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Time-Resolved Fluorescence and ^{1}H NMR Studies of Tyrosyl Residues in Oxytocin and Small Peptides: Correlation of NMR-Determined Conformations of Tyrosyl Residues and Fluorescence Decay Kinetics†

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ABSTRACT: Steady-state and time-resolved fluorescence properties of the single tyrosyl residue in oxytocin and two oxytocin derivatives at pH 3 are presented. The decay kinetics of the tyrosyl residue are complex for each compound. By use of a linked-function analysis, the fluorescence kinetics can be explained by a ground-state rotamer model. The linked function assumes that the preexponential weighting factors (amplitudes) of the fluorescence decay constants have the same relative relationship as the ^{1}H NMR determined phenol side-chain rotamer populations. According to this model, the static quenching of the oxytocin fluorescence can be attributed to an interaction between one specific rotamer population of the tyrosine ring and the internal disulfide bridge.

Steady-state fluorescence studies of peptides and polypeptides in aqueous solution have used all three aromatic amino acids, phenylalanine, tyrosine, and tryptophan, as intrinsic probes [see reviews by Schiller (1981), Longworth (1983), and Creed (1984a,b)]. By contrast, time-resolved fluorescence investigations have emphasized the use of tryptophan (Beechem & Brand, 1985). Less has been done with tyrosine, and phenylalanine has been virtually ignored, since both amino acids have considerably weaker absorption than tryptophan and small quantum yields when incorporated into peptides and proteins (Longworth, 1971). Furthermore, in those molecules containing both tyrosine and tryptophan, tryptophan emission strongly masks tyrosine emission, although the fluorescence of tyrosine has been resolved in careful steady-state (Eisinger, 1969; Eisinger et al., 1969) and time-resolved (Brochon et al., 1974; Lakowicz & Cherek, 1981) studies.

We are interested in how the time-resolved fluorescence kinetics of the aromatic amino acids, especially tryptophan and tyrosine, may be used to obtain information about peptide conformations in dilute solution. As recently reviewed by Beechem & Brand (1985), the fluorescence decay kinetics of most single-tryptophan-containing peptides are complex. The mechanism for the observed kinetics of tryptophan and tryptophyl-containing peptides is not clear. Several possibilities have been mentioned in the literature, including indole photochemistry involving exchange of the C-4 indole hydrogen (Saito et al., 1984) and environmental effects resulting from the three principle different rotameric conformers of the indole side chain (Szabo & Rayner, 1980; Robbins et al., 1980; Ross et al., 1981; Chang et al., 1983; Petrich et al., 1983).

It has recently been found by Libertini & Small (1985) that the single tyrosine residue in histone H1 exhibits complex fluorescence decay kinetics. As shown in the preceding paper in this issue (Laws et al., 1986), the fluorescence decay kinetics of simple tyrosine analogues are also complex. Direct comparison of the ^{1}H NMR[1] determined rotamer populations with the fluorescence data, however, indicates that the complex kinetics seen for tyrosine compounds are readily explained in terms of a rotamer model. In the rotamer model, three chemically distinct environments exist for the phenol ring about the C^α–C^β bond.

In the present paper, we extend the results of the preceding paper (Laws et al., 1986) by examining the fluorescence decay kinetics of a single tyrosyl residue in small, cyclic peptides.

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[1] Abbreviation: ^{1}H NMR, proton nuclear magnetic resonance.

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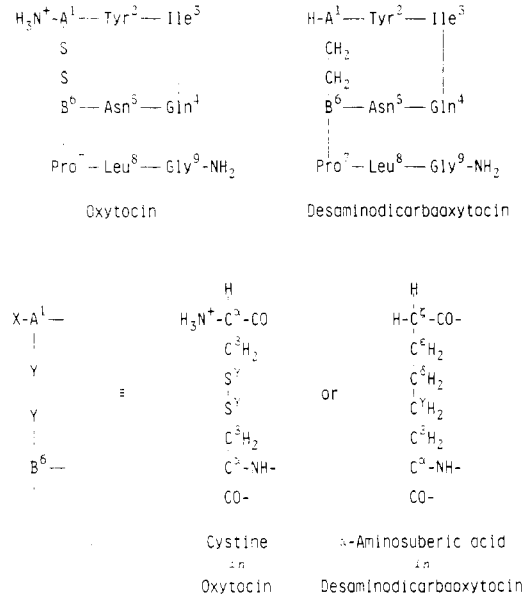

FIGURE 1: Chemical structures for oxytocin and desaminodicarbaoxytocin.

