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Some Remarks about Charge Distribution and Chemical Shifts in Phosphorylated Amino Acids

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In order to follow the changes in the electronic density along the side-chain of some amino acids (AA) that undergo phosphorylation at the side-chain we compare the calculated electronic density of Phosphoserine (PSer) and Phosphothreonine (PThr) obtained from ^{1}H- and ^{13}C-NMR shifts [1, 2] with the electronic density of Ser and Thr as obtained by Del Re *et al.* [3, 4] by means of an approximate MO-LCAO method. Knowledge of such electronic changes can help us to understand the interaction capability of such phosphorylated side-chains with metal ions, membranes, other proteins and with nucleic acids.

To obtain the distributions of electronic charges at carbon (Q_C) and hydrogen (Q_H) atoms along the side-chain of PSer and PThr we follow in a reverse way a method outlined by Del Re [4]. Such a method relates the proton shifts (δ_H) to $Q_{C,H}$ by means of a simple linear relationship. With the aid of a second linear relationship relating the carbon-13 shifts ($\delta_{C\text{-}13}$) to Q_C, suggested to be suitable for amino acid side-chain C-13 carbon atoms [5] (details of the procedure will be published elsewhere), we calculate Q_C. Inserting the obtained Q_Cs in the $\delta_H = f(Q_H, Q_C)$ relationship we obtain the Q_Hs.

In Table I are given the experimental and calculated δ_H shifts of Ser and Thr referred to internal DSS and the corresponding shifts of PSer and PThr. In Table II are the calculated electronic charges of the two phosphorylated side-chains PSer/PThr from the H and C-13 shifts. In this table are also given the Q_Cs and Q_Hs of Ser and Thr deduced from ref. 4. For neutral solutions are given the δ_H (Table I) and the Q_C, Q_H values (Table II) of the neutral $(AA)_n$ and polar $(AA)^{\pm}$ forms of the amino acid carboxy-aminic heads.

From Table II we note that protonation (except the Q_C value of PThr) is generally accompanied by transmission of negative charge from H- and C-atoms to the $-NH_3^+$, -COOH and $-OPO_3H_2$ groups ($Q_{C,H}$ grows upon protonation). Q_C of PThr decreases a little, decreasing the pH value, as already found with other amino acids [6].

Two conclusions can be drawn from our partial results: 1) comparison of Ser and Thr with PSer and PThr tells us that phosphorylation of the side-chain introduces little electronic changes both at the α and β level of the side-chain, 2) the magnitude of the $Q_{C,H}$ and δ_H predicted by our empirical method are of the right order of magnitude but, in view of the approximations made in the derivation, the calculated results cannot be considered as more than limiting values.

Interaction studies of the phosphorylated side-chains with substrates could give us a greater insight into the electronic distribution changes introduced by the phosphorylation process. In fact, even if absolute values of $Q_{C,H}$ are not very useful, changes in $Q_{C,H}$s as we go from not-phosphorylated to phosphorylated side-chain in the same amino acid can be in agreement with results of more accurate calculations.

1 L. Pogliani and D. Ziessow, *Org. Magn. Res.*, *10*, 26 (1977); *ibid.*, *11*, 319 (1978).
2 L. Pogliani and D. Ziessow, *Org. Magn. Res.*, *17*, 214 (1981) and references therein cited.
3 G. Del Re, *J. Chem. Soc.*, 4031 (1958).

TABLE 1. Experimental Proton Shifts in Acid, Neutral and Basic Aqueous Solutions and the Corresponding Calculated Shifts for Ser, Thr, PSer and PThr.

Compound	H	Acid		Neutral			Basic	
		δ_{obs}	δ_{calc}	δ_{obs}	δ_{calc} $(AA)_n$	δ_{calc} $(AA)^{\pm}$	δ_{obs}	δ_{calc}
Ser	α	4.22	4.32	3.84	3.39	4.09	3.35	3.20
	$\bar{\beta}$*	4.05	4.06	3.95	3.87	4.05	3.74	3.86
PSer**	α	4.46	4.45	4.07	4.08	4.09	3.48	3.51
	$\bar{\beta}$	4.54	4.48	4.25	4.31	4.21	3.89	3.91
Thr	α	4.42	4.14	3.58	3.23	4.07	3.10	3.05
	β	4.03	4.01	4.05	3.94	4.13	3.95	3.94
	γ	1.36	1.31	1.32	1.30	1.31	1.20	1.30
PThr**	α	4.16	4.12	3.62	3.58	3.57	3.08	3.12
	β	4.86	4.92	4.44	4.49	4.47	4.08	4.04
	γ	1.48	1.31	1.40	1.42	1.42	1.24	1.28

*$\delta\bar{\beta} = 1/2(\delta\beta_1 + \delta\beta_2)$. **The calculated shifts of PSer and PThr are back-calculated values from the electronic charges $Q_{C,H}$.

