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TRYPTAMINE-ADENOSINE 5'-MONOPHOSPHATE INTERACTIONS AS STUDIED BY NUCLEAR MAGNETIC RESONANCE AND RELAXATION

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Summary

The conformation of tryptamine-adenosine 5'-monosphate and of their 1 : 1 complex in neutral aqueous solution at 297 K has been investigated by proton NMR and relaxation. The dependences of the proton chemical shift as a function of the tryptamine and AMP concentrations yield an association constant of $6.5 \pm 0.5\ l \cdot mol^{-1}$. The reorientation correlation time of the complex $\tau_R = (2.5 \pm 0.1) \cdot 10^{-10}$ s has been determined from the deuteron and ESR linewidth measurements on specifically labelled AMP. The proton longitudinal relaxation shows that the adenine and indole rings are head-to-head stacked 0.31 ± 0.01 nm apart as confirmed by proton chemical shift measurements. In this complex, the AMP ribose ring takes the 3'-endo (N) conformation and the orientation of the adenine base is *anti*, whereas the tryptamine aminoethyl residue, in the *gauche* conformation, is most likely bound to the phosphate by coulombic interactions.

Introduction

Specific interactions betweeen protein side chains and nucleic acids play a key role in the recognition processes occurring in the living cell. They take place in most of the essential steps of the metabolism such as nucleic acid-nuclease interactions, genetic expression and regulation as well as in the control of enzymatic activity by activators or inhibitors [1—3]. These interactions must be very specific and much work has been devoted to the determination of the possible types of interactions between amino acid side chains and

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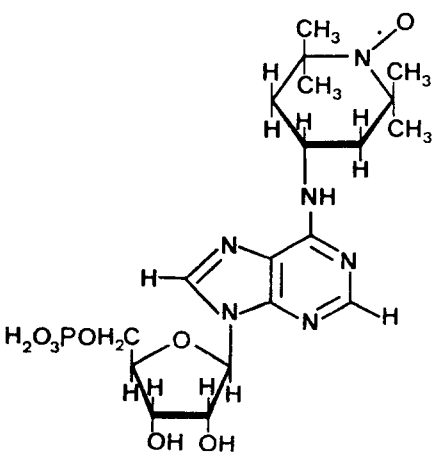
nucleic acid moieties, namely: (1) electrostatic interactions between basic amino acids and nucleic acids [4,5]; (2) interactions between aromatic amino acids and nucleic bases by stacking [6,7] or by hydrogen bonding [8], and (3) specific hydrogen bonding between aspartic or glutamic acids and bases [9].

This work deals with the specific interactions occurring between the indole ring of tryptophan or derivatives and adenosine 5′-monophosphate (AMP). Instead of tryptophan, tryptamine was chosen for this study because of its larger solubility in water at neutral pH. Moreover the absence of carboxyl group prevents the electrostatic repulsion with the phosphate group of AMP at neutral pH. Since this type of interaction was evidenced [10] several methods have been used to obtain more information about this complex, i.e. differential ultraviolet spectroscopy [11], ^{1}H-NMR [12] and ^{13}C-NMR [13] at comparatively high concentrations, but up to now no precise information has been obtained on the geometry of the complex. The use of induced chemical shifts for this determination has to be complemented by another method, since the complexation gives rise to large conformational changes in the nucleotide itself. For that purpose we have used the proton relaxation which appears to be a very convenient method in the investigation of the dynamical behavior and the conformation of nucleotides [14—20]. The proton NMR experiments were completed by some measurements on other nuclei such as ^{2}H, ^{13}C and ^{31}P as well as by ESR experiments on nitroxide spin-labelled AMP to obtain some subsidiary dynamic and conformational information on the molecules under study.

Materials and Methods

Tryptamine hydrochloride (Fluka AG) was recrystallized twice from C_2H_5OH/diethyl ether. After dissolution in $^{2}H_2O$ and adjustment to $p^{2}H = 7.8$ (meter reading), all paramagnetic impurities were removed by treatment with Chelex 100. The filtered solution was freeze-dried twice from $^{2}H_2O$ and stored at −30° C in the dark. Adenosine 5′-monophosphate sodium salt was prepared by the same method. Nitroxide spin-labelled AMP was prepared by the aminolysis of 6-chloropurine riboside monophosphate with nitroxide spin-labelled amine according to the procedure of Gaffney [21].



Nitroxide spin-labelled AMP.

Specific deuteration of AMP at the 8 position was obtained by heating a 0.1 M solution of AMP in ^2H_2O ($p^2H \approx 8$) at 95°C for 3 h. Over 95% exchange was obtained under these conditions. All solutions were prepared by dissolving the dry compounds in (99.95% ^2H) ^2H_2O. In all cases the pH was 7.8 (meter reading) and the temperature was regulated at 297 K.