For this investigation we have selected the hormone oxytocin and two oxytocin analogues. Oxytocin, a lactation and uterine contraction hormone, contains nine residues with Tyr-2 included within the six-residue disulfide loop as shown in Figure 1. One oxytocin analogue, desaminodicarbaoxytocin, also displayed in Figure 1, has the disulfide bridge replaced with an ethylene bridge and lacks the N-terminal amino group (Jošt & Rudinger, 1967). The second analogue, desaminooxytocin (not shown), lacks the N-terminal amino group (Ferrier et al., 1965). Of these three peptides, only oxytocin has been extensively characterized in aqueous solution by NMR (Glickson et al., 1972; Richard-Brewster et al., 1973; Bradbury et al., 1974; Hruby, 1974; Glickson, 1975; Wyssbrod et al., 1977). In addition, the crystal structure of desaminooxytocin has been recently reported (Pitts et al., 1985). In these cyclic peptides, we expect that the phenol ring is more highly restricted for rotation about the C^{α}–C^{β} bond compared with simple tyrosine derivatives. As discussed in the preceding paper (Laws et al., 1986), if a rotamer model with interconversion on a longer time scale than the decay of the excited state is the major factor governing the complex decay kinetics of tyrosine, we then expect that the fluorescence decay data can be readily interpreted in terms of ^{1}H NMR determined rotamer populations.

Materials and Methods

Chemicals. Oxytocin was synthesized by the solid-phase method, essentially as reported by Live et al. (1977). The desaminooxytocin synthesis was similar, except [(*p*-methylbenzyl)thio]propionic acid was used instead of *N-tert*-butoxycarbonyl-*S*-(*p*-methylbenzyl)-L-cysteine, for the final coupling in position 1. Desaminodicarbaoxytocin ([1,6-aminosuberic acid]oxytocin; [Asu^{1,6}]oxytocin) was purchased from Peninsula Laboratories, Inc., Belmont, CA (lot 004789). The linear precursors of the cyclic pentapeptides, *cyclo*(-D-Tyr-Pro-Gly-D-Ala-Pro-) and *cyclo*(-D-Trp-Pro-Gly-D-Ala-Pro-), were synthesized by the solid-phase method starting with glycine on the 1% chloromethylated styrene divinylbenzene resin. The resin was cleaved with anhydrous HF, and the peptide was cyclized by the mixed anhydride procedure (Munekata et al., 1977; Wieland, 1983). The syntheses of the parent cyclic pentapeptides, *cyclo*(-Gly-Pro-Gly-D-Ala-Pro-) and *cyclo*(-D-Phe-Pro-Gly-D-Ala-Pro-), were originally reported by Pease and co-workers (Pease & Watson, 1978; Pease, 1979; Bach et al., 1982). Full details for the syntheses of the D-tyrosine- and D-tryptophan-containing cyclic pentapeptides will be reported in a later paper.

Table I: Fluorescence Decay Parameters of Oxytocin and Its Analogues[a]

compd	α_1[b]	τ_1 (ns)	α_2[b]	τ_2 (ns)	Φ_f/Φ_τ[c]
oxytocin	0.75	0.69	0.25	1.89	0.62 ± 0.05
desaminooxytocin	0.70	0.90	0.30	1.88	0.72[d]
desaminodicarbaoxytocin	0.26	1.06	0.74	3.07	0.95 ± 0.05

[a] Decay data were obtained at 5 °C in 0.001 M HCl with 284- and 302-nm excitation and emission, respectively, with less than 5-nm band-passes. [b] Amplitudes were normalized to a sum of 1.0. [c] Φ_f/Φ_τ represents the ratio of the relative quantum yield determined by steady-state emission to the relative quantum yield calculated from the decay parameters. Steady-state emission spectra (280-nm excitation, 10 °C, pH 3.1) were integrated between 290 and 370 nm. The results were compared with the pH 4.7 integrated spectrum of tyrosine to yield Φ_f. Average lifetimes ($\sum_i \alpha_i \tau_i / \sum_i \alpha_i$) were compared to the 3.76-ns lifetime of the tyrosine zwitterion (Laws et al., 1986) to yield Φ_τ. It was assumed that the Φ_f/Φ_τ ratio was not significantly affected by the temperature difference of 5 °C since all the data were normalized by tyrosine parameters measured at the same respective temperatures. The effective normalized quantum yield ratio is, therefore, $\Phi_f/\Phi_\tau = [(\Phi_f/\Phi_\tau)_{compd}/(\Phi_f/\Phi_\tau)_{Tyr}]$. [d] Single determination.

