(4) J. E. Coleman, *J. Biol. Chem.*, **243**, 4574(1968).
(5) R. L. Ward, *Biochemistry*, **8**, 1879(1969).
(6) E. Shefter and P. Sackman, *J. Pharm. Sci.*, **60**, 282(1971).
(7) P. W. Taylor, R. W. King, and A. S. V. Burgen, *Biochemistry*, **9**, 2638(1970).
(8) *Ibid.*, **9**, 3894(1970).
(9) S. L. Bradbury, *J. Biol. Chem.*, **244**, 2002(1967).
(10) R. W. King and A. S. V. Burgen, *Biochim. Biophys. Acta*, **207**, 278(1970).
(11) J. A. Verpoorte, S. Mehta, and J. T. Edsall, *J. Biol. Chem.*, **242**, 4221(1967).
(12) P. W. Taylor and A. S. V. Burgen, *Biochemistry*, **10**, 3859(1971).
(13) P. W. Taylor, J. Feeney, and A. S. V. Burgen, *ibid.*, **10**, 3866(1971).
(14) M. E. (R.) Fabry, S. H. Koenig, and W. E. Schillinger, *J. Biol. Chem.*, **245**, 4256(1970).
(15) M. Eigen, *Ber. Bunsenges. Phys. Chem.*, **67**, 753(1963).
(16) M. Eigen and R. G. Wilkens, *Advan. Chem. Ser.*, **No. 49**, 55(1965).
(17) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, **37**, 307(1962).
(18) M. Eigen and L. de Maeyer, in "Technique in Organic Chemistry," vol. VIII, Part II, A. Weissberger, Ed., Wiley-Interscience, New York, N.Y., 1963, p. 895.

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Drug–Biomolecule Interactions: Proton Magnetic Resonance Studies of Complex Formation between Bovine Neurophysins and Oxytocin at Molecular Level

JOHN H. GRIFFIN *§, JACK S. COHEN *x, PAUL COHEN ‡, and MARYSE CAMIER ‡

Abstract □ Proton magnetic resonance spectroscopy was used to monitor individual amino acid residues in bovine neurophysin, in the nonapeptide hormone oxytocin, and in the complex formed between them. For neurophysin I alone, a normal titration curve for the C-2 proton resonance of the lone histidine residue was obtained with an apparent ionization constant of 6.9. Addition of oxytocin to a solution of neurophysin I at pH 6.5 resulted in several changes in the spectrum. The effect on the histidine C-2 proton resonance signal indicated a slow exchange process between two states, probably representing a conformational change in the protein. The apparent pK of the histidine residue in the hormonal complex was shifted to 6.7, indicating a slightly more positive (less electron dense) environment for the histidine residue. Resonances of the single tyrosine residue of oxytocin were observed to broaden significantly, but not to shift appreciably, on the addition of neurophysin II. These observations may indicate involvement of the tyrosyl residue of oxytocin in the hormone–"carrier protein" interaction.

Keyphrases □ Neurophysins, bovine—complex formation with oxytocin, PMR □ Oxytocin—complex formation with bovine neurophysins, PMR □ Hormonal interactions—bovine neurophysins–oxytocin, PMR □ PMR—monitoring bovine neurophysins–oxytocin complex formation □ Drug–biomolecule interactions—PMR studies of complex formation between bovine neurophysins and oxytocin at the molecular level □ Interactions—drugs with biomolecules, symposium

High-resolution proton magnetic resonance (PMR) has proved useful in providing information about selected residues in proteins during denaturation (1–3) and inhibitor–enzyme interactions (4, 5). (For reviews, see Refs. 6 and 7.) A few studies of protein–polypeptide or protein–protein interactions using PMR techniques have been reported (8–10).

The neurohypophyseal nonapeptides oxytocin and 8-arginine (or 8-lysine) vasopressin are biologically important hormones known to associate noncovalently with the proteins neurophysin I and neurophysin II (11, 12). These proteins are found in the neurosecretory granules of the pituitary glands of several species associated with the hormonal peptides (13). The physical and chemical properties of the neurophysins, which have monomeric molecular weights near 10,000, have been investigated (14, 15), and some amino acid sequences for bovine neurophysins I and II have been proposed (16, 17). The study of the molecular interactions between neurophysins and peptide hormones has been approached using classical equilibrium methods (18–22) and spectroscopic techniques (23–26).