^1H-NMR experiments were performed with at 250 MHz with a Cameca TSN 250 spectrometer. All chemical shifts are given relative to internal $5 \cdot 10^{-4}$ M *tert*-butanol. The other usual internal references for aqueous solutions (sodium 2,2-dimethyl-2-dilapentane-5-sulfonate (DSS) or sodium tetramethyl-silyl-propionate d_4 (TSP)) cannot be used because of their interaction with aromatic rings [22]. The ^1H and ^{13}C longitudinal relaxation rates R_1 were obtained by the inversion recovery method. The delay between the 180°, τ, 90° sequences was taken to be at least five times larger than the value of R_1^{-1} estimated from preliminary experiments. The ^{31}P and ^2H NMR spectra were recorded with a XL100 Varian spectrometer operating at 40 MHz and 15.35 MHz, respectively. The ^{13}C experiments were run with a CFT 20 Varian spectrometer at 20 MHz. Some subsidiary ESR experiments were performed by means of an E9 Varian X band spectrometer operating at 9.2 GHz with a 100 kHz magnetic field modulation.

Results and Discussion

Conformation of free tryptamine and AMP

Before examining the conformation of the tryptamine-AMP complex, we shall report the main results of our NMR and relaxation study on the free components of the complex under similar experimental conditions to have a better understanding of conformation changes occurring upon complexation. The principles and methods of interpretation of relaxation data in terms of conformation and molecular motions may be found in Refs. 14, 17, 23—27.

The populations of the *trans* (T) and *gauche* (G^+, G^-) rotamers of the C_3-C_β-C_α-N residue of tryptamine about the C_α-C_β bond has been estimated from the vicinal coupling constant of α and β protons ($^3J_{\alpha\beta} = 6.5$ Hz) by means of the Karplus relation, with the parameters of Kopple et al. [28] or Pachler [29] which yield $P_T \approx 0.4$, $P_{G^+} = P_{G^-} \approx 0.3$. The isotropic reorientation correlation time τ_R of the whole molecule was derived from the longitudinal relaxation rate of the indole ring carbons 13 (Table I). The interconversion rates $W_1(T \rightarrow G^+, G^-)$, $W_2(G^+, G^- \rightarrow T)$, $W_3(G^+ \rightleftharpoons G^-)$ of the aminoethyl group were obtained from the ^1H and ^{13}C longitudinal relaxation rates in the α and β methylene groups [24—27]. A fair agreement between the experimental and computed relaxation rates (Table I) was achieved by assuming that $(CH_2)_\beta$ undergoes 90° jumps among two equivalent sites about C_3-C_β, the rate of which is approx. 10^{10} s^{-1}, whereas the reorientation of $(CH_2)_\alpha$ occurs by 120° jumps among three sites about C_α-C_β with $W_1 \approx 1.5 \cdot 10^9$ s^{-1}, $W_2 = 2.0 \cdot 10^9$ s^{-1} and $W_3 \leqslant 10^8$ s^{-1} (Fig. 1). The equilibrium positions of C_α-C_β about the C_3-C_β bond were obtained from the H2 and H4 initial relaxation rates R_1^0 which are strongly dependent upon dipolar interactions with α and β methylene protons. From the computed dependence of $(R_1^0)_{H2}$ and $(R_1^0)_{H4}$ upon the rotational angle φ about C_3-C_β, it appears that the aminoethyl group of tryptamine oscillates among two

TABLE I

^1H AND ^{13}C EXPERIMENTAL AND COMPUTED RELAXATION RATES OF FREE TRYPTAMINE

Exp. experimental; calcd., calculated. *N* denotes the number of attached protons

	(R_1^0) (s^{-1})						
	H2	H4	H5	H6	H7	Hα	Hβ
^1H(297 K; $\tau_R = 3.5 \cdot 10^{-11}$ s)							
Exp.	0.21	0.30	0.25	0.28	0.18	0.66	0.72
Calcd.	0.218	0.297	0.288	0.288	0.136	0.645	0.674
	$(NT_1)^{-1}$ (s^{-1})						
	Indole methine carbons					Cα	Cβ
^{13}C(303 K; $\tau_T = 2.66 \cdot 10^{-11}$ s)							
Exp.	0.57					0.42	0.42
Calcd.	0.57					0.418	0.424