Spectroscopy. The fluorescence and ^{1}H NMR instrumentation and data analysis methods are described in the preceding paper (Laws et al., 1986).

Results

Absorption and Steady-State Emission Spectra. The absorption spectra of oxytocin and its analogues closely resemble the absorption of tyrosine in the 250–300-nm region. Oxytocin and desaminooxytocin show an additional weak, broad band underlying the tyrosine absorption. This extinction extends to the red of the tyrosine band and can be identified with the disulfide of the cystine residue (Wetlaufer, 1962); this weak, broad band is not present in desaminodicarbaoxytocin where the disulfide bridge is replaced by the ethylene bridge. The steady-state emission spectra of the three peptides show the characteristic emission band for tyrosine (Longworth, 1971). The quantum yields are smaller than that of the tyrosine zwitterion, as previously found for oxytocin (Cowgill, 1964; Cowgill, 1967a). Relative to the tyrosine zwitterion, the quantum yield of desaminodicarbaoxytocin (0.65) is considerably larger than that for oxytocin (0.17) and for desaminooxytocin (0.23), in reasonable agreement with values reported by Bodanszky et al. (1981), indicating that the disulfide bond is a quencher of the tyrosine excited state as suggested earlier by Cowgill (1967b) for oxytocin and as commonly found in other polypeptides (Longworth, 1968).

Time-Resolved Fluorescence. The fluorescence decay data of oxytocin, desaminooxytocin, and desaminodicarbaoxytocin are given in Table I. A free-floating analysis for the sum of two exponentials is required for an adequate statistical fit in all cases. The analysis results for oxytocin and desaminooxytocin are similar, suggesting that the N-terminal amino group does not strongly affect the emission properties of the phenol ring. The parameters describing the fluorescence decay of desaminodicarbaoxytocin, compared with oxytocin, reflect the increase in quantum yield in the absence of the disulfide bridge: the decay constants are longer and the relative weighting of the decay constants changes. Comparison of the steady-state quantum yield, relative to the tyrosine zwitterion, with the quantum yield calculated from the fluorescence decay parameters, expressed as the ratio Φ_f/Φ_τ in Table I, also reveals a dramatic difference between the phenol excited-state prop-

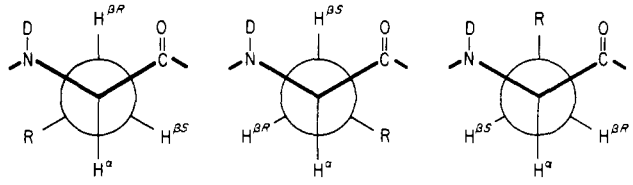

FIGURE 2: Newman projections about the C^{α}–C^{β} bond, detailing the possible positions of the phenol group in relation to the C^{α} substituents. The rotamer assignments I, II, and III, and the corresponding values of the torsion angle χ^1, are indicated for the L configuration of tyrosine. The peptide bond linkages are not shown. The atom D indicates the state of the compound in D_2O, the solvent used for the NMR studies.

Table II: ^{1}H NMR Coupling Constants and Calculated Phenol Group Rotamer Populations

	coupling constants[a]		populations[b]		
compd	$^3J_{(H^{\alpha}-H^{\beta R})}$	$^3J_{(H^{\alpha}-H^{\beta S})}$	p_I	p_{II}	p_{III}
oxytocin[c]	7.9	6.9	0.48	0.39	0.12
desaminodicarbaoxytocin[d]	10.07	5.19$_5$	0.68	0.24	0.08

[a] Values given are in Hz. [b] Sum of the populations = 1.0, with errors in p_I and p_{II} of ±0.03 and in p_{III} of ±0.05. [c] Data, taken at 25 °C, are from Wyssbrod et al. (1977). The temperature coefficients for the coupling constants and the populations are essentially 0 (Wyssbrod et al., 1977; Meraldi et al., 1975; Boicelli et al., 1977). [d] Data taken at 22 °C.

erties in oxytocin and desaminodicarbaoxytocin. The ratio Φ_f/Φ_τ should be unity if all the excited-state depopulation mechanisms are dynamic. A reduction in this ratio is indicative of a static process. Accordingly, static quenching is linked to the presence of the disulfide bridge in oxytocin.

