S10-026

Density Functional Calculations Modelling Tyrosine Oxidation in Oxygenic Photosynthetic Electron Transfer

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Keywords: Y_D, Y_Z FTIR, B3LYP

Introduction

Two tyrosine amino acid residues, D2-Tyr160(Y_D) and D1-Tyr161(Y_Z) are oxidised by the electron transfer reactions of PSII(1). In the reduced state the phenolic OH groups of both tyrosine residues have been proposed to act as hydrogen bond donors to one of the imidazole nitrogens of a nearby histidine residue i.e D2-His189 for Y_D and D1-His190 for Y_Z(*Synechocystis* numbering). Upon oxidation it is suggested that the hydroxyl hydrogen transfers to the imidazole nitrogen of the histidine, see reference 2 for a review. Some studies have however suggested that both tyrosines are deprotonated in the reduced state existing as tyrosinate residues(3).

Fourier Transform Infra Red(FTIR) difference spectroscopy has been used to monitor the oxidation of both tyrosine residues mentioned above(4, 5). The presence of a band assigned to the COH bending mode for both tyrosines in the reduced state appeared to provide direct evidence that both are protonated prior to oxidation(4,5). There is however disagreement on the correct band assignment and interpretation of the FTIR difference spectra of both Y_D and Y_Z. In a recent paper Barry and coworkers(6) have suggested that the data presented in references 4 and 5 do not arise from PSII tyrosines but are due to buffer treatments used in the sample preparations. To help clarify this situation we report here on density functional calculations(B3LYP) performed on model tyrosine hydrogen bonded complexes, in the reduced and oxidised state. These allow us to accurately predict the relevant vibrational frequencies and modes of the model complexes used. In such a fashion we clarify the experimental assignments as well as providing a deeper insight into the factors governing the vibrational characteristics of tyrosine and tyrosyl free radicals.

Methods

The density functional calculations were performed using the B3LYP functional combined with the EPR-II basis set. The tyrosine model complexes are shown in fig 1. The principal models used were p-cresol (MePH) , p-cresol/imidazole(MePH-IM), p-cresyl/imidazolium ion(MeP-$IMH^+\bullet$) and p-cresyl/imidazole(MeP-IM$\bullet$). Experimental studies on tyrosine and p-cresol(4) indicate that the 7a and δCOH bending vibrations are essentially identical for both suggesting that further extension of the models beyond p-cresol can be expected to have little influence on the calculated values. The calculated harmonic frequencies were scaled by 0.980(7a) and 0.983(δCOH) to allow comparison with experimental anharmonic values. These scaling factors were derived by comparing experimentally determined 7a and δCOH vibration frequencies for gas phase phenol with calculations performed at the level reported here.

Results and Discussion

The optimised bond distances for each model studied are given in fig 1.

Upon oxidation of MePH-IM and MePH-IM-HB proton transfer occurs simultaneously, without an energy barrier, from the phenol to the imidazole which then back hydrogen bonds