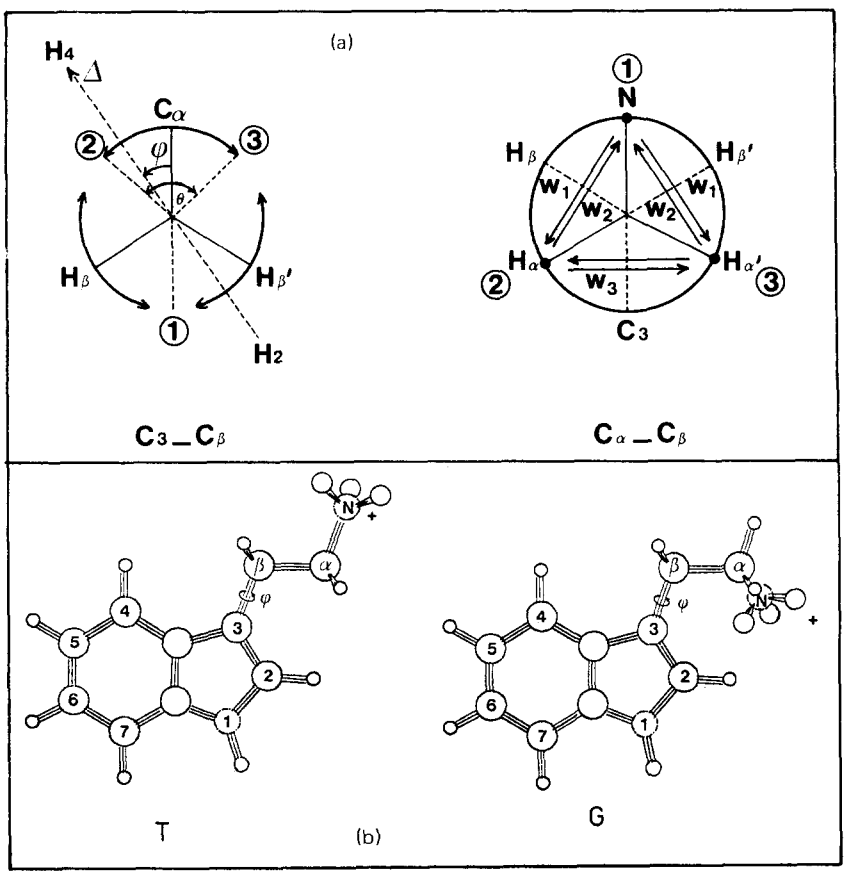


Fig. 1. (a) Models for the internal motions of tryptamine about C3-Cβ and Cα-Cβ bonds. Δ, projection of the indole ring onto a plane perpendicular to C3-Cβ. The angle φ defines the mean orientation of the Cα-Cβ bond for 90° rotational jump about C3-Cβ. $\varphi = 0$ corresponds to the shortest Cβ-H2 distance. (b) *Trans* (T) and *gauche* (G) rotamers of the tryptamine aminoethyl group.

positions 45° apart from the indole plane, the $C_\beta H_\beta$ bonds being oriented toward H4 (Fig. 1).

The conformations of AMP in aqueous solutions has been investigated by conventional NMR [30,31] as well as by nuclear relaxation methods [16,17,20, 32]. Some discrepancies appearing in the determination of the *syn* ⇌ *anti* equilibrium of the base, which may be due in part to temperature and concentration effects (see for instance Ref. 20), prompted us to reexamine this problem under the present experimental conditions. The preferential orientations of the base have been derived from the proton longitudinal relaxation rates of all non-exchangeable protons, according to a least-square method reported previously [17] and by measuring the changes in these rates upon substitution of H8 by a deuteron. The main orientations of the base are indicated in Table II by the torsional angle Υ increasing clockwise about $\overrightarrow{N9C1'}$ from the position where the H8—H1′ distance is minimal. This table shows that for the N form of the ribose ring there is almost an equal population of the *syn* and *anti* conformers of the base, whereas the *syn* conformer is predominant for the S conformer of the ribose ring.

Stoichiometry and conformation of the AMP-tryptamine complex

The stoichiometry of the complex has been obtained by plotting the proton chemical shifts in tryptamine and AMP as a function of the molar fractions ([AMP]/([AMP] + [tryptamine])) and ([tryptamine]/([AMP] + [tryptamine])), the total concentration of substrates being constant (Job plots [33]). Figs. 2 and 3 show that the maximum of the Job plots occurs exactly for the 0.5 molar fraction of AMP and tryptamine. A 1 : 1 complex is therefore formed as in the case of the tryptophan-AMP system [34,35].

The tryptamine-AMP association constant as well as the value of any observable Δ in the complex was obtained by the Benesi-Hildebrand method [36].