This article presents PMR observations on highly purified bovine neurophysins, the hormone oxytocin, and the molecular complex formed between them. In particular, high-resolution PMR methods allow the study of the possible involvement of the lone histidine residue of neurophysin I or the single tyrosyl residue of oxytocin in the hormonal complexes[1].

[1] A preliminary account of some of this work has appeared (27).

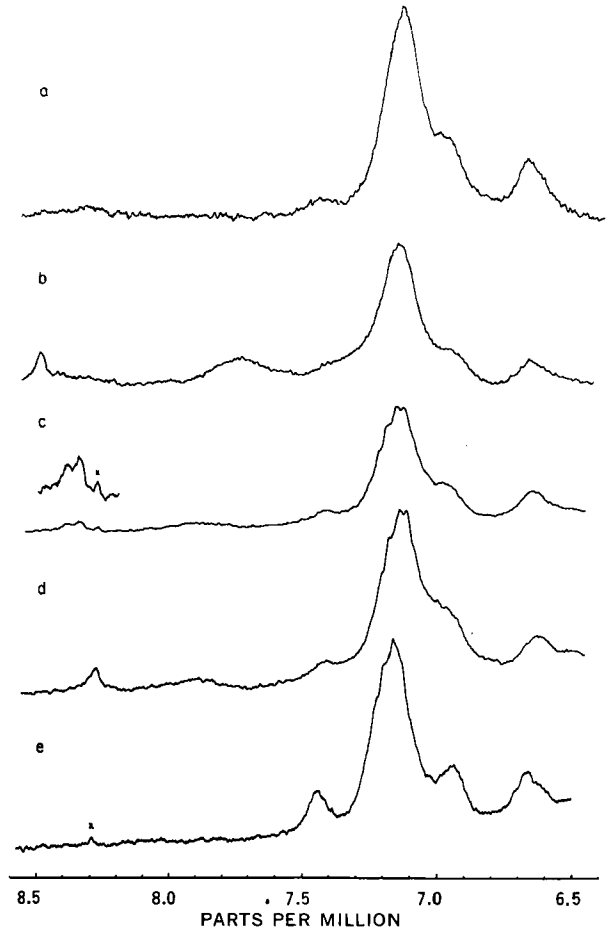

Figure 1—*Aromatic region of PMR spectra recorded in D_2O, 0.1 M NaCl, of neurophysin II at pH 6.52* (a) *and of neurophysin I at pH 2.21* (b), *5.77* (c), *6.09* (d), *and 8.85* (e). *The number of scans varied between 9 and 37. The insert in* (c) *is the same spectrum at higher gain. The peaks marked × are not due to neurophysin.*

EXPERIMENTAL[2]

Samples of neurophysins for PMR studies were lyophilized several times from D_2O and were dissolved in 0.1 *M* NaCl in D_2O[3] (100%) at a concentration of 30 mg in 0.5 ml. Measurements of pH were performed directly in the NMR sample tube as detailed previously (3), and the pH values quoted are direct meter readings[4].

Spectra were recorded on a spectrometer[5] with a probe temperature of 22 ± 1°. Time-averaging was carried out with a computer[6]. Other details of the PMR methods were described elsewhere (3). Chemical shift values are quoted in parts per million downfield from external 6% tetramethylsilane in carbon tetrachloride. Concentration measurements were checked spectrophotometrically[7]. For neurophysin, ϵ was taken as 3400 M^{-1} cm^{-1} at 260 nm (15); for oxytocin, an ϵ at 280 nm was taken as 1400 M^{-1} cm^{-1}.

As a control experiment for viscosity effects, neurohypophyseal proteins (mol. wt. ≃ 50,000) that eluted before the neurophysins during filtration[8] were used. This protein fraction was devoid of hormonal binding ability by equilibrium dialysis experiments carried out with radioactive oxytocin (22). The PMR spectrum of oxytocin was recorded in the presence of 15 mg of this protein and showed a negligible line-broadening effect (<10%).

