SUPPORTING INFORMATION

Probing Electrostatic Interactions along the Reaction Pathway of a Glycoside Hydrolase: Histidine Characterization by NMR Spectroscopy

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Supplemental Table S1: Chemical shifts and $^{1}J_{NH}$ couplings for the histidine residues in CexCD (30 ºC, pH 6.5).

Residue	Protein Form	$^{13}C^{\delta 2}H$ (ppm)	$^{13}C^{\varepsilon 1}H$ (ppm)	$^{15}N^{\delta 1}H$ (ppm)	$^{15}N^{\varepsilon 2}H$ (ppm)	$^{1}J_{N\delta 1H}$ (Hz) [b]	$^{1}J_{N\varepsilon 2H}$ (Hz) [b]
His80	apo	- /7.12	137.9/7.42	202.5 [a] / -	182.4 [a] / -	-	-
	2FCb	129.3/6.83	138.6/6.64	172.1/13.80	246.9/ -	98±1	-
	XbIso	- /6.63	137.5/6.71	172.4/13.85	243.6/ -	- [c]	-
	XbIm	- /6.74	140.1/7.04	169.9/13.32	244.1/ -	97±2	-
His85	apo	118.4/6.91	139.2/8.52	247.6/-	167.7/11.83	-	-
	2FCb	118.4/6.88	139.0/8.43	247.5/ -	167.5/11.80	-	101±2 [d]
	XbIso	- /6.85	138.5/8.35	- / -	167.0/11.75	-	-
	XbIm	- /6.79	138.9/8.50	248.1/ -	166.6/11.71	-	97±4
His107	apo	118.6/6.28	135.8/8.27	184.8/16.74	170.7/13.52	89±2	96±2
	2FCb	118.8/6.36	136.0/8.30	184.6/16.68	170.6/13.50	92±3	101±2
	XbIso	- /6.39	135.9/8.27	183.2/16.46	170.2/13.47	94±2	99±2
	XbIm	- /6.37	135.9/8.26	183.0/16.42	170.2/13.52	90±2	99±2
His114	apo	118.8/3.79	135.7/8.28	181.7 [a] / -	177.4 [a] / -	-	-
	2FCb	118.8/3.76	135.7/8.23	181.4 [a] / -	177.4 [a] / -	-	-
	XbIso	- /3.76	135.6/8.30	181.0 [a] / -	177.1 [a] / -		
	XbIm	- /3.76	135.6/8.30	181.0 [a] / -	177.1 [a] / -		
His205	apo	120.0/7.59	138.2/7.70	182.8 [a] / -	171.2 [a] / -	-	-
	2FCb	119.5/7.23	136.4/7.75	188.0/17.64	164.3/10.22	85±5	102±2
	XbIso	- /7.69	138.9/7.74	182.4/16.80	173.7/13.79	- [c]	- [c]
	XbIm	- /7.32	137.8/7.51	179.6/16.00	179.6/14.29	97±2	98±4

[a]Specific assignments of the $^{15}N^{\delta 1}$ and $^{15}N^{\varepsilon 2}$ chemical shifts for protonated imidazoles could not be obtain from a multiple bond ^{15}N-HSQC spectrum (Fig. 3), and thus the downfield chemical shift was arbitrarily tabulated as that from the $^{15}N^{\delta 1}$ nucleus.

[b]Except where indicated, $^{1}J_{NH}$ couplings were measured with IPAP spectra.

[c]Not determined due to overlap or weak signals.

[d]Measured from a ^{15}N-HSQC spectrum without ^{15}N decoupling.

Supplemental Table S2: Predicted pKa values for CexCD and 2FCb-CexCD.

His	Form[a]	Exp.	Calc. PropKa[b]	Calc. WHAT IF[c]	Decomposition[d,e]		
		pKa	pKa	pKa	Desolvation	H-bond or Background	Ionizable
H80	apo	7.9	5.2		-5.0	1.6 (D123)	-1.2 (K47) 1.5 (D123) 1.8 (E233)
				9.8	-2.3	0.9	4.9
	2FCb	< 2.8		6.7	-3.0	1.2	2.2
H85	apo	< 4	0.90		-3.3	0.04 (E127)	-2.4 (R136)
				4.4	-1.3	-0.5	-0.1
	2FCb	< 2.8		4.3	-1.3	-0.1	-0.6
H107	apo	>10.4	4.9		-4.9	1.60 (E52)	1.7 (E52)
				8.4	-3.4	2.7	2.8
	2FCb	>10.1		8.9	-3.4	3.2	2.8
H114	apo	8.1	7.0		-0.3	0.8 (D64)	0.0
				6.5	-1.0	0.6	0.7
	2FCb	8.1		6.6	-1.0	0.6	0.7
H205	apo	>10.4	8.8		-5.4	2.1 (E233) 1.6 (D235)	1.7 (E233) 2.4 (D235)
				11.3	-2.0	-0.1	7.0
	2FCb	>10.1		< 0	-2.5	-2.7	< 0

[a]Using the pdb files for apo-CexCD (2EXO.pdb) and 2FCb-CexCD (1EXP.pdb)

[b]Calculated using the PropKa webserver (http://propka.chem.uiowa.edu/) (*1*)

[c]pKa calculations were carried out with the WHAT IF pKa routines as described previously (*2*). The structural co-ordinate pdb files for apo-CexCD (2EXO.pdb) and 2FCb-CexCD (1EXP.pdb) were regularized using WHAT IF (*3*), and non-protein atoms, except those of the bound inhibitor, were removed. Charge and radii parameters for protein atoms were taken from the OPLS force field (*4*). Ligand topology files and charges on ligand atoms were calculated using the PRODRG web-server (*5*). Radii were assigned manually based on the OPLS force field (*4*). The WHAT IF pKa calculation routines use an algorithm developed by Hooft *et al*. (*6*) to find the optimal hydrogen-bond network for every protonation state needed in the pKa calculation scheme (*7*). The electrostatic interaction energies for the calculation then were obtained by solving the linear form of the Poisson-Boltzmann equation using the DelphiII program (*8*) with the following parameters: temperature 298.15K, ionic strength 0.144 M, ion exclusion radius 2.0 Å, solvent dielectric constant 80, protein dielectric constant.

[c]PropKa pKa values calculated with an intrinsic pKa 6.5 of histidine, corrected for desolvation, for sidechain and backbone hydrogen bonds, and for interactions with ionizable groups in presumed charged states.

[d]WHAT IF pKa values calculated with an intrinsic pKa 6.3 of histidine, corrected for desolvation and for interactions with background (non-titratable) and ionizable groups (at pH = pKa).

Supporting References

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