^{1}H NMR. Figure 2 identifies the three main conformations for the phenol ring about the C^{α}–C^{β} bond. Table II presents the ^{1}H NMR coupling constants and calculated rotamer populations for oxytocin and desaminodicarbaoxytocin. In oxytocin, rotamers I and II have essentially equal populations that are 3 times that of rotamer III. In desaminodicarbaoxytocin, removal of the N-terminal amino group and changing the bridge atoms from sulfur to carbon result in a loss of rotamer II population in favor of rotamer I. The ^{1}H NMR spectrum of desaminooxytocin is more complicated, however, due to the extra hydrogen on the C^{α} of residue 1. Therefore, the phenol rotamer populations of desaminodicarbaoxytocin have not been determined, although removal of the N-terminal amino group is expected to make only a minor perturbation.

Discussion

The fluorescence decay kinetics of tryptophan in peptides are known to be complex (Donzel et al., 1974; Grinvald & Steinberg, 1976; Ross et al., 1981; Cockle & Szabo, 1981). From the present results for oxytocin and its two analogues it is clear that the fluorescence decay kinetics of tyrosine in peptides can also be complex. This might be expected since tyrosine model compounds (Gauduchon & Wahl, 1978; Laws et al., 1986) and the single tyrosine in histone H1 (Libertini & Small, 1985) also exhibit multiexponential decay kinetics. Although complex kinetics can arise from both ground-state and excited-state processes, the data analysis in the preceding paper (Laws et al., 1986) indicated that the multiexponential decay kinetics of simple tyrosine derivatives can be accounted for by ground-state rotamer populations. However, since peptides can have conformations in which the aromatic side chain is near other side chains or the peptide backbone, excited-state reactions might be facilitated. For example, it has been shown by Rayner et al. (1978) that the phenol ring of tyrosine can undergo excited-state proton transfer when a better proton acceptor than water is present. In peptides, a proton acceptor in the vicinity of the phenol hydroxyl group could facilitate excited-state proton transfer, as suggested by the results for histone H1 (Libertini & Small, 1985). Therefore, both ground-state and excited-state mechanisms may be important in peptides.

The most probable excited-state process for tyrosine in water is excited-state proton transfer, as discussed in the preceding paper (Laws et al., 1986). Since the pK_a of the phenol hydroxyl proton is near 10 and the present data were obtained at pH 3, ground-state phenolate is not expected. The absence of ground-state phenolate is borne out by absorption spectra; phenolate exhibits an intense, broad absorption band that extends to the red of the phenol absorption (Wetlaufer, 1962). The weak, longer wavelength absorption to the red of the first singlet state absorption band of tyrosine in oxytocin can be entirely ascribed to the disulfide bridge (Wetlaufer, 1962). Close examination of the emission spectra of oxytocin and its analogues reveals no characteristic fluorescence from phenolate; the emission from phenolate broadens the lower energy (red) side of the tyrosine emission band (Rayner et al., 1978). Moreover, the decay kinetics of these peptides resemble those observed for tyrosine model compounds where it was found that excited-state proton transfer in water is too slow to compete with other kinetic processes (Laws et al., 1986). Furthermore, oxytocin and its analogues do not contain a strong proton acceptor to facilitate excited-state proton transfer, especially at pH 3. Thus the fluorescence data provide no evidence that the tyrosyl residue in oxytocin undergoes excited-state proton transfer or any other excited-state process. Although we cannot entirely rule out excited-state processes, we conclude that the complex fluorescence decays of oxytocin and its two analogues are most likely the result of ground-state processes alone.

