AN IMPROVED T_1 RELAXATION TIME DETERMINATION OF THE SYN-ANTI EQUILIBRIUM OF NUCLEOTIDES IN SOLUTION

C. CHACHATY and G. LANGLET

Service de Chimie Physique, C.E.N. de Saclay, B.P. n° 2, 91190 – Gif-sur-Yvette, France

Received 7 July 1976

1. Introduction

In the last few years, several attempts have been made to determine the average conformation of nucleosides and nucleotides in solution by nuclear relaxation methods: the measurement of the Overhauser effect [1–3] and of the relaxation time T_1 [4–6]. It has been shown that the proton relaxation in these molecules depends on a single correlation time, that of the isotropic reorientation of the whole molecule [5–6]. We have therefore suggested a simple method to estimate the syn and anti character of the orientation of the base about the glycosidic bond by minimizing as a function of the angle defining this orientation, the relative standard deviation $\Delta\tau_c/\bar{\tau}_c$ about the mean experimental value of the correlation time $\bar{\tau}_c$ deduced from the relaxation of non exchangeable protons [6]. It has been found that in the case of several 5′-purine ribonucleotides the average orientation of the base corresponds to Υ = 60–80° in the syn range, Υ being the dihedral angle $H_{1'}C_{1'}N_9\,C_8$ counted clockwise about the $\overrightarrow{N_9C_{1'}}$ axis. However several potential energy calculations indicate that the position of the potential wells are generally different for the N and S pseudorotational conformers of the ribose ring [7–8]. The aim of the present work is to deduce from the minimization of $\Delta\tau_c/\bar{\tau}_c$ or $\Delta\nu_c/\bar{\nu}_c$ ($\nu_c = \tau_c^{-1}$) the preferential orientation of the base associated with the N and S conformers. For that purpose several computer programs have been written in APL* and their use will be illustrated by the examples of adenosine, inosine, guanosine and uridine 5′-monophosphates (5′-AMP, 5′-IMP, 5′-GMP, 5′-UMP) in neutral aqueous solution.

2. Experimental

The samples dissolved in H^2HO were freed from divalent ions by chelex 100 and the P_{2_H} was adjusted to 8 ± 0.5. After lyophylization the nucleotides were diluted to 2×10^{-2} M and sealed under nitrogen after addition of EDTA 10^{-3} M to scavenge any paramagnetic impurity. The T_1 measurements were performed at 250 MHz by the inversion recovery method with a Cameca TSN 250 spectrometer. Most of the experiments were carried out at the vicinity of 40°C so that the H^2HO peak was located between the $H_{2'}$ and $H_{3'}$ resonance without perturbing them. The experimental data reported in table 1 are the mean of at least three measurements done on different samples, the reproducibility being about ± 5%.

3. Results and discussion

The initial slope of the logarithmic plot of the longitudinal magnetization recovery of a proton i gives the relation rate:

$$T_{1i}^{-1} = \tfrac{3}{2}\gamma_H{}^4 \hbar^2 \left[n\, F_i + (1-n)\, G_i \right] \tau_{C_i} \qquad (1)$$

where

$$F_i = \sum_j \langle r_{ij}^{-6}(\Upsilon_N)\rangle\ ,\ G_i = \sum_j \langle r_{ij}^{-6}(\Upsilon_S)\rangle$$

---

* The listings of these programs are available on request.