TABLE II

PROTON RELAXATION RATES AND CONFORMATIONS OF FREE AMP 10^{-2} M AT 297 K

10^{-2} M free AMP was used at 297 K. Exp., experimental; calcd., calculated.

		H8	H1′	H2′	H3′	H4′	H5′ H5″
(R_1^0) (s^{-1})	Exp.	0.53	0.39	0.97	1.00	1.06	3.56
(normal AMP)	Calcd. *	0.514	0.404	0.979	1.03	1.013	3.241
$\langle r_{8i} \rangle$	Calcd. *	$\langle r_{81'} \rangle = 0.288$ nm, $\langle r_{82'} \rangle = 0.269$ nm					
			H1′	H2′	H3′	H4′	H5′ H5″
(R_1^0) (s^{-1})	Exp.		0.28	0.71	1.10	1.10	3.33
AMP deuterated in position 8	Calcd. *		0.256	0.757	0.91	1.01	3.23
$\langle r_{8i} \rangle$	Exp. **	$\langle r_{81'} \rangle = 0.304$ nm, $\langle r_{82'} \rangle = 0.264$ nm					
Main orientations of the base	$\Upsilon 1N = 75°$		$P_{1N} = 0.23$				
	$\Upsilon 2N = 180°$		$P_{2N} = 0.21$		$\tau_R = 1.09 \cdot 10^{-10}$ s		
	$\Upsilon 1S = 55°$		$P_{1S} = 0.55$				

* Calculated from the method of Ref. 17.

** Obtained from the change of the relaxation rates of H1′ and H2′ when H8 is substituted by a deuteron.

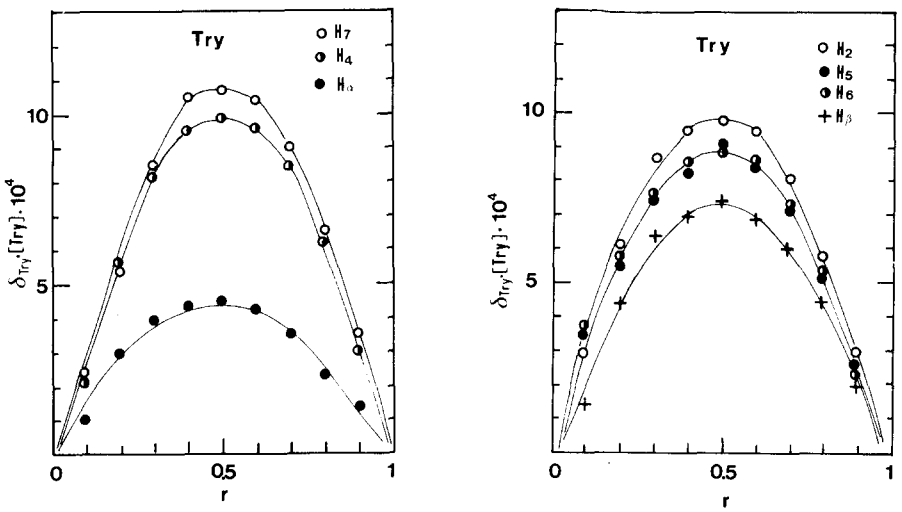


Fig. 2. Job plots of the chemical shifts (upfield) of tryptamine protons. r = [tryptamine]/([tryptamine] + [AMP]).

Here Δ is the proton chemical shift, relaxation rates $(R_1^0)_i$, vicinal coupling $J_{1'2'}$ in AMP, the deuteron and ESR linewidths (nitroxide spin-labelled AMP) or the correlation time τ_R deduced thereof.

A and B being the components of the complex under study, it is readily shown that for [B] >> [A]:

$$\frac{1}{[B]} = \frac{\Delta_C^A}{\Delta_{obs}^A} K - K \qquad (1)$$

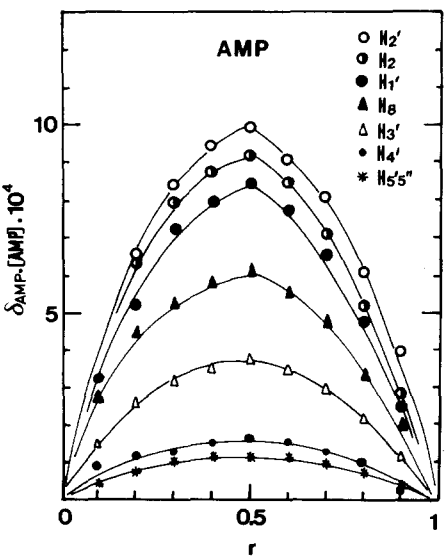


Fig. 3. Job plot of the chemical shifts of AMP protons. (r = [AMP]/([AMP] + [tryptamine])). All the shifts are upfield except that of H5'.