One ground-state process is obviously important in oxytocin. Comparison of the relative quantum yields based on steady-state data and lifetime parameters for oxytocin and desaminodicarbaoxytocin indicates that since the Φ_f/Φ_τ ratio of oxytocin is less than 1 (Table I), the disulfide bridge is linked to a static quenching process. A static quenching mechanism requires that a fraction of the possible emitters be eliminated as the result of a nonradiative, ground-state complex with a characteristic equilibrium constant (Noyes, 1961). The quenching mechanism could involve, for example, charge transfer, vibrational relaxation, or highly efficient energy transfer, depending on the chemical and physical nature of the complex. Static quenching will not change the fluorescence decay time constants of the unaffected population; its effect is to reduce the quantum yield. The large reduction in the relative quantum yield of oxytocin and desaminooxytocin, compared to desaminodicarbaoxytocin, suggests that a significant fraction of the tyrosine population is in a complex with the disulfide bridge. An interaction between the disulfide bridge and the tyrosine side chain has been detected by circular dichroism, and these studies have also suggested an interaction between the N-terminal amino group and the tyrosine side chain (Frič et al., 1974; Hruby et al., 1978). The amino group interaction may explain the small difference in the quantum yields of oxytocin and desaminooxytocin, although this difference is close to experimental error.

The multiexponential fluorescence decay of oxytocin and its two analogues could arise from a mixture of different

chemical and structural (conformational) species. The experimental conditions for data acquisition (pH 3) are such that only one chemical (ionic) form is present in solution. A number of structural species are possible, however, involving both phenol side-chain rotamer populations (Figure 2) and peptide backbone conformations. Indeed, NMR data suggest that in aqueous solution there is a considerable degree of flexibility in the backbone of oxytocin [for a review, see Glickson (1975)]. But regardless of the interconversions among backbone conformations, the general spatial relationship and interaction of the phenol group with neighboring moieties, such as the disulfide bridge and the two adjacent peptide linkages, depend primarily upon the rotameric state about the C^{α}–C^{β} bond.

The origin of the complex fluorescence decay kinetics for the tyrosyl residue in these oxytocin compounds could be due to ground-state heterogeneity induced by the three main phenol rotamers having different environments. This model requires that the interconversion between rotamers occur on the same time scale or on a slower time scale than the depopulation of the excited state. The results of the preceding paper (Laws et al., 1986) suggest that the exchange between the rotamers is slow when the α-amino and α-carboxyl groups of tyrosine are in peptide bonds. The slow-exchange rotamer model therefore predicts that, in the absence of excited-state reactions, the fluorescence decay of the oxytocin tyrosyl residue should be the sum of three exponentials with each rotamer having its own characteristic lifetime and that the relative weighting (amplitudes) of the fluorescence decay constants be equivalent to the rotamer populations determined by ^{1}H NMR (Laws et al., 1986).

It is conceivable that one or two of the oxytocin phenol rotamers is involved with the disulfide bond, eliminating that (those) rotamer(s) as a possible emitter. (All three rotamers cannot be statically quenched; oxytocin does exhibit tyrosine fluorescence.) The quantum yield of a rotamer is the product of its relative amplitude (population) and fluorescence lifetime. To account for the significant decrease in the steady-state quantum yield of oxytocin by static quenching, it is required that a nonradiative rotamer have either (1) a large population with a somewhat appreciable lifetime or (2) a small population with an otherwise long lifetime. The slow-exchange rotamer model for oxytocin with either one or two rotamers complexed with the disulfide then predicts either a double or single exponential decay, respectively. Since the fluorescence decay kinetics of the oxytocin tyrosyl residue are not single exponential, but double, only one rotamer can be involved in a ground-state complex with the disulfide bridge. The rotamer model, therefore, requires that the remaining two exponentials describing the decay of tyrosine fluorescence in oxytocin have relative weights (amplitudes) equivalent to the NMR-determined populations of the other two rotamers. Moreover, the fluorescence decay kinetics for the tyrosyl residue in desaminodicarbaoxytocin should be described by the sum of three exponentials with amplitudes reflecting all three rotamer populations.

Examination of the crystal structure detailing the backbone of desaminooxytocin (Pitts et al., 1985) indicates that rotamer I or III could have contact with the disulfide bridge. If rotamer I is quenched, the amplitude ratio for the oxytocin decay parameters should be about 3 to 1, on the basis of the rotamer populations given in Table II for rotamers II and III. If rotamer III is statically quenched, the ratio should be slightly greater than 1. The ratio of the amplitudes for the decay of oxytocin in Table I, however, is 3 to 1. Therefore, when all the physical chemical data are taken into consideration, rotamer I is the best assignment for the quenched species. In addition, this analysis assigns rotamer II in oxytocin to the short lifetime; the correlation of the short lifetime with rotamer II is consistent with the results of tyrosine model compounds (Laws et al., 1986).

