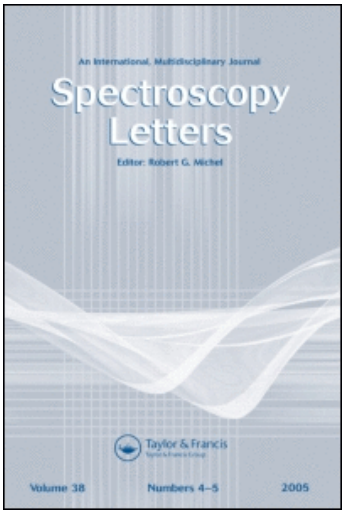

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C-13 Spin-Relaxation Study of Some Amino Acids, Their Phospho-Derivatives and Some Small Peptides

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C-13 Spin-Relaxation Study of some Amino Acids, their Phospho-derivatives and some small Peptides.

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Abstract

Carbon C-13 spin-lattice relaxation times , T1, of Serine, Threonine, Phosphoserine and Phosphothreonine have been measured in D_2O at neutral pD. Changes in over-all motions under phosphorylation has been detected . Segmental motions are not affected by phosphorylation. With the aid of T1(C-13) values of other amino acids an empirical relationship between NT1 and molecular weights (Mw) is tested that seems valid also for small peptides. Such relationship allows to interpret results from T1 measurements with the aid of a simple spherical model.

In the course of a NMR study on amino acids Ser and Thr and their corresponding phospho derivatives, phosphoserine (PSer) and phosphothreonine (PThr) (1,2) the biological significance of which has been recently confirmed (3-5), we detected an influence of the phospho group on the over-all conformation of the side-chain.

Aim of the present letter is to investigate by the aid of T1(C-13) relaxation time the possibility of the phospho group to act like an 'anchor' on the whole molecule with a consequent decrease of over-all molecular rotational diffusion and/or segmental motions. By the aid of NT1(C_α) values of other amino acids we look also for a relationship that relates molecular weights (Mw) and experimental NT1 values similar to the one already found in smaller cyclic peptides (6).

Present T1(C-13) relaxation study on PSer, PThr, Ser and Thr in natural abundance in D_2O (0.5 M) was performed using the inversion-recovery method on a Varian XL-200-FT spectrometer.

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Table 1. NT1(C-13) values of different carbon atoms some amino acids in D_2O taken from literature and in Ser, Thr, PSer and PThr in D_2O. In parenthesis are the corresponding Mw values.

	α	β	γ	δ	ε	ref.
Gly (Mw=75)	6.0					13
Ala (Mw=89)	3.1, 3.4	5.4, 6.3				7,13
-Amino-butyryc acid (Mw=103)	3.4	2.4	4.2			13
Pro (Mw=115)	4.3	7.5	8.8	6.6		7
Ile (Mw=131)	2.2	2.1	3.2	9.6		7
Leu (Mw=131)	1.9	2.8	2.6	5.1		7
Nle (Mw=131)	2.3	2.0	3.0	4.0	13.5	7
Lys (Mw=146)	0.7-1.8	1.8	2.4	2.4	3.0	7
Ser (Mw=105)	3.5	3.6				
Thr (Mw=119)	2.2	2.3	3.9			
PSer(Mw=185)	1.0	2.0				
PThr(Mw=199)	1.3	1.3	3.9			
Glt (Mw=307)	0.65	0.4	0.3			

C-13 NT1 values of α, β and γ carbons of of Ser,Thr, PSer and PThr together with NT1 values of other amino acids are collected in table 1.

To achieve a deeper insight in molecular processes the correlation times τ_{eff} of our compounds should be known. In our case, assuming dipolar relaxation mechanism and over-all isotropic molecular motions and rapid with respect to the ^{13}C -NMR time scale (7.8) we obtain, after solving for $\langle r_{CH} \rangle = 1.09$ Å :

$$\tau_{eff} = 4.7 \cdot 10^{-11} / NT1 \quad sec.$$

From NT1 values of our compounds in table 1 follows: a) α and ß carbons have similar τ_{eff} values that represent an estimate of the over-all isotropic diffusion of the whole molecule, b) the lower $\tau_{eff}(\gamma)$ value means a greater mobility of the methyl group, i.e., some degree of segmental motions, otherwise, the similarity of this value in Thr and PThr tell us that methyl group is not affected by the presence of the phospho group at ß level and c) $\tau_{eff}(Ser) < \tau_{eff}(Thr) < \tau_{eff}(PSer) < \tau_{eff}(PThr)$: such a decrease in over-all mobility with molecular weight can be better explained if we consider the NT1 values of the other amino acids given in table 1.