Δ^A_{obs} is the difference observed for Δ in the presence and in the absence of B, Δ^A_C the value of Δ in the complex and K the association constant of the AB complex. K and Δ^A_C are therefore obtained from the horizontal axis intercept and the slope of $1/\Delta^A_{obs}$ vs. $1/[B]$ (Fig. 4). From the proton chemical shifts in AMP and tryptamine, it was found that $K = 6.5 \pm 0.5\ l \cdot mol^{-1}$. This value was confirmed by the deuteron and ESR linewidths (see below).

The conformation of the ribose ring and of the phosphoester exocyclic group of AMP in the tryptamine-AMP complex was derived from the dependence of $J_{1'2'}$, $J_{4'5'5''}$ and $J_{PH5'5''}$ as a function of the tryptamine concentration. The two latter coupling constants equal to 3.5 Hz and 5 Hz, respectively, remain virtually the same as in free AMP, showing that in the complex the H4'-C4'-C5'-O5' and C4'-C5'-O5'-P residues are predominantly in the *trans* conformation [31]. On the other hand $J_{1'2'}$ decreases from 5.9 Hz in AMP to approx. 2 Hz in the complex, indicating that the 3'-endo conformer of the ribose becomes predominant with $P_N = (9.3 - J_{1'2'})/9.3 \approx 0.8$ [31]. A similar effect is reported in the case of the tryptophan-AMP complex [34].

These main conformers were adopted in the determination of the geometry

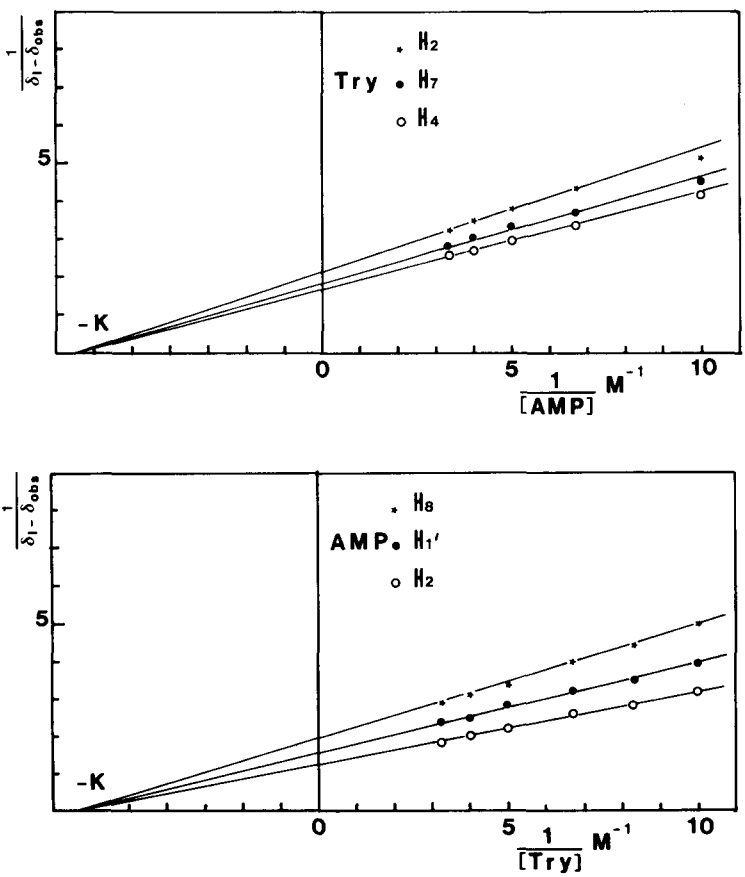


Fig. 4. Benesi-Hildebrand plots of the proton chemical shifts of AMP and tryptamine.

of the complex by proton longitudinal relaxation. The position of the tryptamine indole group with respect to the adenine ring of AMP was defined by the position of the $\vec{A'B'}$ vector joining the centers of the six and five-membered rings of the former, relative to the XYZ reference frame fixed to the center A of the adenine six-membered ring, the X-axis being directed along $\vec{AB}$ (Fig. 5). Assuming that in the complex, the indole and adenine rings are parallel, the proton relaxation rates have been computed as a function of the interplanar distance z and of the angle ψ between $\vec{AB}$ and $\vec{A'B'}$, the latter rotating about the axis $X = R$, $Y = 0$, R being an adjustable parameter. Two series of calculations were performed taking the ordinate of the N1 from tryptamine positive or negative for $\psi = 0$. The angle Υ defining the orientation of the base about the AMP N9C1′ bond was varied over the sterically allowed ranges namely $60° < \Upsilon_{syn} < 80°$ and $180° < \Upsilon_{anti} < 240°$.