Table III: Fluorescence Decay Parameters Based on a Rotamer/Linked-Amplitude Analysis

compd	α_1[a]	τ_1 (ns)	α_2[a]	τ_2 (ns)	α_3[a]	τ_3 (ns)
desaminodicarbaoxytocin	0.68	3.05	0.24	0.78	0.08	2.45
desaminodicarba-oxytocin[b]	0.70	3.05	0.22	0.7	0.08	1.9

[a] Amplitudes were normalized to a sum of 1.0. [b] τ_2 and τ_3 were held constant at the values obtained for a two-component analysis for oxytocin (Table I); all other parameters iterated freely, and there was no amplitude linkage.

According to this interpretation, certain predictions follow for the desaminodicarbaoxytocin analogue. If rotamer I is in a nonradiative complex in oxytocin, replacement of the disulfide with an ethylene bridge should allow rotamer I to emit. Therefore, three lifetimes are expected with preexponential terms equivalent to the ^{1}H NMR determined rotamer populations. Although the results in Table I show that the desaminodicarbaoxytocin data can be adequately fit in a free-floating analysis by the sum of two exponentials, the results are still consistent with the above rotamer model. The relative amplitude of 0.26 for the shorter lifetime of desaminodicarbaoxytocin correlates well with the 24% population of rotamer II. Again, this population/lifetime assignment is consistent with what we have previously observed with the tyrosine model compounds (Laws et al., 1986). In this case, rotamers I and III of desaminodicarbaoxytocin apparently have similar lifetimes which are difficult to resolve since rotamer III has only 8% of the phenol distribution (Table II).

The desaminodicarbaoxytocin data was further analyzed by using a function linking the amplitude terms to the ^{1}H NMR determined rotamer populations (Ross et al., 1986). This is a null hypothesis approach that will fail to resolve the expected three components if the slow-exchange rotamer model is inappropiate. The result of the linked-amplitude analysis is given in Table III. When the amplitudes are forced to be proportional to the rotamer populations given in Table II, the longer lifetime of the two-component analysis is split, with rotamer III having a slightly shorter lifetime than rotamer I; the shortest lifetime is essentially unchanged. Furthermore, this association was unique: *statistically adequate analysis required that only rotamer II be correlated with the shortest lifetime.*

The slow-exchange rotamer model was tested further by analyzing the oxytocin data for three components. Two approaches were used: first, the amplitudes and lifetimes were unrestricted; and second, the amplitudes were linked according to the ^{1}H NMR determined rotamer populations. When three components could be extracted in the unrestricted analysis, there was no correlation among the decay parameters between repeated data sets. The linked-function analysis for three components always resolved only two time constants. This result indicates that two time constants are sufficient to describe properly the fluorescence decay of oxytocin, consistent with the static quenching of one rotamer.

The desaminodicarbaoxytocin data can be analyzed for three components by using a conceptually different, independent approach. If rotamer I is in a ground-state complex and rotamers II and III are essentially unaffected by the disulfide bridge, then replacement by the ethylene bridge should affect

only rotamer I. Assuming that the two lifetimes for rotamers II and III have not changed, they can be fixed at the values found for the unrestricted two-component analysis of oxytocin (see Table I). A three-component analysis with two fixed decay constants, one free-floating decay constant, and three unrestricted amplitudes with no linkages should yield amplitudes consistent with the ^{1}H NMR determined rotamer populations and recover the lifetime due to rotamer I. The result of this analysis is shown in Table III. A third component of 3 ns is recovered, agreeing with the linked-amplitude analysis results discussed above. Although no assumptions were made about the relative weighting of the amplitudes, remarkable agreement is obtained with the rotamer populations. The fitting statistics with this approach were the same as those obtained for both the unrestricted, two-component and the linked-amplitude, three-component analyses.

It might be assumed that adding additional parameters will generally improve fitting statistics. This will be true if there is systematic error, or if an incorrect model was assumed. But it is also true that an equivalent statistical fit can be obtained with fewer parameters than predicted by the correct model, if some parameters have similar values (for example, desaminodicarbaoxytocin). Constraining parameters on the basis of additional information about the system, as in a linked-function or a fixed-parameter analysis, limits the parameter search space. Improper constraints will, therefore, generally result in poor fitting statistics. In this way, it is possible to discriminate among similar, related models. A constrained analysis for the adequate and sufficient model will have fitting statistics as good as those obtained from a free-floating analysis for fewer, but arbitrary, parameters.