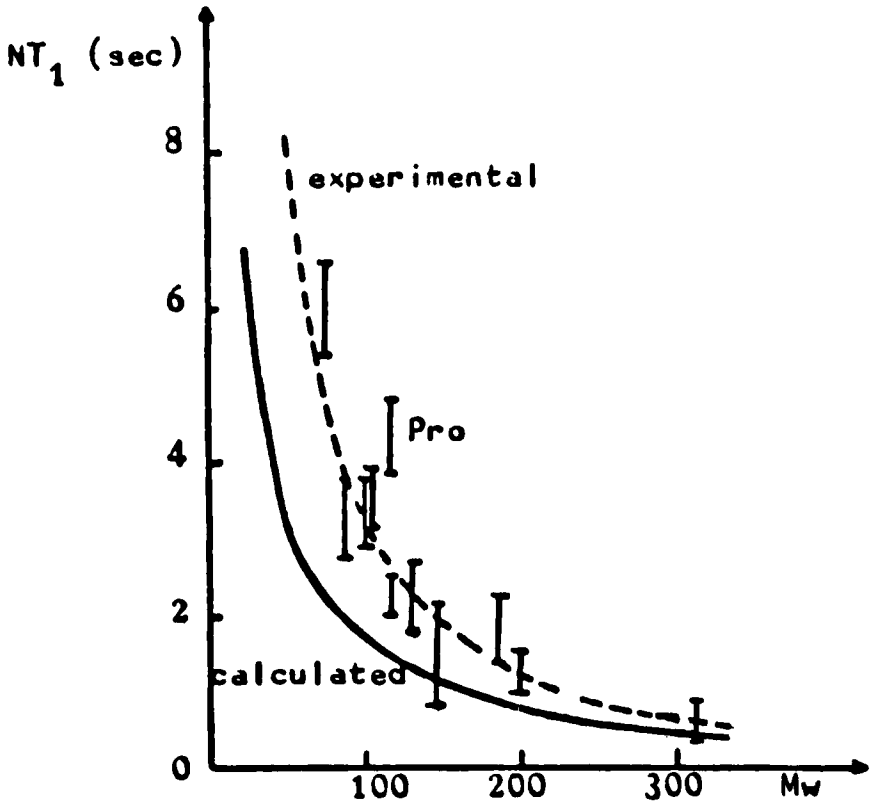

Fig.1. Observed (----) and calculated (———) correlation between molecular weights (Mw) and NT1 values of α-carbons of compounds given in table 1.

The following relationship has been proposed between NT1 and Mw (6), after solving for the different constants at T=300°K and η=0.0101 P (i.e., viscosity of the solution being determined by the viscosity of the solvent):

$$1 / NT1 = 6.4\ 10^{-3} \cdot f_r \cdot Mw \quad sec^{-1}$$

where f_r is a microviscosity factor that for large molecules undergoing brownian motions results to be equal to 1.

Plotting NT1/Mw values of table 1 we obtain the experimental curve of fig.1 with larger T1 values than the calculated ones with f_r=1 (calculated curve). The empirical relationship for the microviscosity factor that fits experimental data being:

