tryptophan zwitterion are both in close proximity to the indole ring. The zfs parameters of these two models bracket those of the di- and tripeptide models, in which one or more amino acid residues are present between the tryptophan residue and either the amino- or carboxyl-terminal end of the zwitterionic peptide. The increased distance—and increased number of conformational possibilities—for at least one of the charges is likely to result in a broader charge distribution with respect to the indole moiety. Coupled with this is an increase in the number of ways that the ionic components of the solvent can interact with this charge and thereby further reduce its specific effect on the indole ring (experiments were carried out in 0.1 M K_xPO_4 to reduce the magnitude of electrostatic effects). Gly-Trp-Gly should be a particularly good model compound in this respect, since (a) both charges are removed along the chain by one amino acid residue, (b) specific side-chain interactions of the glycine residues with the indole moiety are negligible (glycine has no side chain), and (c) rotation about the N-C and C-C′ bonds (ϕ,ψ angles) is largely unhindered in glycine, leading to an increased number of conformational possibilities.

Having chosen the baseline for the Zimm-Bragg analysis as the average of the tryptophan-containing dipeptides and Gly-Trp-Gly because of their apparent random structure in dilute solution, the theoretical Zimm-Bragg curves were plotted using glucagon as the model system for (effectively) 100% helical structure in the region of the single tryptophan residue. The evidence that this region of the glucagon molecule is indeed helical has been discussed in detail by Ross et al.,[15] and will be reviewed briefly here for completeness of the present arguments.

(i) The circular dichroism of dilute glucagon solutions indicates that some 20% of the molecule exists in an α-helical conformation,[23] although the CD does not specify the location of the helical segment.

(ii) Of the exchangeable protons in monomeric glucagon, 20–30% (8 ± 2) exchange much more slowly than those in model polypeptides with random conformations.[24] The lower limit of six slowly exchanging protons could correspond to the hydrogen-bonded amide protons of a ten-residue α-helical segment ($n - 4$ hydrogen bonds).

(iii) Trp-25 in glucagon exhibits impaired rotational freedom compared to Gly-Trp, Gly-Trp-Gly, and other models for random conformational behavior (including ACTH, vide infra).[25] The tryptophan residue is not "buried" in the glucagon structure, however, as its fluorescence spectrum is characteristic of tryptophan residues exposed to solvent.[25-27]

(iv) Theoretical predictions of glucagon structure indicate that the ten amino acid segment between residues 18 and 27 has a high potential for helix formation.[28]

(v) The zfs parameters, ODMR line widths, and phosphorescence maxima of glucagon indicate a structured environment for Trp-25 as compared to either tryptophan-containing model peptides or to des-Met_{27}-Asn_{28}-Thr_{29}-glucagon,[15] the 1–26 peptide fragment which is believed to be structureless on the basis of CD and other evidence.[29] Met-27 or a similar amino acid with reasonable helical folding potential is apparently required for helix formation, since although CD indicates an absence of structure for the 1–26 peptide, the 1–27 peptide obtained by cyanogen bromide cleavage of glucagon (in which Met-27 is replaced by homoserine lactone-27) has CD spectra similar to native glucagon in dilute aqueous solution.[30] Furthermore, the increase in the zfs parameter $|E|$ in glucagon relative to model compounds is indicative of decreased symmetry in the electron distribution.[31]

(vi) X-ray diffraction studies show that almost the entire glucagon molecule is helical in the crystal, with the exception of the short 1–5 segment at the N terminus.[32] The finding of virtually complete helical structure in the crystal undoubtedly reflects the tendency of native glucagon to acquire additional helical character as the concentration of glucagon solutions is raised above 10^{-4} M and the molecules begin to aggregate.[33]

If the interpretation we have made in assigning the proper zero and 100% levels for the Zimm-Bragg analysis is correct, we conclude that while the region in the immediate vicinity of Trp-25 in glucagon is helical, the evidence strongly suggests that the region around Trp-9 in ACTH has no elements of defined structure. This is in agreement with current views depicting the ACTH molecule as a structureless random coil, as evidenced by deuterium exchange,[34] optical rotatory dispersion,[35-37] circular dichroism and fluorescence spectroscopy,[27] and excitation energy transfer.[38] As far as our hypothesis is concerned, ACTH represents an important control experiment: a polypeptide of a different sequence than glucagon and with known random structure, having ODMR properties closely resembling those of the dipeptide and tripeptide model compounds.