$<r_{ij}^{-6}(\Upsilon)>^{-1/6}$ being the motional average of the interproton distances for the N and S conformers, the n and $1-n$ weigths of which are given by the $J_{1'2'}$ coupling constant [9]. The terms $<r_{i5'}^{-6}(\Upsilon)>$ and $\sum_j <r_{5'j}^{-6}(\Upsilon)>$ are weighted by the probabilities P_{gg}, P_{gt} and P_{tg} of the rotamers of the phosphoester exocyclic group, which are given by $J_{4'5'}$ and $J_{4'5''}$ [10]. The different ways of minimizing $\Delta\tau_c/\bar{\tau}_c$ or $\Delta\nu_c/\bar{\nu}_c$ assuming that the relaxation of all nonexchangeable protons depends on the same correlation time or frequency, may be conveniently discussed with the help of the expression of $\Delta\nu_c^2$:

$$\Delta\nu_c^2 = \frac{1}{k-1}\Bigg[n^2\Big[\sum_i A_i^2 F_i^2 - \frac{1}{k}(\sum_i A_i F_i)^2\Big] + (1-n)^2\Big[\sum_i A_i^2 G_i^2 - \frac{1}{k}(\sum_i A_i G_i)^2\Big] + 2n(1-n)\Big[\sum_i A_i^2 F_i G_i - \frac{1}{k}(\sum_i A_i F_i \times \sum_i A_i G_i)\Big]\Bigg] \qquad (2)$$

Where $A_i = 3/2\, T_{1i}\, \gamma_H{}^4 \hbar^2$, k being the number of nonexchangeable protons the relaxation of which depends on Υ namely H_8, $H_{1'}$, $H_{2'}$, $H_{3'}$ for purine nucleotides, H_6, H_5, $H_{1'}$, $H_{2'}$, $H_{3'}$ for pyrimidine nucleotides. Equation (2) may be also written

$$\Delta\nu_c^2 = n^2\, \Delta\nu_N^2(\Upsilon_N) + (1-n)^2\, \Delta\nu_S^2(\Upsilon_S) + 2n(1-n)\, \Delta\nu^2(\Upsilon_N, \Upsilon_S) \qquad (3)$$

The correlation between the syn or anti orientation of the base and the N and S conformation of the ribose ring have been examined by minimizing $\Delta\tau_c/\bar{\tau}_c$ or $\Delta\nu_c/\bar{\nu}_c$ as a function of Υ_N and Υ_S simultaneously for given values of n, P_{gg}, P_{gt} and P_{tg}. Fig.1 shows the diagram $\Delta\nu_c/\bar{\nu}_c = f(\Upsilon_N, \Upsilon_S)$ delineated for a model purine nucleotide the conformation of which has been chosen as typical of the 5′-nucleotide under study with $n = 0.4$, $P_{gg} = 0.7$, $P_{gt} = P_{tg} = 0.15$, $\Upsilon_{1N} = 60°$, $\Upsilon_{2N} = 195°$, $\alpha = P_{60°}/(P_{60°} + P_{195°}) = 0.6$, $\Upsilon_{1S} = 90°$, $\beta = P_{90°} = 1$. All calculations have been made with the internuclear distances given in refs. [11] and [12] with pseudorotational angles $\Theta_N = 10°$, $\Theta_S = 160°$ and a maximum torsion angle $\tau_m = 40°$. This diagram shows several zones of minima partially spread over sterically disallowed domains which have been delimited with a subsidiary APL program where the minimum distance d_{min} between atoms of different groups are given. We have generally taken $d_{min} = 0.22$ or 0.24 nm. It may be pointed out that the graphs $<\Delta\nu_c/\bar{\nu}_c>_N = 1/2\pi \int_0^{2\pi} f(\Upsilon_N, \Upsilon_S)d\Upsilon_S$ and $<\Delta\nu_c/\bar{\nu}_c>_S = 1/2\pi \int_0^{2\pi} f(\Upsilon_N, \Upsilon_S)d\Upsilon_N$ show respectively two pronounced minima $\Upsilon'_{1N} = 70°$, $\Upsilon'_{2N} = 195°$ and $\Upsilon'_{1S} = 95°$, $\Upsilon'_{2S} = 155°$. Three of these angles are close to those given above and correspond therefore approximately to the preferential orientations of the base.

