Variations of Electronic Charges in Substituted Amino Acids deduced from NMR Parameters

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Electron distribution in biological molecules is becoming more and more important as a means of rationalising biological events at the molecular level [1, 2]. Years ago Del Re *et al.* [3, 5] deduced the distribution of electronic charges at carbon (Q_C) and hydrogen (Q_H) atoms in α-amino acids. They succeeded in relating them to the corresponding proton shifts (δ_H) by means of a simple linear relationship. Similar relationships have been suggested [6, 7] between C-13 shifts and Q_C in amino acids.

The present work explains the method recently used [8] to derive charge densities in β-substituted amino acids, the NMR data of which are known. Starting points are electronic distribution and NMR shifts of the corresponding parent amino acid. Investigated substituted amino acids are phosphoserine (PSer) and phosphothreonine (PThr), parent amino acids are: Ala, Ser and Thr. NMR shifts of Ala, Ser, Thr, PSer and PThr in D_2O solutions referred to internal DSS at different pD values are taken from the literature [9, 10] and references cited therein. The electronic structures of Ala, Ser and Thr are from reference 4 (distribution of electronic charges in neutral solutions in Ala, Ser and Thr being the mean of $Q_{C,H}$ in neutral and dipolar ion forms).

In order to calculate electronic changes caused by phosphorylation we calculate Q_C in PSer and PThr through the linear relationship:

$$\delta(\text{C-13}) = L + M \cdot Q_C \quad (1)$$

M and L values are deduced from known C13 shifts and from Q_C values in Ser and Thr at three different pD values. For PSer the following values are obtained: $L\alpha = 69.15$, $M\alpha = -126.39$, $L\beta = 96.99$ and $M\beta = -771.43$; for PThr: $L\alpha = 53.68$, $M\alpha = -162.69$, $L\beta = 119.31$, $M\beta = -546.62$, $L\gamma = 53.68$ and $M\gamma = 300.00$ ppm/electron. Resulting Q_C's of PSer and PThr together with corresponding proton shifts are entered in the following relationship [4]:

$$\delta_H = A \cdot Q_C + B \cdot Q_H + C \quad (2)$$

TABLE I. Experimental and Calculated Proton Shifts of Ala, Ser, PSer, Thr and PThr in Acid, Neutral and Basic Aqueous Solutions.

Compound	H	Acid		Neutral		Basic	
		δ_{obs}	δ_{calc}	δ_{obs}	δ_{calc}	δ_{obs}	δ_{calc}
Ala	α	4.20	4.60	4.11	4.11	3.32	3.50
	β	1.59	1.53	1.47	1.43	1.22	1.33
Ser	α	4.22	4.32	3.84	3.74	3.35	3.20
	$\bar{\beta}$	4.05	4.06	3.95	3.96	3.74	3.86
PSer	α	4.46	4.50	4.07	4.09	3.48	3.49
	$\bar{\beta}$	4.54	4.53	4.25	4.26	3.89	3.95
Thr	α	4.42	4.14	3.58	3.65	3.10	3.05
	β	4.03	4.01	4.05	4.04	3.95	3.94
	γ	1.36	1.31	1.32	1.305	1.20	1.30
PThr	α	4.16	4.22	3.62	3.63	3.08	3.10
	β	4.86	4.84	4.44	4.46	4.08	4.12
	γ	1.48	1.54	1.40	1.42	1.24	1.27

$\delta H\bar{\beta} = 0.5(\delta H\beta 1 + \delta H\beta 2)$

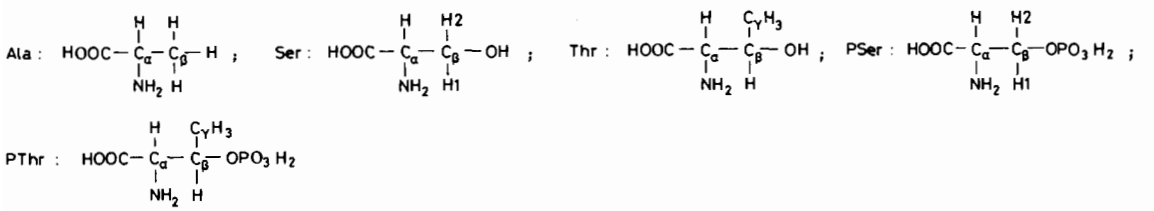

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TABLE II. Calculated Electronic Charges $Q_{C,H}$ from H-1 and C-13 Shifts of PSer and PThr. $Q_{C,H}$ of Ala, Ser and Thr are taken from ref. 4.