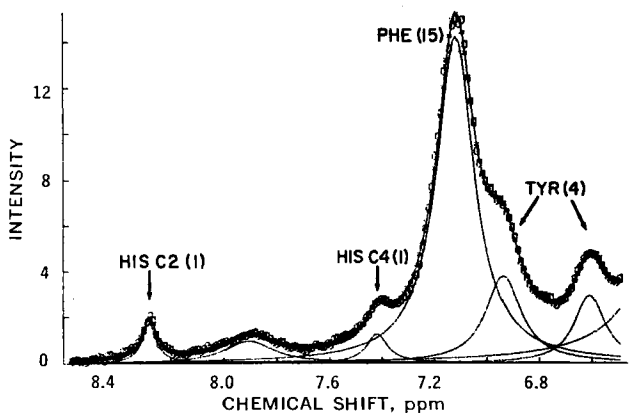

Figure 2—*PMR spectrum of neurophysin I from Fig. 1d fitted with Lorentzian curves. Key: O, observed; *, calculated; and ——, components. The assignments of the peaks are shown in the CALCOMP plot with the relative number of protons in parentheses.*

RESULTS AND DISCUSSION

Neurophysins—Aromatic protons absorb in a region of the PMR spectrum that is well resolved from the resonances of the much more numerous aliphatic protons. Imidazole C-2 ring proton resonances are even better resolved as a result of their unique chemical environment between two nitrogen atoms. The aromatic region of some PMR spectra of neurophysins I and II are shown in Fig. 1. The resonance in the low field region between 8.5 and 7.4 ppm in Figs. 1*b*–1*e* that shifts with pH may be attributed to the C-2 ring proton (8, 28, 29) of the single histidine residue of neurophysin I, which is absent from neurophysin II (30) (Fig. 1*a*).

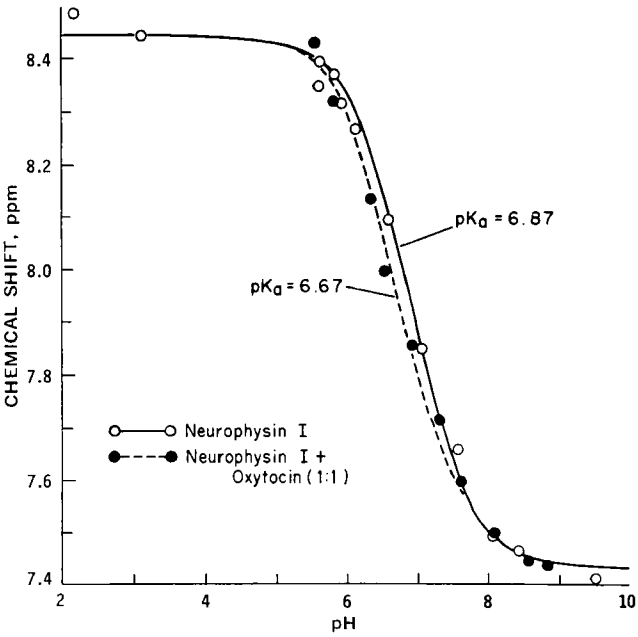

Figure 3—*Titration curves of the C-2 proton resonance of the single histidine residue of neurophysin I and of the neurophysin I–oxytocin complex. The lines are least-squares fits to the data using the theoretical expression for a simple proton association equilibrium (29).*

[2] Details of the preparation of highly purified bovine neurophysin have been described elsewhere (22). The synthetic peptide oxytocin was a gift from Dr. Guttmann, Sandoz, Basle, Switzerland.

[3] Aldrich Chemical Co.

[4] These values are not strictly pH because measurements were made in D_2O; they are not pD because measurements were made with a glass electrode containing water. It has been shown experimentally that the isotope effects on the glass electrode and on the ionization process itself almost cancel, so that no correction is made in these studies. [See D. H. Sachs, A. N. Schechter, and J. S. Cohen, *J. Biol. Chem.*, **246**, 6576(1971), and references cited therein.]

[5] Varian Associates HR 220.

[6] Varian C1024.

[7] Cary 15 spectrophotometer.

[8] Sephadex G-75.