The reorientation correlation time τ_R of the complex assumed to be rigid was derived from the linewidth $\Delta\nu_{1/2}$ of the deuteron in position 8 of the base of AMP, which is related to the quadrupolar transverse relaxation time T_{2q} by:

$$T_{2q}^{-1} = \pi\Delta\nu_{1/2} = \frac{3}{80}\left(\frac{eQq}{\hbar}\right)^2\left[3\tau_R + \frac{5\tau_R}{1+\omega_0^2\tau_R^2} + \frac{2\tau_R}{1+4\omega_0^2\tau_R^2}\right] \tag{2}$$

where $eQq/\hbar = 1.1 \cdot 10^6$ rad $\cdot$ s^{-1} is the deuteron quadrupole coupling constant [37] and ω_0 the Larmor angular frequency. The deuteron linewidth in the complex was obtained by the Benesi-Hildebrand method at increasing concentration of tryptamine, yielding $\Delta\nu_{1/2} = 37 \pm 1$ Hz i.e. $\tau_R \approx 2.6 \cdot 10^{-10}$ s. This value was confirmed by the measurement of the ESR linewidth of the nitroxide spin-labelled AMP under the same conditions, τ_R being given by Ref. 38.

$$\tau_R = \frac{4T_2(0)^{-1}}{b^2}\left[\left(\frac{I_0}{I_+}\right)^{1/2} + \left(\frac{I_0}{I_-}\right)^{1/2} - 2\right] \tag{3}$$

where I_0, I_+, I_- are the respective amplitudes of the $M_N = 0, +1, -1$ lines of the nitroxide ESR triplet and $T_2(0)$ the electron transverse relaxation time derived from the width of the $M_N = 0$ line. $b = 55$ MHz is a constant dependent upon

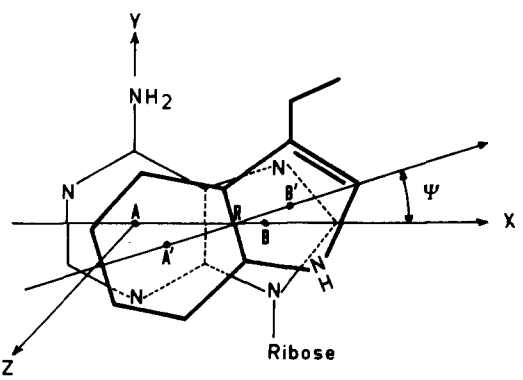


Fig. 5. Orientation of the tryptamine indole ring with respect to the AMP adenine base. R, the abscissa of the rotation axis of tryptamine about the z direction.

the principal values of the nitrogen hyperfine coupling tensor, readily obtained from the ESR spectrum of the frozen solution. At 297 K the ESR yields $\tau_R = 2.5 \cdot 10^{-10}$ s in excellent agreement with the value given above.

To find the most likely conformation of the tryptamine-AMP complex, a large number of calculations were performed, varying ψ from 0 to 360°, z and R in the $-0.4 < z < 0.4$ nm, $-0.2 < R < 0.2$ nm ranges and Υ in the domains defined above. It is assumed that the orientation of the aminoethyl group of tryptamine with respect to the indole ring corresponds to $\varphi = 0$, the C3-Cβ-Cα-N residue being in the *gauche* or *trans* conformation (Fig. 1b).

In spite of the multiple parameters involved in the determination of the geometry of the AMP-tryptamine complex, the number of conformation seemingly consistent with the proton relaxation data (Table III) is quite limited. It appears first that in the complex, the AMP takes the N conformation of the ribose ring with the *anti* orientation of the base ($\Upsilon = 180 \pm 10°$) as confirmed by deuteration in position 8 of the base. The indole ring is 0.31 ± 0.1 nm above the adenine base from AMP, on the same side as O1′ with respect to this plane. The relaxation of the tryptamine H2 is consistent only with a *gauche* conformation of the aminoethyl group. Furthermore, the $\vec{AB}$ vector is nearly coincident with the projection of $\vec{A'B'}$ on the adenine ring, which is therefore head-to-head stacked to the indole ring. This orientation has been clearly evidenced from the dependence upon the rotational angle ψ of the tryptamine H2, H4 and H7 and of AMP H2, H8 and H1′ proton relaxation rates which are the most sensitive to the relative position of the two molecules (Fig. 6). The angle ψ has been obtained from the minimization of the standard deviation.

