SUPPORTING INFORMATION

NMR Determination of pK_a Values for Asp, Glu, His, and Lys Mutants at Each Variable Contiguous Enzyme – Inhibitor Contact Position of Turkey Ovomucoid Third Domain

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All supplementary data were collected and analyzed under conditions equivalent to those described in the journal article.

Table S1. Fitted ^{1}H NMR Chemical Shifts, Measured pH_{mid} Values, and Hill Coefficients for Residue P_3'-Arg^{21} in Various OMTKY3 Variants with Single Amino Acid Replacements.

OMTKY3 variant	proton observed	δ_{A-}(ppm)	$\Delta\delta$ (ppm)[a]	pH_{mid}	Hill coefficient	deviation of the pH_{mid} from the pK_a of P_1'—Glu/Asp (error)
P_6-Lys^{13}Glu	$^1H^{\varepsilon}$	7.23	0.08	3.33(0.11)	0.93(0.22)	-0.14 (0.13)
P_5-Pro^{14}Asp	$^1H^{\varepsilon}$	7.24	0.07	3.51(0.04)	1.18(0.11)	-0.05 (0.07)
P_4-Pro^{15}Asp	$^1H^{\varepsilon}$	7.24	0.07	3.54(0.09)	1.26(0.29)	0.05 (0.10)
P_4-Ala^{15}Glu	$^1H^{\varepsilon}$	7.23	0.06	3.31(0.08)	1.34(0.26)	-0.21 (0.11)
P_4-Ala^{15}His	$^1H^{\varepsilon}$	7.24	0.07	3.55(0.07)	1.09(0.20)	-0.09 (0.09)
P_2-Thr^{17}Glu	$^1H^{\varepsilon}$	7.26	0.09	3.89(0.07)	1.07(0.18)	-0.02 (0.10)
P_2-Thr^{17}His	$^1H^{\varepsilon}$	7.22	0.07	3.75(0.07)	0.94(0.14)	-0.10 (0.12)
P_2-Thr^{17}Val	$^1H^{\varepsilon}$	7.23	0.07	3.71(0.07)	0.95(0.14)	-0.21 (0.10)
P_1-Leu^{18}Gly	$^1H^{\varepsilon}$	7.22	0.07	3.37(0.07)	1.06(0.15)	0.09 (0.08)
P_1-Leu^{18}Ala	$^1H^{\varepsilon}$	7.24	0.08	3.54(0.07)	0.86(0.12)	-0.01 (0.09)

P_1-Leu^{18}Asp	$^1H^{\varepsilon}$	7.23	0.06	3.53(0.05)	1.23(0.16)	0.33 (0.07)
P_1-Leu^{18}Glu	$^1H^{\varepsilon}$	7.23	0.07	3.47(0.06)	0.97(0.12)	0.08 (0.07)
P_1-Leu^{18}His	$^1H^{\varepsilon}$	7.24	0.06	3.33(0.06)	1.08(0.13)	-0.04 (0.07)
P_1'-Glu^{19}Asp	$^1H^{\varepsilon}$	7.36	0.20	3.21(0.02)	1.01(0.05)	0.08 (0.05)

[a]$\Delta\delta = \delta_{A^-} - \delta_{HA}$

Table S2. ^{1}H NMR Chemical Shifts, pH_{mid} Values, and Hill Coefficients for P_2-Asp^{17} P_1'-Glu^{19}, and P_3'-His^{21} in P_3'-Arg^{21}His OMTKY3.

residue	proton	δ_{A^-}(ppm)	$\Delta\delta$ (ppm)[a]	pH_{mid}	Hill coefficient
P_2-Thr	$^{1}H^{N}$	8.24	-0.07	6.07(0.06)	1.28(0.15)
		—	-0.11	3.44(0.03)	2.10(0.19)
P_1'-Glu^{19}	$^{1}H^{N}$	8.33	0.31	6.58(0.03)	1.11(0.05)
		—	0.37	3.42(0.08)	1.34(0.06)
P_1'-Arg^{21}	$^{1}H^{\varepsilon}$	7.62	-1.01	6.72(0.02)	0.92(0.02)
	$^{1}H^{\delta}$	6.84	-0.37	6.70(0.03)	0.88(0.02)
		—	-0.08	3.28(0.07)	1.29(0.18)

[a] $\Delta\delta = \delta_{A^-} - \delta_{HA}$

Table S3. Fitted ^{1}H NMR Chemical Shifts, Measured pH_{mid} Values, and Hill Coefficients of P_2-Thr^{17} and P_1'-Glu^{19} in Selected OMTKY3 Variants.

OMTKY3 variant	residue	proton	δ_{A^-}(ppm)	$\Delta\delta$ (ppm)[a]	pH_{mid}	Hill coefficient
P_2'-Tyr^{20}Asp	P_2-Thr^{17}	$^{1}H^{N}$	8.12	-0.20	4.11(0.04)	1.26(0.12)
	P_1'-Glu^{19}	$^{1}H^{N}$	8.05	0.32	3.98(0.03)	1.37(0.10)
P_2'-Tyr^{20}His	P_2-Thr^{17}	$^{1}H^{N}$	8.10	-0.17	3.47(0.04)	1.21(0.11)
	P_1'-Glu^{19}	$^{1}H^{N}$	8.16	0.56	3.45(0.03)	0.82 (0.05)
Wild type	P_2-Thr^{17}	$^{1}H^{N}$	8.15	-0.24	3.54(0.02)	1.00(0.04)
	P_1'-Glu^{19}	$^{1}H^{N}$	8.23	0.60	3.54(0.01)	1.04 (0.02)
P_3'-Arg^{21}Glu	P_2-Thr^{17}	$^{1}H^{N}$	8.15	-0.39	3.80(0.03)	0.75(0.04)
	P_1'-Glu^{19}	$^{1}H^{N}$	8.89	1.28	3.91(0.02)	0.76(0.02)

[a] $\Delta\delta = \delta_{A^-} - \delta_{HA}$

Table S4. ^{1}H NMR Chemical Shifts, Measured pK_a Values, and Hill Coefficients of Titratable Residues in Wild Type OMTKY3.

residue	proton	δ_{A^-}(ppm)	$\Delta\delta$ (ppm)[a]	pK_a	Hill coefficient	variation from model pK_a values[b]
P_{12}-Asp^{7}	$^1H^{\beta}$	2.72	-0.24	2.78(0.05)	0.84(0.08)	-1.1
P_{9}-Glu^{10}	$^1H^{\gamma}$	2.40	-0.25	4.18(0.02)	1.04(0.40)	-0.1
P_{6}-Lys^{13}	$^1H^{\varepsilon}$	1.98	-0.56	9.65(0.05)	0.80(0.07)	-0.9
P_{1}'-Glu^{19}	$^1H^{N}$	8.23	0.60	3.54(0.02)	1.04(0.03)	-0.8
P_{9}'-Asp^{27}	$^1H^{\beta}$	2.55	-0.46	2.34(0.03)	0.89(0.04)	-1.6
P_{25}'-Glu^{43}	$^1H^{\gamma}$	2.42	-0.40	4.62(0.07)	0.89(0.11)	0.3
P_{34}'-His^{52}	$^1H^{\varepsilon}$	7.87	-0.92	7.53(0.02)	0.92(0.02)	0.5

[a] $\Delta\delta = \delta_{A^-} - \delta_{HA}$

[b] Standard pK_a values assumed here were: Asp (3.84), Glu (4.32), His (6.50), and Lys (10.5); see the text.