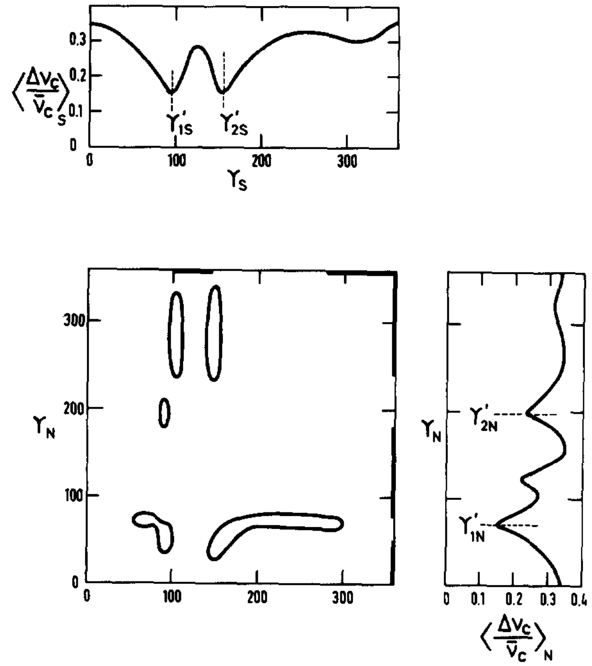

Fig.1. Simultaneous minimization of $\Delta\nu_c/\bar{\nu}_c$ as a function of Υ_N and Υ_S for the model purine nucleotide indicating the domains $\Delta\nu_c/\bar{\nu}_c \leq 0.1$. The heavy lines indicate the sterically disallowed orientations of the base for the N and S conformers.

These ones may be also obtained by minimization of $\Delta\tau_c/\bar{\tau}_c$ or $\Delta\nu_c/\bar{\nu}_c$ as a function of Υ_N and Υ_S with $n = 1$ and $n = 0$ respectively (fig.2). Equation 3 shows indeed that $\Delta\nu_c^2$ comprising the terms $\Delta\nu_N^2(\Upsilon_N)$ and $\Delta\nu_S^2(\Upsilon_S)$ will show minima for the four angles mentionned above. Assuming as suggested by potential energy curves [7–8] that there are only one or two preferred orientations of the base for each pseudorotational conformer, equations similar to (3) may be derived for the N and S forms:

$$\begin{aligned}\Delta\nu_N^2(\Upsilon_N) &= \alpha^2\Delta\nu_N^2(\Upsilon_{1N}) + (1-\alpha)^2\Delta\nu_N^2(\Upsilon_{2N}) + \\ &\quad 2\alpha(1-\alpha)\Delta\nu_N^2(\Upsilon_{1N},\Upsilon_{2N}) \qquad (4)\\ \Delta\nu_S^2(\Upsilon_S) &= \beta^2\Delta\nu_S^2(\Upsilon_{1S}) + (1-\beta)^2\Delta\nu_S^2(\Upsilon_{2S}) + \\ &\quad 2\beta(1-\beta)\Delta\nu_S^2(\Upsilon_{1S},\Upsilon_{2S})\end{aligned}$$

The subscripts 1 and 2 referring respectively to angles found generally in the syn and anti ranges. The conformation of the nucleotides is then obtainable by minimizing $\Delta\nu_c^2$, $\Delta\nu_c/\bar{\nu}_c$ or $\Delta\tau_c/\bar{\tau}_c$ as a function of α, β and n simultaneously (eq. 3 and 4). The determination of n by this way is however somewhat inaccurate since for given values of α and β, the dependence of n on the standard deviations about $\bar{\nu}_c$ or $\bar{\tau}_c$ shows a shallow minimum the position of which may be quite sensitive to experimental uncertainties. It is therefore preferable to take the value of n given by $J_{1'2'}$ and to minimize the standard deviations by varying α and β simultaneously as shown in fig.2 on the example of the model nucleotide, the overall probability of the syn orientation being given by:

$$P_{syn} = \alpha n + (1-n)\beta \qquad (5)$$

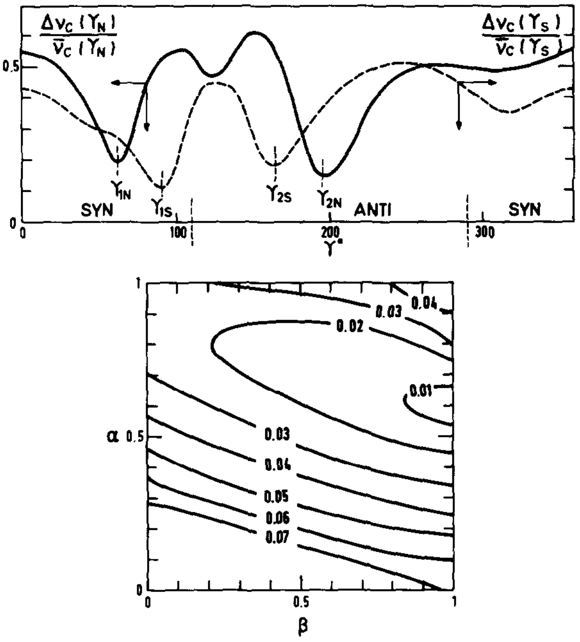

Fig.2. Upper diagram: plot of $\Delta\nu_c/\bar{\nu}_c$ versus Υ_N and Υ_S for the model nucleotide. Lower diagram: simultaneous minimization of $\Delta\nu_c/\bar{\nu}_c$ as a function of the probabilities α and β of the syn orientation of the base for the N and S conformers taking $n = 0.4$.

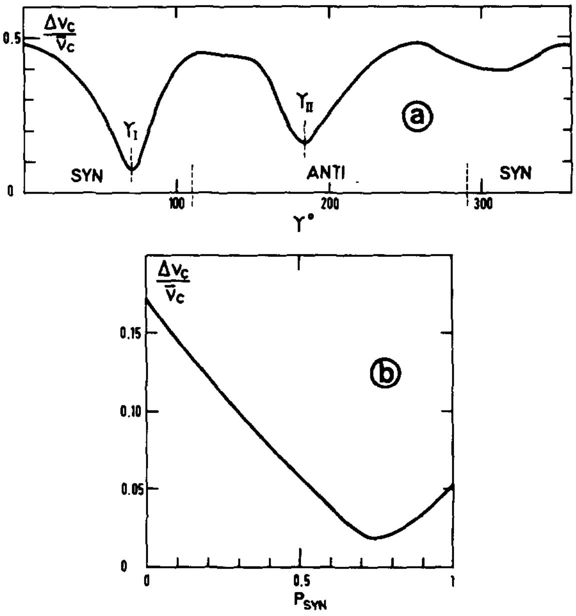

Fig.3. (a) Angular dependence of $\Delta\nu_c/\bar{\nu}_c$ on $\Upsilon = \Upsilon_N = \Upsilon_S$ computed for the model nucleotide taking $n = 0.4$. (b) Dependence of $\Delta\nu_c/\bar{\nu}_c$ on P_{syn}.

The experimental relaxation times, normalized for a reorientation correlation time $\tau_c = 10^{-10}$ sec are compared in table 1 to the values calculated for the conformations derived from the minimization of $\Delta\nu_c/\bar{\nu}_c$ or $\Delta\tau_c/\bar{\tau}_c$, with an agreement better than 10%. It is seen that for the nucleotides under study, the preferential orientations of the base associated with the N conformer are $60° < \Upsilon_{1N} < 70°$ (syn) and $190° < \Upsilon_{2N} < 200°$ (anti) whereas the S conformer shows a single preferential orientation of the base at $\Upsilon_{1S} \simeq 90°$ (syn), the minimum of $\Delta\nu_c/\bar{\nu}_c$ located at 160–170° does not correspond to an actual orientation. P_{syn} is always higher than 70–80% confirming our previous estimate [6] given by the dependence of $\Delta\tau_c/\bar{\tau}_c$ (or $\Delta\nu_c/\bar{\nu}_c$) on the angle Υ common to the N and S conformers which shows two pronounced minima $\Upsilon_{1N} < \Upsilon_I < \Upsilon_{1S}$, $\Upsilon_{2S} < \Upsilon_{II} < \Upsilon_{2N}$ (table 1 and fig.3).