$$\sigma = \frac{1}{\bar{x}}\left(\frac{\Sigma(x^2 - n\bar{x}^2)}{n}\right)^{1/2} \tag{4}$$

where x is the ratio of the longitudinal relaxation rates of the above-mentioned protons computed as a function of ψ and the observed ones. Fig. 7 shows that the minimum of σ occurs at $\psi \approx 0$. In this conformation, the tryptamine amino group which is protonated under our experimental conditions ($p^2H = 7.8$,

TABLE III

EXPERIMENTAL AND COMPUTED PROTON RELAXATION RATES IN THE 1 : 1 TRYPTAMINE-AMP COMPLEX

Proton relaxation rates are in s^{-1}. $T = 297$ K. Exp., experimental; calcd., calculated.

	$(R_1^0)_i$						
	H2	H4	H5	H6	H7	Hα	Hβ
Tryptamine							
Exp.	0.96	1.70	1.47	1.22	0.85	3.60	4.35
Calcd.	1.04	1.26	1.40	1.54	0.89	5.93	6.13
	H2	H8	H1′	H2′	H3′	H4′	H5′ H5″
AMP							
Exp.	0.38	1.25	0.83	1.67	1.67	2.0	5.0
Calcd.	0.37	1.12	0.84	1.39	1.80	1.82	5.6

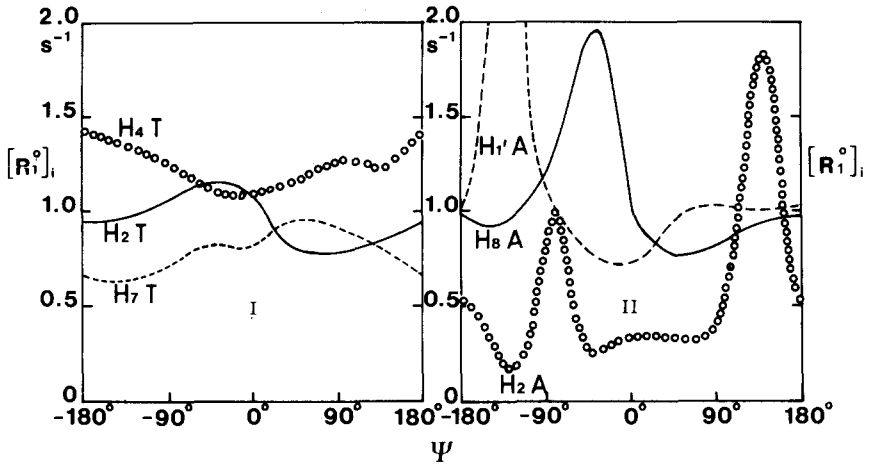


Fig. 6. Computed dependence of the relaxation rates of H2, H4, H7 from tryptamine (I) and of H2, H8, H1′ from AMP (II) upon the angle ψ defined in Fig. 5. Here $R = 0.105$ nm, $z = 0.31$ nm. The AMP is in the N, *anti* ($\Upsilon = 180°$) conformation. The *gauche* conformer of the aminoethyl group is assumed for tryptamine.

$pK_{tryp} \approx 9.0$) experiences most likely a coulombic interaction with the ionized phosphate group (Fig. 8).

The availability of the theoretical iso-shielding curves of nucleic bases [39] makes possible a semi-quantitative interpretation of the proton chemical shift differences which occur upon the formation of the tryptamine-AMP complex. Our geometrical model for this complex corresponds to a distance of 0.55–0.6 nm of H2′ and H3′ to the indole ring. The comparatively large upfield shift of 0.8 ppm observed for H2′ upon complexation (Table IV) may be therefore assessed to a change in the average orientation of the adenine base which moves to 100% *anti*. The calculated contributions to this shift are 0.6 and 0.1 ppm from the adenine and indole rings, respectively. A similar calculation performed

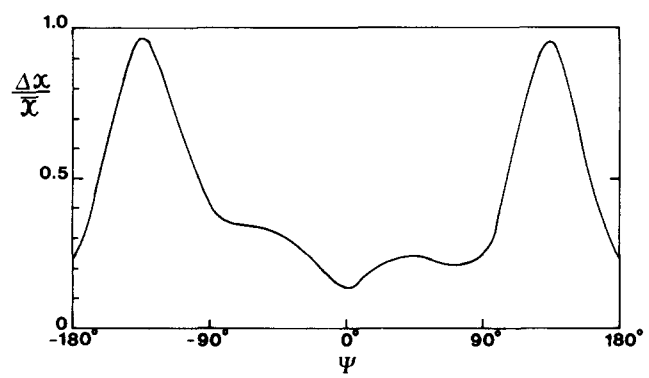


Fig. 7. Minimization of the standard deviation between the computed relaxation rates of H2, H4, H7 (tryptamine), H2, H8, H1′ (AMP) and the experimental ones as a function of the relative orientation of the indole and adenine rings. Same assumption as in Fig. 6.