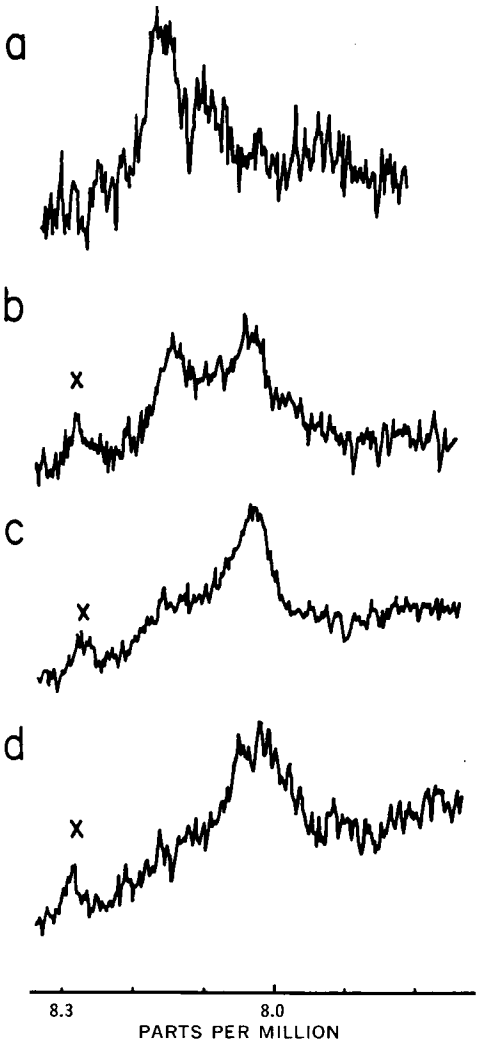

Figure 4—*PMR spectra showing the histidine C-2 proton resonance of neurophysin I at pH 6.51 in the absence of oxytocin* (a) *and at neurophysin–oxytocin molar ratios of 5:1* (b), *2.5:1* (c), *and 1:1* (d). *Solutions contained 0.1* M *NaCl in* D_2O. *The number of scans varied between 19 and 98. The peaks marked* × *are not due to neurophysin.*

Between pH 3.5 and 5.5, the resonance lines broaden due to aggregation of the protein as precipitation occurs near the isoelectric point, pH 4.4 (15, 22). Above pH 5, the protein redissolves and the lines correspondingly sharpen again. However, they remain fairly broad (5–20 Hz) considering the size of this protein compared to others studied previously (6, 31). This may result from dimer formation (15).

Another cause of line broadening may be the existence of a fairly rigid structure due to the presence of seven disulfide bonds. Reduction of internal motion causes the resonances to be broader by increasing the efficiency of dipolar relaxation between closely adjacent protons (32). The precise contribution of the two factors to the observed linewidth is difficult to estimate. A dilution by a factor of 5 to a concentration of 12 mg/ml produced no significant measurable reduction in linewidth.

The assignment of peaks in the low field region of a spectrum of neurophysin I (Fig. 1*d*) is shown in Fig. 2, in which the resonance peaks are fitted with Lorentzian functions (29). The broad resonance at chemical shift 7.75 ppm is attributed to protons that exchange slowly with water, since it disappeared entirely over 24 hr due to exchange with deuterium.

The pH titration data of the histidine C-2 proton resonance of neurophysin I are shown in Fig. 3. These data fit a theoretical curve based on a simple proton association equilibrium and give a pK value of 6.9, indicative of a normally titrating histidine residue (29). One unusual feature of the titration of the histidine C-2 proton resonance in this case was the consistent observation of a doubling of the peak at pH values between 5.6 and 5.9 (Fig. 1*c*). It was difficult to obtain spectra at slightly lower pH values due to precipitation of the protein (15); above pH 6.0, the phenomenon was no longer observable.

This doubling phenomenon could represent a slow equilibrium of the histidine residue between two environments, as previously suggested for similar observations involving histidine resonances (1, 3). Changes in the environment of the histidine residue may possibly be related to aggregation processes known to occur in this pH range (15).

Binding of Oxytocin to Neurophysins—Aliquots of oxytocin were added to a solution of neurophysin I at pH 6.5, and the effects on the PMR spectrum of the protein were monitored. A doubling of the histidine C-2 proton resonance was noted (Fig. 4). Since oxytocin does not contain a histidine residue, this phenomenon must be attributed to the effect of complex formation on the lone histidine in neurophysin I.

The presence of two separate resonances (Fig. 4*b*) at intermediate neurophysin–oxytocin molar ratios indicates a slow conformational equilibrium between two environments (1), for which the lifetime in one state must be greater than 13 msec (*i.e.*, $\gg 1/\pi\Delta\nu$, where $\Delta\nu$ is the separation between the two resonances, 25 Hz). This slow equilibrium could be the oxytocin binding process or a protein conformational change that exposes the histidine residue to two different environments. The latter possibility seems more likely in view of previous knowledge of the rates of protein conformational changes (33). No other significant changes in most regions of the spectrum were observable up to a molar ratio of 1:1.