$Q_{C,H}$	Acid			Neutral			Basic		
	Ala	Ser	PSer	Ala	Ser	PSer	Ala	Ser	PSer
$Q_{C\alpha}$	0.057	0.072	0.077	0.048	0.0615	0.0625	0.041	0.054	0.056
$Q_{C\beta}$	−0.104	0.046	0.040	−0.1075	0.0425	0.039	−0.111	0.039	0.035
$Q_{H\alpha}$	0.052	0.054	0.055	0.049	0.0505	0.053	0.045	0.047	0.049
$Q_{H\beta}$	0.041	0.054	0.058	0.0405	0.0535	0.056	0.040	0.050	0.054
	Ala	Thr	PThr	Ala	Thr	PThr	Ala	Thr	PThr
$Q_{C\alpha}$	0.057	0.068	0.076	0.048	0.059	0.057	0.041	0.052	0.044
$Q_{C\beta}$	−0.104	0.095	0.084	−0.1075	0.091	0.086	−0.111	0.088	0.079
$Q_{C\gamma}$		−0.106	−0.110		−0.106	−0.108		−0.107	−0.110
$Q_{H\alpha}$	0.052	0.053	0.053	0.049	0.050	0.050	0.045	0.046	0.047
$Q_{H\beta}$	0.041	0.050	0.057	0.0405	0.0505	0.054	0.040	0.050	0.052
$Q_{H\gamma}$		0.041	0.043		0.041	0.042		0.041	0.041

to deduce Q_H values in PSer and PThr at the α- and β-level. To refer proton shifts to internal DSS (as Del Re referred proton shifts to water peak) we adopt the following values for A, B and C: A = 9.92, B = 133.93 and C = −2.93. Moreover for Ser, Thr, PSer and PThr it was assumed C = −3.63 and C(Hγ) = −3.13, as already suggested for β-substitution [4].

In Table I are collected experimental and calculated proton shifts of Ala, Ser, Thr, PSer and PThr in D_2O at three different pD regions in units of ppm referred to internal DSS. δ_{Hcalc} of PSer and PThr are back-calculated values from obtained $Q_{C,H}$ values truncated at the third decimal figure. In Table II are collected electronic charges of PSer and PThr obtained from carbon and proton shifts and the corresponding $Q_{C,H}$ values of Ala, Ser and Thr [4]. We remember that the $pk_{1,2}$ values of the phospho group in PSer and PThr are 0.5 and 6.5 and the corresponding pk values of the carboxyl and amino groups are 2.2 and 9.1 (9.7 in Ala).

From Table II it follows that protonation of functional groups is accompanied by transmission of positive charge to C and H atoms ($Q_{C,H}$ grows upon protonation) as phosphorylation is associated with transmission of negative charge towards the Cβ atom.

Two conclusions can be drawn from this study: a) comparison among Ala, Ser, Thr, PSer and PThr shows that the OH group introduces consistent charge changes at the α- and β-level, as phosphorylation of the OH group induces small charge changes especially at the β-level; b) $Q_{C,H}/\delta_H$ and Q_C/δ(C-13) relationships can be used to obtain approximate proton and carbon shifts in ppm referred to internal DSS in molecules the electron distributions of which are known. Concerning conductivity in Pproteins, from our conclusions it seems that polarization along the side-chain is little affected when the OH group is replaced by a phospho group and from this it follows that phosphorylated and non-phosphorylated side-chains have similar roles in conduction phenomena. It is well known that proteins show semiconductor properties [2, 11] and side-chains behave like impurities adding perturbation bands between valence and conduction band. At this level the phospho group itself could act like an added impurity (adding impurity levels between the two bands) and the resulting 'overdoped' protein could show an enhanced conductivity.

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