Supporting Information

Protein conformational gating of enzymatic activity in xanthine oxidoreductase

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Reference 27:
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Evaluation of the protein dielectric constant ε_p. The optimal ε_p value depends on the protein model used. The more atomic details are included explicitly in the description of the molecular system, the lower the ε_p value becomes. The ε_P size is a reflection of what is not included explicitly (e.g., $\varepsilon_p = 1$ for the system with all the possible factors influencing electrostatic interactions being considered explicitly) (1,2). As demonstrated by Warshel and coworkers (1,2) $\varepsilon_p \approx 4$ can be used when protein flexibility is taken into account explicitly. Furthermore, it is unlikely that the ε_p for charge-dipole and charge-charge interactions are identical (1). It has been suggested that $\varepsilon_p \approx 20$ and 40 should be used for interactions of charged-uncharged groups and charged-charged groups, respectively (1,3).

In the present study, we do not consider flexibility of protein structure explicitly. However, we consider protonation states of all titratable sites in the whole protein explicitly, i.e., the flexibility of protein charge where all the titratable sites are simultaneously equilibrated. It is important to note that the protonation states of the titratable groups are modulated in response to the flavin redox state. Hence, the ε_p value in the present study could be lower than that used in other studies, in which changes of atomic charges as a consequence of titration are not modeled explicitly. Based on a number of studies, we obtained best results for the redox potential values for redox-active groups (e.g. flavin (4-6), chlorophyll (7), pheophytin (8), heme (9), and tyrosine (10)) and pK_a values for titratable groups (11-14) in a number of proteins, using a dielectric constant of $\varepsilon_p = 4$ inside the protein. Hence, $\varepsilon_p = 4$ appears to be optimal in the current computational model used in the present study.

SUPPORTING REFERENCES

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Table S1. Atomic partial charges of titratable phosphoacid (the proximal and distal phosphate sites in the pyrophosphate region) of FAD and NAD.

atom	protonated	deprotonated
P	1.43	1.27
O1	–0.42	–0.84
O2	–0.42	–0.84

Table S2. Atomic partial charges of the molybdopterin center.

atom	Mo(VI)
N1	–0.66
C2	0.79
N2	–0.78
H2A	0.31
H2B	0.31
N3	–0.80
H3	0.41
C4	0.72
O4	–0.57
N5	–0.44
H5	0.30
C6	0.13
C7	0.12
N8	–0.39
H8	0.29
C9	–0.17
C10	0.43
C1'	–0.03
S1'	–0.27
C2'	–0.04
S2'	–0.44
C3'	0.22
O3'	–0.28
MO	0.87
S	–0.43
O1	–0.59
HO1	0.35
O2	–0.36

Table S3. Atomic partial charges of NAD^+/NADH.

	NAD^+	**NADH**
N1	0.37	0.03
C6	-0.04	-0.13
H6	0.18	0.15
C5	-0.1	-0.23
H5	0.18	0.10
C4	0.04	0.24
H4	0.00	0.00
H42	0.00	0.00
H4D	0.16	0.00
C3	-0.16	-0.27
C2	0.02	-0.03
H2	0.15	0.11
C7	0.66	0.64
O7	-0.44	-0.52
N7	-0.84	-0.83
H71	0.41	0.37
H72	0.41	0.37

Table S4 (a) Protein components differentiating the $E_{sq/hq}$ value for XDH/XO in mV. Protein components that increase/decrease the $E_{sq/hq}$ difference by ≥ 20 mV are listed. For clarity, other residues are omitted from the table.

	$E_{sq/hq}$(XDH)			$E_{sq/hq}$(XO)			Difference ($\Delta E_{sq/hq}$) [a]			
	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	location
main components increasing $\Delta E_{sq/hq}$										
Lys433	40	0	40	154	-2	152	-114	2	-112	loop [d]
Glu263	-174	-3	-177	-101	-3	-104	-73	0	-73	
Asp429	-151	14	-137	-53	-13	-66	-98	27	-71	loop [d]
Asp430	-83	-25	-108	-44	-2	-46	-39	-23	-62	loop [d]
Asp360	-275	39	-236	-230	40	-190	-45	-1	-46	
Arg426	92	-4	88	126	-1	125	-34	-3	-37	loop [d]
Asn71	-16	2	-14	13	2	15	-29	0	-29	
Glu267	-35	3	-32	-13	3	-10	-22	0	-22	
FeS-II[e]			-151			-129			-22	
Gln144	-8	-6	-14	12	-5	7	-20	-1	-21	
main components decreasing $\Delta E_{sq/hq}$										
Lys422	257	4	261	152	3	155	105	1	106	loop [d]
P (proximal)[f]			14			-81			95	
Lys1228	124	3	127	76	4	80	48	-1	47	
Lys271	52	1	53	20	2	22	32	-1	31	
Lys343	86	4	90	64	3	67	22	1	23	
Ala338	3	27	30	5	5	10	-2	22	20	

[a] $E_{sq/hq}$(XDH) – $E_{sq/hq}$(XO).

[b] Side chain.

[c] Backbone.

[d] The loop region (Gln423–Lys433) close to the flavin ring [5].

[e] The iron-sulfur cluster proximal to the flavin ring.

[f] A phosphate titratable site of the FAD diphosphate region proximal to the flavin ring.