Taking all the evidence together, we might also conclude, from an examination of Figures 1–3, that although glucagon peptides with eight or more amino acid residues terminating in Thr-29 are helical in the region of Trp-25, the shorter glucagon peptides ending in Leu-26 and the PTH peptides also show some elements of structure. It is not likely that this is helical structure per se since the peptides are too short to form stable α helices in solution,[18,39] except possibly the PTH heptapeptide. On the other hand, Bundi et al.[40] have recently presented good evidence for nonrandom structure localized in the 20–24 peptide segment (Arg-Val-Gln-Trp-Leu) of otherwise randomly coiled human PTH. The nonrandom character persists in the 18–24, 19–24, and 20–24 peptide fragments, two of which were studied here by ODMR techniques. The results obtained in the two laboratories provide mutual support for the idea that the PTH peptides as well as the glucagon peptides 22–26 and 23–26 have elements of nonrandom structure in dilute solution.

The tendency for these short peptides to form partially ordered structures may explain in part why the Zimm-Bragg s parameters needed to fit the glucagon data are considerably larger than those deduced experimentally from the fitting of data obtained with large homopolypeptides. The largest s value measured so far is 1.92 for poly(L-leucine),[41] and other experimentally obtained s values fall in the range of 0.57–1.36 for homopolypeptides of other amino acids.[42] The steepness of the transition observed in the glucagon peptide series may well be the result of partially preformed structures with a very high potential for assuming a helical conformation.[43] Once a sufficient number of residues are present such that a *stable* helix can exist ($n \geq 7$–8 residues), the conversion to the fully helical conformation is readily effected.

It has been suggested that tryptophan and the other aromatic amino acids could serve as efficient nucleation centers for the formation of ordered—but not necessarily helical—structures in proteins.[44] Because of their large surface areas,

and in the case of tryptophan, its permanent electric dipole moment, conditions are such that attractive interactions between the aromatic ring and other side chains could lead to quasistable structures. While not *necessarily* significant, it is interesting that glucagon and the glucagon and PTH peptides studied here all contain a Trp-Leu sequence. The tryptophanyl side chain is the most hydrophobic of all the amino acids, and the leucyl side chain is also strongly hydrophobic.[45] Molecular models of glucagon in the fully α-helical conformation show that the side chains of Trp-25 and Leu-26 can overlap to the point where one face of the indole ring system is completely covered by the leucyl side chain.

We cannot explain why the change in the zfs parameter $|E|$ is better approximated by a Zimm-Bragg curve with a higher s and lower n_c than either the ODMR line width or the phosphorescence data. Preliminary calculations indicate that the shifts in the position of the phosphorescence maxima may arise from the nonuniform electric field which results from ordering the polypeptide chain in an α-helical conformation (Stark effect).[46] Provided the field is of sufficient magnitude, all three parameters of the triplet state—zfs, ODMR line widths, and the position of the phosphorescence maxima—would be affected, depending upon the relative orientations of the indole ring and the helix. Theoretically, the zfs parameter $|E|$ should be very sensitive to an asymmetric (electronic) environment,[10,31] but the detailed origin of the difference in $|E|$ between helical and nonhelical structures has so far not been established.

The results with somatostatin and ACTH can be rationalized on the basis of the ideas expressed above. The backbone chain of somatostatin is believed to assume a β-pleated sheet structure in which the dipole moments of the peptide bonds are oppositely directed. The net dipolar field is thus quite small, and a similar situation should exist for random coil structures (ACTH) since random internal rotation also tends to minimize the dipolar field contribution. Thus, in contrast to glucagon, where the peptide bond dipole moments of the helical backbone add to give a net dipole moment of several debyes, Trp-8 in somatostatin should not experience a significant perturbation arising from the presence of β-sheet structure. Similarly, random internal rotation in the vicinity of Trp-9 in ACTH should effectively cancel out the dipole contribution. The data of Figures 1-3 are consistent with views on the conformations of the various hormones as reported by ourselves and by others, and support the suggestion—discussed above—that the optical and magnetic properties of tryptophan in glucagon and in certain glucagon peptides are modified by a Stark field arising from the presence of α-helical structure.

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References and Notes

(1) This research was supported by Grant No. GM 22603 awarded by the National Institutes of Health, Department of Health, Education, and Welfare, and in part by Grant No. GB 18016 awarded by the National Science Foundation.
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(6) Abbreviations used are: EPR, electron paramagnetic resonance; ODMR, optically detected magnetic resonance; ACTH, adrenocorticotropic hormone (1–24); PTH, human parathyroid hormone; zfs, zero-field splitting; EGW, ethylene glycol–water, 1:1 (v/v), buffered at pH 7.4 with 0.1 M K_xPO_4; CD, circular dichroism.
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