The titration curve of the neurophysin I–oxytocin complex at a 1:1 molar ratio (>95% oxytocin binding sites filled) is shown in Fig. 3. The histidine pKa value is shifted 0.20 pH unit to a lower value due to complex formation, representing a somewhat more positive (less electron dense) environment for the histidine residue in the hormone–neurophysin I complex. Such a relatively minor pK shift suggests that the histidine residue does not play a significant role in the hormonal interaction. This conclusion might be expected

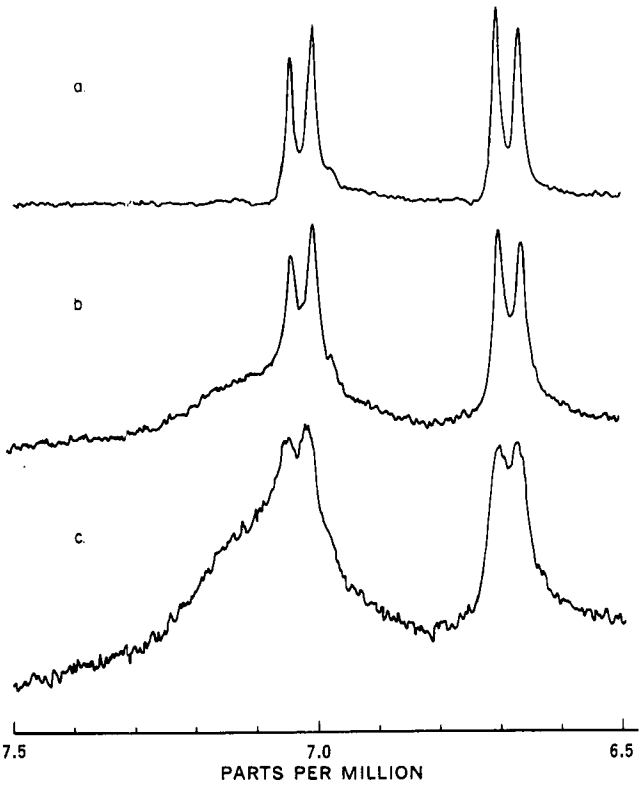

Figure 5—*Aromatic region of PMR spectra of oxytocin at pH 6.60* (a) *and after the addition of aliquots of neurophysin II to yield oxytocin–neurophysin molar ratios at pH 6.79 of 7:1* (b) *and 3:1* (c). *Solutions contained 0.1* M *NaCl in* D_2O.

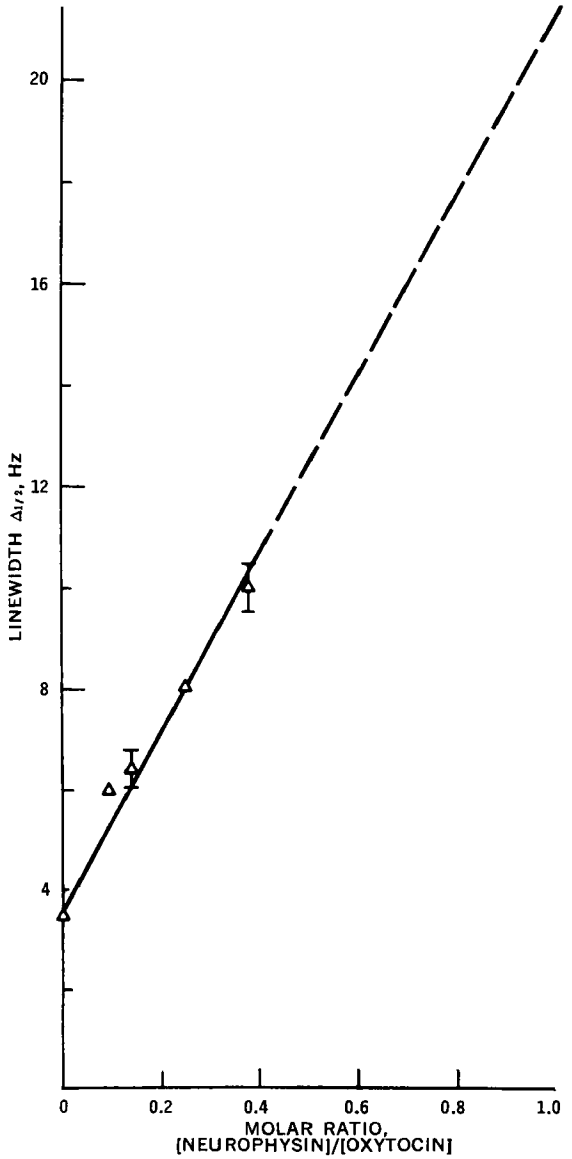

Figure 6—*Plot of the observed full linewidth at half-height of the tyrosyl resonance at 6.7 ppm of oxytocin* versus *the molar ratio of neurophysin–oxytocin.*

since neurophysin II binds oxytocin yet lacks a histidine residue.