Table 1

T_1 Relaxation time data from 5′-nucleotides

Model	Experimental T_1 (sec)		Calculated T_1 (sec)					
5′-Purine			Υ_{1N} = 60°, α = 0.6	H_8	2.09			
Nucleotide			Υ_{2N} = 195°	H_2	70.9			
n = 0.4			Υ_{1S} = 90°, β = 1	$H_{1'}$	2.85	Υ_I = 70°		
P_{gg} = 0.7			P_{syn} = 0.84	$H_{2'}$	0.97	Υ_{II} = 185°		
$P_{gt} = P_{tg}$ = 0.15				$H_{3'}$	1.02	P_{syn} = 0.75		
				$H_{4'}$	0.98			
				$H_{5'}$	0.32			
5′-AMP	H_8	1.84	Υ_{1N} = 64°, α = 0.82	H_8	1.96	Υ_I = 75°	H_8	1.77
n = 0.45	H_2	(13.0)[a]	Υ_{2N} = 190°	H_2	(65.3)[b]	Υ_{II} = 180°	H_2	(64.9)[b]
P_{gg} = 0.7	$H_{1'}$	2.66	Υ_{1S} = 90°, β = 1	$H_{1'}$	2.74	P_{syn} = 0.80	$H_{1'}$	2.78
$P_{gt} = P_{tg}$ = 0.15	$H_{2'}$	0.91	P_{syn} = 0.92	$H_{2'}$	0.89	$\Delta T_1/T_1$ = 0.03	$H_{2'}$	0.87
	$H_{3'}$	1.04	$\Delta T_1/T_1$ = 0.06	$H_{3'}$	1.05		$H_{3'}$	1.02
	$H_{4'}$	(1.02)[a]		$H_{4'}$	0.91		$H_{4'}$	0.98
	$H_{5'}$	(0.33)[a]		$H_{5'}$	0.32		$H_{5'}$	0.32
5′-GMP	H_8	2.01	Υ_{1N} = 60°, α = 0.82	H_8	2.02	Υ_I = 68°	H_8	2.07
n = 0.45	$H_{1'}$	2.55	Υ_{2N} = 190°	$H_{1'}$	2.71	Υ_{II} = 180°	$H_{1'}$	2.65
P_{gg} = 0.7	$H_{2'}$	0.99	Υ_{1S} = 90°, β = 1	$H_{2'}$	1.07	P_{syn} = 0.84	$H_{2'}$	0.94
$P_{gt} = P_{tg}$ = 0.15	$H_{3'}$	1.04	P_{syn} = 0.92	$H_{3'}$	1.05	$\Delta T_1/T_1$ = 0.08	$H_{3'}$	1.03
	$H_{4'}$	(1.16)[a]	$\Delta T_1/T_1$ = 0.09	$H_{4'}$	0.98		$H_{4'}$	0.98
	$H_{5'}$	(0.36)[a]		$H_{5'}$	0.32		$H_{5'}$	0.32

Table 1 (continued)