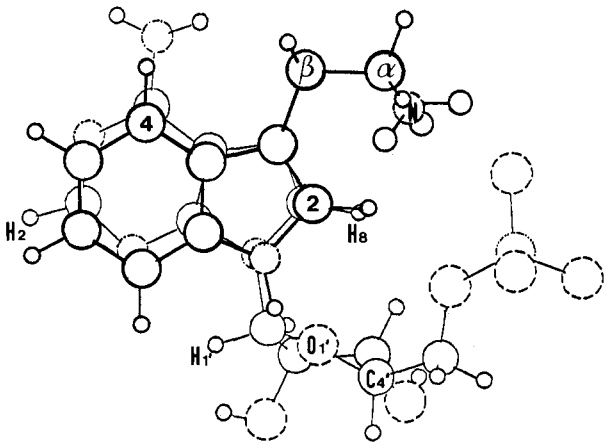


Fig. 8. Representation of the AMP-tryptamine complex. This complex as well as tryptamin represented in Fig. 1. has been plotted by means of an APL computer program [40].

for H3′ yields a 0.25 ppm upfield in reasonable agreement with the observed value of 0.3 ppm. H4′ is quite remote from the aromatic ring centers and its resonance shift cannot be accurately calculated. A shift of the order of 0.1 ppm upfield is expected and actually observed. In free AMP, H5′ and H5″ protons are on the average, quite far from the adenine ring. Upon complexation, these

TABLE IV

PROTON CHEMICAL SHIFTS IN TRYPTAMINE, AMP AND THEIR 1 : 1 COMPLEX

In ppm from *tert*-butanol, negative sign is upfield.

	Shift (ppm)						
	H2	H8	H1′	H2′	H3′	H4′	H5′ H5″
AMP							
Free	7.006	7.363	4.888	3.58	3.269	3.121	2.757
Complex	6.205	6.83	4.23	2.77	3.01	3.02	2.85
Δ	−0.8	−0.54	−0.65	−0.81	−0.26	−0.1	+0.1
	Shift (ppm)						
	H2	H4	H5	H6	H7	Hα	Hβ
Tryptamine							
Free	6.074	6.453	6.043	5.95	6.298	2.091	1.933
Complex	5.29	5.663	5.323	5.23	5.428	1.791	1.413
Δ	−0.78	−0.79	−0.72	−0.72	−0.87	−0.30	−0.52
	Normalized values of the complexation induced shifts						
	H2	H4	H5	H6	H7	Hα	Hβ
Tryptamine							
Experimental	0.99	1	0.91	0.91	1.10	0.38	0.66
Calculated	0.67	1	0.89	0.89	1.11	0.22	0.55

protons are in the neighborhood of the planes of both adenine and indole rings and a downfield shift of 0.1 ppm is calculated in close agreement with the observed one.

The adenine and indole rings being parallel and separated by approx. 0.3 nm, only upfield shifts of the relevant protons from their resonances in the free molecules are expected. We did not succeed to fit accurately the calculated shifts induced in the indole ring by adenine to the experimental ones, so that only relative values are given in Table IV, assuming a head-to-head stacking. A satisfactory agreement is then obtained except for H2 from tryptamine which may be due to the proximity of the AMP phosphate group. The shifts of adenine protons have not been calculated because of the absence of data on the indole iso-shielding curves. However, the large differences between the AMP H2 and H8 shifts observed upon complexation are consistent with the head-to-head stacking model since the shifts induced by a five-membered aromatic ring are in general significantly smaller than by a six-membered ring. Moreover the values of these shifts are of the same order as those expected from the iso-shielding curves of adenine, likely not very different from the indole ones. We have considered likewise the H1′ shift, mainly due to the proximity of the indole ring. Under the same assumption one finds a 0.53 ppm upfield shift which may be compared to the experimental value of 0.67 ppm (Table IV).

This discussion shows that the model proposed for the conformation of the tryptamine-AMP complex is reasonably well confirmed by chemical shift measurements, although a quantitative agreement has not been achieved because of too many contributions which cannot be taken into account. In the study of the interactions between two molecules, the proton relaxation appears therefore as an essential support to conventional NMR methods.

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