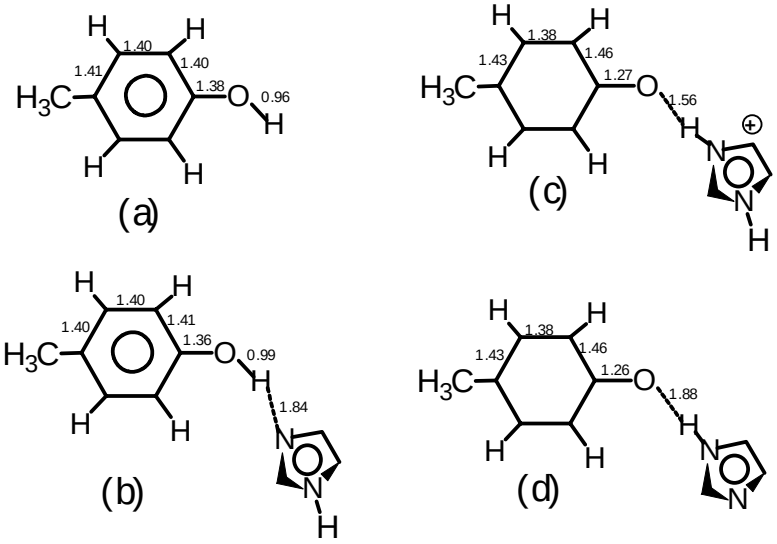

Figure 1 (a) MePH, (b) MePH-IM, (c) MeP-IMH$^{+\bullet}$, (d) MeP-IM$^{\bullet}$

with the oxygen atom of the phenoxyl radical in MeP-IMH$^{+}\bullet$ and MeP-IMH-HB$^{+}\bullet$. The scaled harmonic frequencies calculated for MePH, MePH-IM and MePH-IM-HB, for the 7a CO stretching and the δCOH bending vibrational modes, are presented in Table 1 where they are compared with experimental values for p-cresol and a p-cresol/imidazole complex (4). The trends in frequency changes observed experimentally are well reproduced by the calculations. In essence the increased frequency value of both modes on hydrogen bond formation of the phenol OH group with imidazole is quantitatively reproduced. The frequency values for the two redox active tyrosines of Photosystem II , Y_D and Y_Z (Table 1) are also close to the MePH-IM model providing supporting evidence that both correspond to protonated tyrosine residues hydrogen bonded to an imidazole group of a nearby histidine residue. In Table 1 the shifts in frequency observed on isotopic substitution are also presented. The predicted frequency values are in excellent agreement with those reported for both Y_D and Y_Z. These calculations provide confirmation of the assignments given in references 4 and 5 and indicate that both Y_Z and Y_D are *protonated* tyrosines which are hydrogen bonded to nearby imidazole groups of neighbouring histidine residues. One electron oxidation of MePH-IM leads to simultaneous transfer of the phenol proton to the hydrogen bonded imidazole as found previously for phenol-imidazole hydrogen bonded complexes(7). The geometry of the resultant positively charged radical complex, MeP-IMH$^{+}\bullet$ is given in fig 1c. The scaled harmonic frequency values calculated for the 7a CO stretching mode are given in Table 2 and compared with experimental determinations for $Y_Z\bullet$ and $Y_Z\bullet$. The shifts in frequency for the various isotopomers are also given in Table 2. It is clear from this Table that the MeP-IMH$^{+}\bullet$ model impressively reproduces the experimental values measured for the *in vivo* radicals. The calculated harmonic frequency data of Table 2 show that the MeP-IM$\bullet$ model does not exhibit good agreement with the experimental determinations for $Y_D\bullet$ and especially $Y_Z\bullet$.

Table 1 Comparison of calculated and experimental frequencies, cm^{-1}, for the reduced forms. Intensity (km/mol) and isotope shift values are given in square and round brackets respectively. Experimental values are as given in references 4 and 5.

System	Normal Isotopes		$^{13}C(4)$		$^{13}C_6$		$^{2}H_4$	
	7a	**δCOH**	**7a**	**δCOH**	**7a**	**δCOH**	**7a**	**δCOH**
THEORY								
MePH	1261[98]	1173[131]						
MePH-IM	1283[91]	1247[152]	1261(-22)	1232(-15)	1251(-32)	1222(-25)	1236(-47)	-
EXPERIMENTAL								
p-cresol/CCl_4	1255	1175	ND	ND	ND	ND	ND	ND
p-cresol/imidazole	1271	1251	ND	ND	ND	ND	1226(-45)	ND
Y_D	1275	1250	ND	1226(-24)	ND	1220(-30)	1230(-45)	ND
Y_Z	1279	1255	ND	1234(-21)	ND	1230(-25)	1233(-46)	ND

ND, Not determined experimentally; $^{13}C(4)$ labelling at C4 atom position; $^{13}C_6$ labelling at all six phenol ring carbons; $^{2}H_4$ labelling at all four phenol ring hydrogens.

Table 2 Comparison of experimental and calculated 7a frequency and isotope frequency shifts for the oxidised forms. Experimental values are as given in references 4 and 5.

System	ν_{7a}	$\Delta\nu_{7a}\ ^{13}C(4)$	$\Delta\nu_{7a}\ ^{2}H_4$	$\Delta\nu_{7a}\ ^{13}C_6$
THEORY				
MeP-IMH^{+}•	1515	-27	-17	-41
MeP-IM•	1493	-29	-24	-40
EXPERIMENTAL				
Y_Z•	1512	-27	-16	-36
Y_D•	1503	-26	-17	-35

$^{13}C(4)$ labelling at C4 atom position; $^{13}C_6$ labelling at all six phenoxyl ring carbons; $^{2}H_4$ labelling at all four phenoxyl ring hydrogens.

The upward shift in frequency, on hydrogen bond formation, for the 7a mode of phenoxyl type radicals is directly proportional to the hydrogen bond strength. The weaker hydrogen bond donation in MeP-IM• results therefore in a lower perturbation of the 7a mode form. A reasonable interpretation of the above data is that Y_Z• and Y_D• correspond to the situation modelled by MeP-IMH $^+$• or at least to a situation where the charge is located close to the tyrosyl free radical. The frequency value and the isotope shifts are in quite good agreement with experimental determinations. The phenol proton released on Y_Z oxidation is retained close to the tyrosyl radical retaining a strong hydrogen bond link between the D1-His190 and the Y_Z• radical and is readily available for back transfer on reduction of Y_Z• by the manganese complex. For Y_D• the lower 7a mode value compared with Y_Z• suggests a weaker hydrogen bonding interaction between D2-His189 and Y_D• than with Y_Z• and D1-His190. We conclude that the experimental FTIR data presented by Hienerwadel et al(4) and Berthomieu et al(5) are a true reflection of tyrosine oxidation for Y_D and Y_Z respectively and find no evidence to support the assignments from Barry and coworkers(6).

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