5′-IMP	H_8	1.90	$\Upsilon_{1N} = 60°, \alpha = 1$	H_8	2.03	$\Upsilon_I = 72°$	H_8	1.91
$n = 0.45$	H_2	(9.7)[a]	$\Upsilon_{1S} = 88°, \beta = 1$	H_2	(63.0)[b]	$\Upsilon_{II} = 184°$	H_2	(74.4)[b]
$P_{gg} = 0.70$	$H_{1'}$	2.46	$P_{syn} = 1$	$H_{1'}$	2.63	$P_{syn} = 0.96$	$H_{1'}$	2.62
$P_{gt} = P_{tg} = 0.15$	$H_{2'}$	0.96	$\Delta T_1/T_1 = 0.08$	$H_{2'}$	0.90	$\Delta T_1/T_1 = 0.08$	$H_{2'}$	0.88
	$H_{3'}$	1.08		$H_{3'}$	1.07		$H_{3'}$	1.06
	$H_{4'}$	(1.14)[a]		$H_{4'}$	0.98		$H_{4'}$	0.98
	$H_{5'}$	(0.31)[a]		$H_{5'}$	0.32		$H_{5'}$	0.32
5′-UMP	H_5	2.35	$\Upsilon_{1N} = 70°, \alpha = 0.60$	H_5	2.16	$\Upsilon_I = 75°$	H_5	2.16
$n = 0.45$	H_6	0.62	$\Upsilon_{2N} = 200°,$	H_6	0.67	$\Upsilon_{II} = 185°$	H_6	0.66
$P_{gg} = 0.70$	$H_{1'}$	2.40	$\Upsilon_{1S} = 90°, \beta = 1$	$H_{1'}$	2.52	$P_{syn} = 0.68$	$H_{1'}$	2.50
$P_{gt} = P_{tg} = 0.15$	$H_{2'}$	0.75	$P_{syn} = 0.82$	$H_{2'}$	0.65	$\Delta T_1/T_1 = 0.07$	$H_{2'}$	0.67
	$H_{3'}$	0.97	$\Delta T_1/T_1 = 0.08$	$H_{3'}$	0.98		$H_{3'}$	0.91
	$H_{4'}$	(1.05)[a]		$H_{4'}$	1.08		$H_{4'}$	1.08
	$H_{5'}$	(0.31)[a]		$H_{5'}$	0.32		$H_{5'}$	0.32

[a] Not taken into account in the minimization of $\Delta\tau_c/\bar{\tau}_c$ or $\Delta\nu_c/\bar{\nu}_c$.

[b] Not taken into account in the calculation of the relative standard deviation $\Delta T_1/T_1$ between the experimental and calculated T_1.

It may be pointed out lastly that although the oxygen and paramagnetic ions have been carefully removed from the samples the relaxation time of H_2 in 5′-AMP and 5′-IMP does not exceed 15 sec instead of 60–70 sec as expected from the average conformation of these nucleotides (table 1). That seems an indication of the persistence of an intermolecular base stacking even at high dilution as previously suggested [10].

References

[1] Hart, P. A. and Davis, J. P. (1971) J. Am. Chem. Soc. 93, 753–760.

[2] Schirmer, R. E., Davis, J. P., Noggle, R. H. and Hart, P. A. (1972) J. Am. Chem. Soc. 94, 7903–7911.

[3] Gueron, M., Chachaty, C. and Tran Dinh, S. (1973) Ann. N.Y. Acad. Sci. 222, 307–323.

[4] Lüdemann, H. D., Westhof, E. and Röder, O. (1974) Eur. J. Biochem. 49, 143–150.

[5] Imoto, T., Akasaka, K. and Hatano, H. (1975) Chem. Phys. Lett. 32, 86–89.

[6] Chachaty, C., Zemb, T., Langlet, G., Tran Dinh, S., Buc, H. and Morange, M. (1976) Eur. J. Biochem. 62, 45–53.

[7] Berthod, H. and Pullman, B. (1973) FEBS Lett. 30, 231–234.

[8] Yathindra, N. and Sundaralingam, M. (1973) Biopolymers 12, 297–314 and 2075–2082.

[9] Altona, C. and Sundaralingam, M. (1973) J. Am. Chem. Soc. 95, 2333–2344.

[10] Tran Dinh, S. and Chachaty, C. (1973) Biochim. Biophys. Acta 335, 1–13.

[11] Kraut, J. and Jensen, L. H. (1963) Acta Cryst 16, 79–88.

[12] Saenger, W. (1973) Angew. Chem. Int. Ed. 12, 591–601.