(b) Influence of the atomic charges of residues in the 423-433 loop (including Lys422) on the $E_{sq/hq}$ value for XDH/XO in mV.

	$E_{sq/hq}$(XDH)			$E_{sq/hq}$(XO)			Difference ($\Delta E_{sq/hq}$) [a]		
residues	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total
Lys422	257	4	261	152	3	155	105	1	106
Gln423	0	-2	-2	2	-7	-5	-2	5	3
Ala424	1	3	4	-1	0	-1	2	3	5
Ser425	1	4	5	-1	2	1	2	2	4
Arg426	92	-4	88	126	-1	125	-34	-3	-37
Arg427	29	-8	21	21	2	23	8	-10	-2
Glu428	-41	2	-39	-19	-7	-26	-22	9	-13
Asp429	-151	14	-137	-53	-13	-66	-98	27	-71
Asp430	-83	-25	-108	-44	-2	-46	-39	-23	-62
Ile431	9	0	9	-6	0	-6	15	0	15
Ala432	1	9	10	0	-2	-2	1	11	12
Lys433	40	0	40	154	-2	152	-114	2	-112
total	155	-3	152	331	-27	304	-176	24	-152

[a] $E_{sq/hq}$(XDH) – $E_{sq/hq}$(XO).

[b] Side chain.

[c] Backbone.

Table S5. $E_{sq/hq}$ for XDH; (a) protein components increasing the $E_{sq/hq}$ value in response to NAD^+ binding in mV. Protein components that increase/decrease the $E_{sq/hq}$ difference by ≥ 20 mV are listed. For clarity, other residues are omitted from the table.

				with NAD^+			Difference [a]		
	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total
NAD^+						358			358
Lys422	257	4	261	318	5	323	61	1	62
P (NAD^+, proximal)[d]						36			36
Arg394	56	4	60	83	7	90	27	3	30
Lys343	86	4	90	108	6	114	22	2	24

[a] $E_{sq/hq}$(XDH with NAD^+) – $E_{sq/hq}$(XDH).

[b] Side chain.

[c] Backbone.

[d] A phosphate titratable site of the NAD^+ diphosphate region proximal to the nicotinamide ring.

(b) Protein components compensating for the $E_{sq/hq}$ upshift in response to NAD^+ binding in mV.

				with NAD^+			Difference [a]		
	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total
Asp429	-151	14	-137	-280	27	-253	-129	13	-116
Glu263	-174	-3	-177	-257	0	-257	-83	3	-80
Asp360	-275	39	-236	-358	46	-312	-83	7	-76
FeS-II[e]	-151	0	-151	-183	0	-183	-32	0	-32
Asp430	-83	-25	-108	-103	-31	-134	-20	-6	-26
P (FAD, distal)[f]	-75	0	-75	-97	0	-97	-22	0	-22

[e] The iron-sulfur cluster proximal to the flavin ring.

[f] A phosphate titratable site of the FAD diphosphate region distal to the flavin ring.

Table S6. Protein components differentiating the pK_a(N5) value for $FADH^{\bullet}/FAD^{\bullet-}$ in pK_a. Protein components that increase/decrease the pK_a(N5) difference by ≥ 0.4 are listed.

	XDH			XO			Difference [a]		
	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total	side.[b]	b.b.[c]	total
increasing the pK_a(N5) difference[a]									
Lys433	-0.7	0	-0.7	-2.5	0	-2.5	1.8	0	1.8
Asp430	1.5	0.5	2.0	0.7	0	0.7	0.8	0.5	1.3
Asp429	2.6	-0.4	2.2	0.8	0.2	1.0	1.8	-0.6	1.2
Glu263	2.7	0	2.7	1.6	0	1.6	1.1	0	1.1
Asp360	4.3	-0.6	3.7	3.5	-0.6	2.9	0.8	0	0.8
Asn71	0.3	0	0.3	-0.2	0	-0.2	0.5	0	0.5
Gln144	0.2	0.1	0.3	-0.2	0.1	-0.1	0.4	0	0.4
Arg426	-1.8	0.1	-1.7	-2.1	0	-2.1	0.3	0.1	0.4
FeS-II[d]			2.6			2.2			0.4
decreasing the pK_a(N5) difference[a]									
Lys422	-4.3	-0.1	-4.4	-2.5	-0.1	-2.6	-1.8	0	-1.8
P (proximal)[e]			-0.2			1.3			-1.5
Lys1228	-2.3	-0.1	-2.4	-1.4	-0.1	-1.5	-0.9	0	-0.9
Lys271	-0.9	0	-0.9	-0.3	0	-0.3	-0.6	0	-0.6
Ala338	0	-0.8	-0.8	-0.1	-0.1	-0.2	0.1	-0.7	-0.6
Lys343	-1.6	-0.1	-1.7	-1.1	-0.1	-1.2	-0.5	0	-0.5

[a] pK_a(XDH) – pK_a(XO). A positive value here indicates an increased difference between pK_a(N5) in the two enzymes, while a negative value indicates a decreased difference.

[b] Side chain.

[c] Backbone.

[d] The iron-sulfur cluster proximal to the flavin ring.

[e] A phosphate titratable site of the FAD pyrophosphate part proximal to the flavin ring.