TABLE II. Calculated Electronic Charges $Q_{C,H}$ of PSer and PThr (Electro-Charges of Ser and Thr Are Taken from ref. 4) from H and C-13 Shifts.[a]

$Q_{C,H}$	Acid		Neutral				Basic	
			$(AA)_n$	$(AA)^{\pm}$	$(AA)_n$	$(AA)^{\pm}$		
	Ser	*PSer*	Ser	Ser	*PSer*	*PSer*	Ser	*PSer*
$Q_{C\alpha}$	0.072	0.072	0.060	0.063	0.062	0.063	0.054	0.058
$Q_{C\beta}$	0.046	0.034	0.040	0.045	0.031	0.034	0.039	0.018
$Q_{H\alpha}$	0.054	0.055	0.048	0.053	0.053	0.053	0.047	0.049
$Q_{H\bar{\beta}}$	0.054	0.058	0.053	0.054	0.057	0.056	0.050	0.055
	Thr	*PThr*	Thr	Thr	*PThr*	*PThr*	Thr	*PThr*
$Q_{C\alpha}$	0.068	0.052	0.057	0.061	0.065	0.064	0.052	0.073
$Q_{C\beta}$	0.095	0.106	0.088	0.094	0.103	0.101	0.088	0.112
$Q_{C\gamma}$	−0.106	−0.107	−0.107	−0.106	−0.108	−0.108	−0.107	−0.109
$Q_{H\alpha}$	0.053	0.054	0.047	0.053	0.049	0.049	0.046	0.045
$Q_{H\beta}$	0.050	0.056	0.050	0.051	0.053	0.053	0.050	0.049
$Q_{H\gamma}$	0.041	0.042	0.041	0.041	0.042	0.042	0.041	0.041

[a] *PSer*: $HOOC-C\alpha(H)(NH_2)-C\beta(H1)(H2)-OPO_3H_2$; *PThr*: $HOOC-C\alpha(H)(NH_2)-C\beta(H)(C\gamma H_3)-OPO_3H_2$ (neutral forms)

4 G. Del Re, B. Pullman and T. Yonezawa, *Biochem. Biophys. Acta, 75*, 153 (1962).
5 R. Deslauriers and I. C. P. Smith, 'Topics in Carbon-13 Spectroscopy', ed. G. Levy, vol. II, 2 (1975).
6 W. J. Horsley and H. Sternlicht, *J. Am. Chem. Soc., 90*, 3738 (1968).

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Paramagnetic Biomolecule–Metal Interactions: A New Dimeric Complex of Copper(II) with Spin-Labeled Glycin: Synthesis, Spectral and Magnetic Properties

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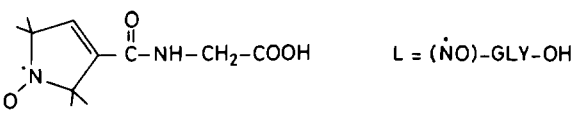
The use of spin-label and spin-probe techniques is of considerable interest in Biology and Biophysics [1, 2]. The presence of different paramagnetic centers in biological systems, as nitroxide radicals and paramagnetic metals, and the resulting spin–spin interactions provide substantial structural and dynamic information on such systems. Studies of model systems containing a metal ion and a labeled biomolecule by application of ESR and magnetic methods are rare. Our investigations concern a new dimeric copper(II) complex with spin-labeled glycin. We report the synthesis, spectral and magnetic properties of this complex.

Experimental

Spin labeling of glycine was performed using ethyl ester glycine and 3,5-dibromo-4-oxo-2,2,6,6-tetramethyl-piperidin-1-oxide according to the method previously described by B. T. Golding [3]. Hydrolysis of this peptidic compound with an original protocol, resulted in a new paramagnetic N-substituted α-amino-acid:



The complex was prepared by adding copper acetate monohydrate to an ethanolic solution of the labeled ligand in the ratio Cu/L = 1/2. The green compound was purified by recrystallization in boiling water.

Results and Discussion

Elemental analysis shows a Cu/L ratio of 1/2 and the molecular weight determined by mass spectrometry is 1142.14. These results are in agreement with a dinuclear species: $[Cu((\dot{N}O)GLY-O)_2]_2 \cdot 3H_2O$.