The protein resonances obscure the oxytocin resonances in the aliphatic region of the PMR spectrum even at low protein to oxytocin molar ratios, and it is virtually impossible to distinguish the resonances of the two components. However, oxytocin contains a single tyrosine residue, and it is possible to follow the effects of the addition of neurophysin II on the tyrosyl aromatic protons. PMR spectra at several molar ratios are shown in Fig. 5. The mean value of the full width at half-height of the two tyrosine resonances in the high field doublet at 6.7 ppm is plotted in Fig. 6 as a function of the molar ratio of the total amount of each species in solution.

The apparent linear increase in linewidth of the oxytocin tyrosyl C-3 and C-5 proton resonances (Fig. 6) could arise from either a slow or a fast exchange process between two states for the tyrosyl residue. In the case of a simple bimolecular process (Scheme I):

$$\text{oxytocin} + \text{neurophysin} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{oxytocin–neurophysin}$$

Scheme I

the exchange of the tyrosyl residue between free and bound states can be described by equations derived for either the fast or the slow exchange limit (34). In the slow exchange limit:

$$\Delta_{1/2\,\text{obs}} = (1 - p)\Delta_{1/2\,\text{free}} + p/\tau \qquad \text{(Eq. 1)}$$

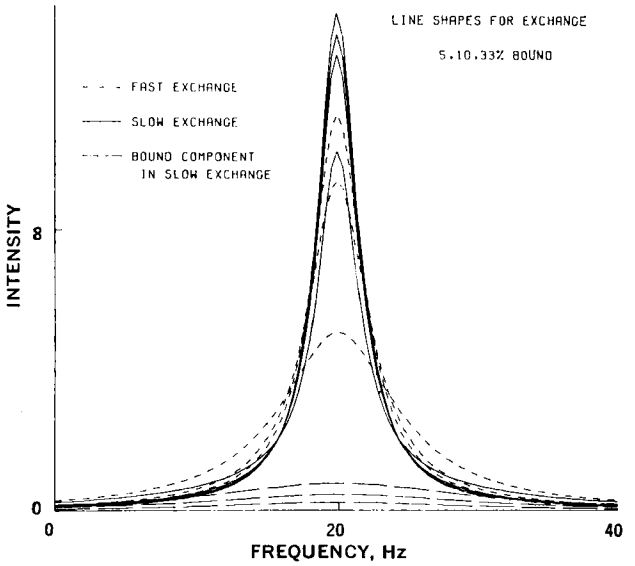

Figure 7—*Simulated peak shapes for fast and slow exchange conditions for a single Lorentzian-shaped peak. In both conditions the linewidth at half-height, $\Delta_{1/2}$, of the free component is 3.5 Hz, while that of the bound component, assumed to have the same chemical shift, is 21.5 Hz. In the slow exchange limit when $\tau \gg P$ (Eq. 1), $\Delta_{1/2obs} = \Delta_{1/2free}$, and the observed peak (solid line) is broadened by summation with the bound component. For slow exchange, the area of each of the two components is proportional to the amounts that are free and bound. For fast exchange the linewidth of the observed peak for different proportions bound was determined from Eq. 2 and is represented by the small dashed line. The actual observed values of $\Delta_{1/2}$ in each case would be:*

percent bound	*fast exchange*	*slow exchange*
0	*3.5 Hz*	*3.5 Hz*
5	*3.4 Hz*	*3.6 Hz*
10	*5.3 Hz*	*3.8 Hz*
33	*9.4 Hz*	*4.3 Hz*

while in the fast exchange limit:

$$\Delta_{1/2\,\text{obs}} = (1 - p)\Delta_{1/2\,\text{free}} + p\Delta_{1/2\,\text{bound}} \qquad \text{(Eq. 2)}$$

where $\Delta_{1/2}$ is the full linewidth at half-height, p is the fraction of oxytocin bound, and $\tau = 1/k_{-1}$, which is the mean lifetime of the complex. These equations apply when the concentration of free oxytocin is much greater than the concentration of the oxytocin–neurophysin complex and when:

$$\Delta_{1/2} = 1/\pi T_2 \qquad \text{(Eq. 3)}$$

where T_2 is the tranverse relaxation time of the observed proton.