The ability of the two conceptually different, three-component analyses (linked amplitudes or fixed lifetimes) to find equivalent, yet unique, solutions for the fluorescence decay of desaminodicarbaoxytocin that agree with the ^{1}H NMR results lends support to our slow-exchange rotamer model for the tyrosine phenol ring. The consistent agreement of relative amplitudes and ^{1}H NMR determined rotamer populations, paralleled by the persistent assignment of rotamer II to the shortest lifetime, is striking. The correlation between the number of exponential components, and their relative weighting, of tyrosyl residues in peptides with rotamer populations determined from ^{1}H NMR is further corroborated by recent preliminary studies on a synthetic cyclic pentapeptide. Bruch et al. (1985) have shown that in organic solvent the phenyl ring in *cyclo*(-D-Phe-Pro-Gly-D-Ala-Pro-) is constrained into only rotamer II with a χ^1 of 180°, in agreement with the crystal structure (Karle, 1981). We have synthesized the corresponding cyclic pentapeptide *cyclo*(-D-Tyr-Pro-Gly-D-Ala-Pro-). The ^{1}H NMR spectrum of the tyrosine-containing peptide in methanol is similar to that of the phenylalanine peptide. We therefore conclude that the phenol ring of tyrosine is also constrained into the same rotamer. Our preliminary time-resolved fluorescence results for this pentapeptide show a single exponential decay for the tyrosine emission. Further studies are in progress to characterize this model cyclic pentapeptide. We have also synthesized the corresponding tryptophan pentapeptide; we anticipate that comparison of the time-dependent fluorescence and ^{1}H NMR results will allow detailed examination of the mechanism(s) involved in the complex fluorescence decay kinetics of tryptophan.

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Identification of Polypeptides of the Phencyclidine Receptor of Rat Hippocampus by Photoaffinity Labeling with [^{3}H]Azidophencyclidine

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ABSTRACT: Polypeptide components of the phencyclidine (PCP) receptor present in rat hippocampus were identified with the photolabile derivative of phencyclidine [^{3}H]azidophencyclidine ([^{3}H]AZ-PCP). The labeled affinity probe was shown to reversibly bind to specific sites in the dark. The number of receptor sites bound is equal to those labeled by [^{3}H]PCP, and their pharmacology and stereospecificity are identical with those of the PCP/σ-opiate receptors. The dissociation constant of [^{3}H]AZ-PCP from these receptors is 0.25 ± 0.08 μM. Photolysis of hippocampus membranes preequilibrated with [^{3}H]AZ-PCP, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, revealed the existence of five major labeled bands of which a M_r 90 000 band and a M_r 33 000 band were heavily labeled. Inhibition experiments, in which membranes were incubated with [^{3}H]AZ-PCP in the presence of various PCP analogues and opiates, indicate that labeling of both the M_r 90 000 band and the M_r 33 000 band is sensitive to relatively low concentrations (10 μM) of potent PCP/σ receptor ligands, while similar concentrations of levoxadrol, naloxone, morphine, D-Ala-D-Leu-enkephalin, atropine, propranolol, and serotonin were all ineffective. Stereoselective inhibition of labeling of the M_r 90 000 band and of the M_r 33 000 band was also observed by the use of dexoxadrol and levoxadrol. The M_r 33 000 band was not as sensitive as the M_r 90 000 band to inhibition by the selective PCP receptor ligands *N*-[1-(2-thienyl)cyclohexyl]piperidine and PCP. Strong inhibition of labeling of the M_r 33 000 band by less selective PCP receptor ligands such as *N*-[1-(3-hydroxyphenyl)cyclohexyl]piperidine and (±)-*N*-allylnormetazocine was also observed. The labeling of the other three polypeptides (M_r 62 000, 49 000, and 40 000) was only mildly affected by dexoxadrol and (±)-*N*-allylnormetazocine, suggesting that this interaction does not have a classical PCP/σ receptor pharmacology. Thus, these labeled bands could be constituents of a second PCP receptor.

Phencyclidine (PCP,[1] angel dust) is a synthetic drug introduced originally in the late 1950s as a general anesthetic (Johnston et al., 1959; Domino, 1964). Because of its prolonged duration of action and its psychotomimetic effects, it

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