$$f_r = 0.30 + 0.035 \cdot Mw(solute)/Mw(D_2O)$$

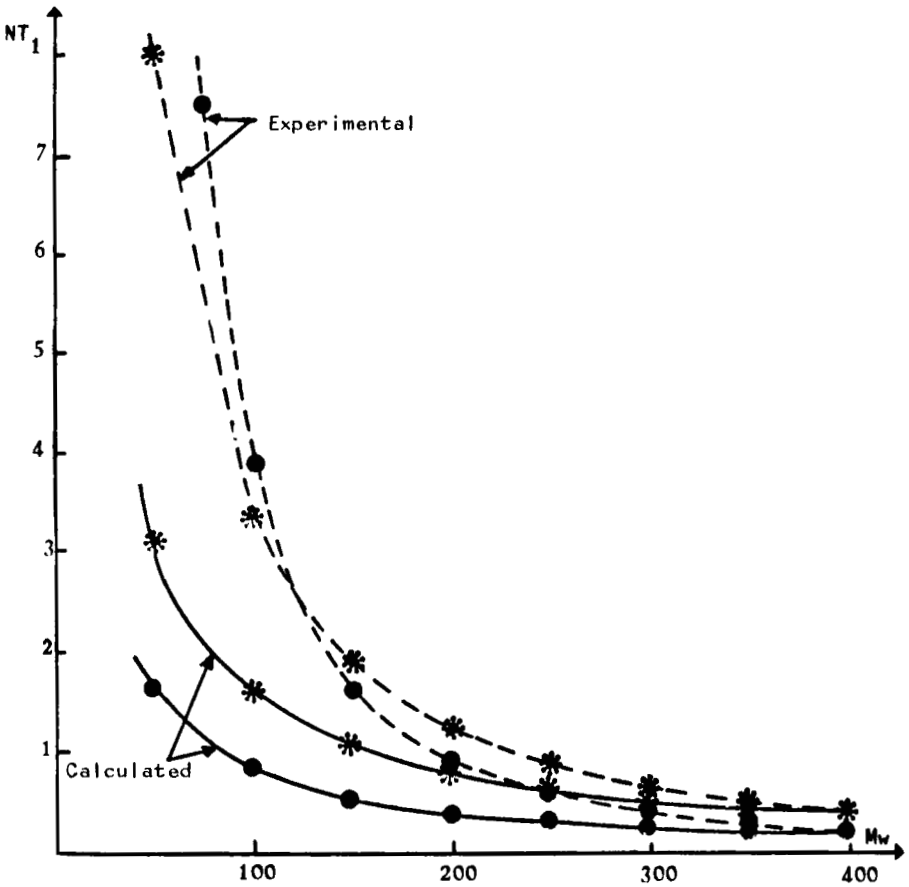

Fig.2 Calculated (——) and experimental (-----) correlation between Mw and NT1 values in cyclic peptides (●) in $(CD_3)_2SO$ solutions and in amino and phosphoamino acids (✱) in D_2O solutions.

A similar plot was already found with diketopiperazines in $(CD_3)_2SO$ solutions (6) the experimental curve of which was fitted by the following microviscosity factor:

$$f_r = -0.04 + 0.2 \cdot Mw(solute)/Mw((CD_3)_2SO)$$

In fig.2 are resumed our results on amino acids and phospho-amino acids in D_2O and results on diketopiperazines in $(CD_3)_2SO$, together with the corresponding calculated curves for $f_r = 1$ (equation that fits data on diketopiperazines is (6): $1/NT1 = 0.0129 \cdot f_r \cdot Mw$) . From fig.2 results that : a) calculated and expe-

rimantal curves seem to converge for Mw≤400, and for Mw≥200, experimental NT1 values are very close to the calculated ones that are based on the assumption of a pure spherical model undergoing isotropic rotational diffusion, b) two experimental curves show similar NT1 values for Mw>100 and c) exception represented by Pro in fig.1 can be rationalized in terms of consistent contributions of internal motions of the ring (9,10). The discrepancy detected between experimental and calculated NT1 values, explicited by the f_r factor, can be explained on the basis of motions dominated in some way, by inertial effects (11,12).

As T1(C-13) measurements of small and middle-sized molecules are relatively easy to perform, it would be interesting to proof if the NT1-Mw relationship is generally valid and to look for a dependence of the f_r factor on dielectric constant of the solvent and on the functional groups of the solute.

T1(C-13) measurements on Glutathione in H_2O (Glt in table 1) fit in a good way our curve of fig.1 . In DMSO it oxydizes rapidly to Oxydized-glutathione (Mw=614) and it cannot fit curve of fig.2 as it is valid only for Mw≲400.

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