Both Eqs. 1 and 2 can describe a linear dependence of the observed half-width on the amount of complex formed, as illustrated in Fig. 7. The possibilities of slow or fast exchange for the oxytocin–tyrosyl binding process can be distinguished. Studies at different temperatures and at different magnetic field strengths, coupled with direct measurement of the longitudinal relaxation time, T_1, should serve to distinguish these possibilities (34).

On the basis of preliminary work, Balaram *et al.*[9] concluded that the lysine vasopressin–neurophysin II complex exhibits slow exchange. However, Alazard *et al.*[10] recently found that oxytocin and lysine vasopressin show a fast exchange process with neurophysin II. This conclusion cannot necessarily be extended to all such complexes, not only because of the different hormones and conditions used but also because of recent evidence that these two peptide hormones bind differently (22).

[9] P. Balaram, A. A. Bothner-by, and E. Breslow, personal communication [see *Biochemistry*, **12**, 4695(1973)].

[10] R. Alazard, P. Cohen, J. H. Griffin, and J. S. Cohen, unpublished results.

If a slow exchange is assumed, evaluation of τ from Eq. 1 using the data shown in Fig. 6 leads to a value of 56 msec, which is consistent with the limiting value (*i.e.*, >13 msec) of the lifetime of the histidine residue in the hormonal complex. This value of τ gives 18 sec^{-1} for the dissociation rate constant, k_{-1}. Since the oxytocin–neurophysin dissociation constant, K_d, which equals k_{-1}/k_1, is approximately 2×10^{-5} M (22), an estimate of k_1, the bimolecular association rate constant, yields 9×10^5 M^{-1} sec^{-1}.

Alternatively, if a fast exchange is assumed, evaluation of $\Delta_{1/2\,bound}$ from Eq. 2 gives an extrapolated value of 22 Hz for the linewidth of the tyrosyl resonances in the complex at a 1:1 molar ratio. At this ratio, saturation of oxytocin binding sites on neurophysin occurs (19, 22). Since the observed linewidth is related through $1/T_2$ (Eq. 3) to the rotational correlation time of the tyrosyl side chain, the large increase in linewidth upon binding indicates a significant restriction in motion of the tyrosyl residue if fast exchange obtains. Involvement of the tyrosyl ring of oxytocin in the binding process, indicated here by PMR studies, is consistent with the apparent lack of binding capacity of 2-isoleucine-oxytocin (19) and of oxytocin iodinated on the tyrosyl ring (22). Experiments carried out with oligopeptides corresponding to the *N*-terminal amino acid sequence of the hormone also reinforce this idea (15, 25, 26).

Complex formation between neurophysin and oxytocin could involve hydrophobic or π–π interactions between neurophysin and the hormone's tyrosyl side chain as well as hydrogen bonding of the tyrosyl phenolic hydroxyl groups. However, π–π interactions seem unlikely in this case, since the stacking of aromatic rings produces measurable (>0.1 ppm) upfield shifts (35), and no significant change in chemical shift of the oxytocin tyrosyl resonances is observed at the highest molar ratios (Fig. 5).

Additionally, hydrogen bonding of the phenolic hydroxyl may not be essential for hormone binding since 2-phenylalanyl-oxytocin is bound almost as well as oxytocin (19). In this case, the tyrosyl residue of oxytocin seems to be bound in the hormone–neurophysin II complex through hydrophobic bonding[11].

REFERENCES

(1) C. C. McDonald and W. D. Phillips, in "Fine Structure of Proteins and Nucleic Acids," G. D. Fasman and S. N. Timasheff, Eds., Dekker, New York, N.Y., 1970, p. 1.

(2) C. C. McDonald, W. D. Phillips, and J. D. Glickson, *J. Amer. Chem. Soc.*, **93,** 235(1971).

(3) H. F. Epstein, A. N. Schechter, and J. S. Cohen, *Proc. Nat. Acad. Sci. USA*, **68,** 2042(1971).

(4) D. H. Meadows, G. C. K. Roberts, and O. Jardetzky, *J. Mol. Biol.*, **45,** 491(1969).

(5) J. D. Glickson, W. D. Phillips, and J. A. Rupley, *J. Amer. Chem. Soc.*, **93,** 4031(1971).

(6) G. C. K. Roberts and O. Jardetzky, *J. Biol. Chem.*, **245,** 1180(1970).

(7) J. S. Cohen, in "Experimental Methods in Biophysical Chemistry," C. Nicolau, Ed., Wiley, London, England, 1973, p. 521.

(8) D. H. Meadows, O. Jardetzky, R. M. Epand, H. H. Ruterjans, and H. A. Scheraga, *Proc. Nat. Acad. Sci. USA*, **60,** 766(1968).

(9) S. Ogawa and R. G. Shulman, *Biochem. Biophys. Res. Commun.*, **42,** 9(1971).

(10) F. M. Finn, J. Dadok, and A. A. Bothner-by, *Biochemistry*, **11,** 455(1972).

(11) J. Chauvet, M. T. Lenci, and R. Acher, *Biochim. Biophys. Acta*, **38,** 226(1960).

(12) L. O. Uttenthal and D. B. Hope, *Biochem. J.*, **116,** 899(1970).

(13) C. R. Dean, D. B. Hope, and T. Kazic, *Brit. J. Pharmacol.*, **34,** 192P(1968).

(14) T. C. Wuu and M. Saffran, *J. Biol. Chem.*, **244,** 482(1969).

(15) E. Breslow, H. L. Aanning, L. Abrash, and M. Schmir, *ibid.*, **246,** 5179(1971).

(16) R. Walter, D. H. Schlesinger, I. L. Schwartz, and J. D. Capra, *Biochem. Biophys. Res. Commun.*, **44,** 293(1971).

(17) J. D. Capra, J. M. Kehoe, D. Kotelchuck, R. Walters, and E. Breslow, *Proc. Nat. Acad. Sci. USA*, **69,** 431(1972).

(18) M. Ginsburg and M. Ireland, *J. Endocrinol.*, **32,** 187(1965).

(19) E. Breslow and L. Abrash, *Proc. Nat. Acad. Sci. USA*, **56,** 640(1966).

(20) R. Rauch, M. D. Hollenberg, and D. B. Hope, *Biochem. J.*, **115,** 473(1969).

(21) E. Breslow and R. Walter, *Mol. Pharmacol.*, **8,** 75(1972).

(22) M. Camier, R. Alazard, P. Pradelles, J. T. Morgat, P. Fromageot, and P. Cohen, *Eur. J. Biochem.*, **32,** 207(1973).

(23) E. Breslow, *Proc. Nat. Acad. Sci. USA*, **67,** 493(1970).

(24) A. J. Furth and D. B. Hope, *Biochem. J.*, **116,** 187(1965).

(25) P. Balaram, A. Bothner-by, and E. Breslow, *J. Amer. Chem. Soc.*, **94,** 4017(1972).

(26) E. Breslow and J. Weis, *Biochemistry*, **11,** 3474(1972).

(27) P. Cohen, J. H. Griffin, M. Camier, M. Caizergues, P. Fromageot, and J. S. Cohen, *FEBS Lett.*, **25,** 282(1972).

(28) J. H. Bradbury and H. A. Scheraga, *J. Amer. Chem. Soc.*, **88,** 420(1966).

(29) J. S. Cohen, R. I. Shrager, M. McNeel, and A. N. Schechter, *Nature*, **228,** 642(1970).

(30) M. D. Hollenberg and D. B. Hope, *Biochem. J.*, **106,** 557(1968).

(31) C. C. McDonald and W. D. Phillips, in "Magnetic Resonance in Biological Systems," A. Ehrenberg, B. Malmstrom, and T. Vanngard, Eds., Pergamon, London, England, 1967, p. 3.

(32) J. H. Bradbury, B. E. Chapman, and N. L. R. King, *Int. J. Prot. Res.*, **3,** 351(1971).

(33) G. G. Hammes, *Advan. Protein Chem.*, **23,** 1(1968).

(34) B. D. Sykes, P. G. Schmidt, and G. R. Stark, *J. Biol. Chem.*, **245,** 1180(1970).

(35) R. Deslauriers and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **40,** 179(1970).

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[11] UV absorption difference spectroscopy provides evidence for this [R. Alazard, J. H. Griffin, and P. Cohen, *J. Biol. Chem.*, **248,** 7975(1973)].