BJ www.biochemj.org

Biochem. J. (2008) **413**, 81–91 (Printed in Great Britain) doi:10.1042/BJ20071373

SUPPLEMENTARY ONLINE DATA

Kinetic, thermodynamic and X-ray structural insights into the interaction of melatonin and analogues with quinone reductase 2

Barbara CALAMINI*, Bernard D. SANTARSIERO*, Jean A. BOUTIN† and Andrew D. MESECAR*[1]

*Center for Pharmaceutical Biotechnology and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The University of Illinois at Chicago, 900 S. Ashland Ave M/C 870, Chicago, IL 60607, U.S.A., and †Pharmacologie Moléculaire et Cellulaire, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Seine, France

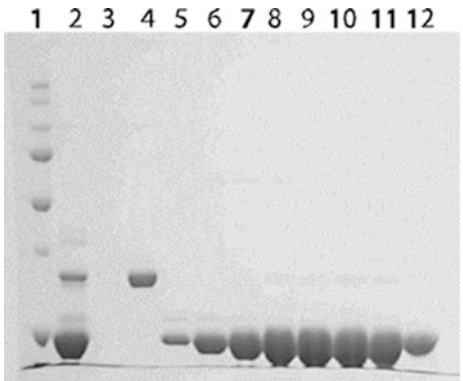

Figure S1 SDS/PAGE gel for the last chromatographic step in the purification of untagged wild-type human QR2

Lane 1, molecular-mass standards ladder; lane 2, Superdex pool; lanes 3 and 4, eluted impurities; lanes 5–12, eluted fractions of QR2.

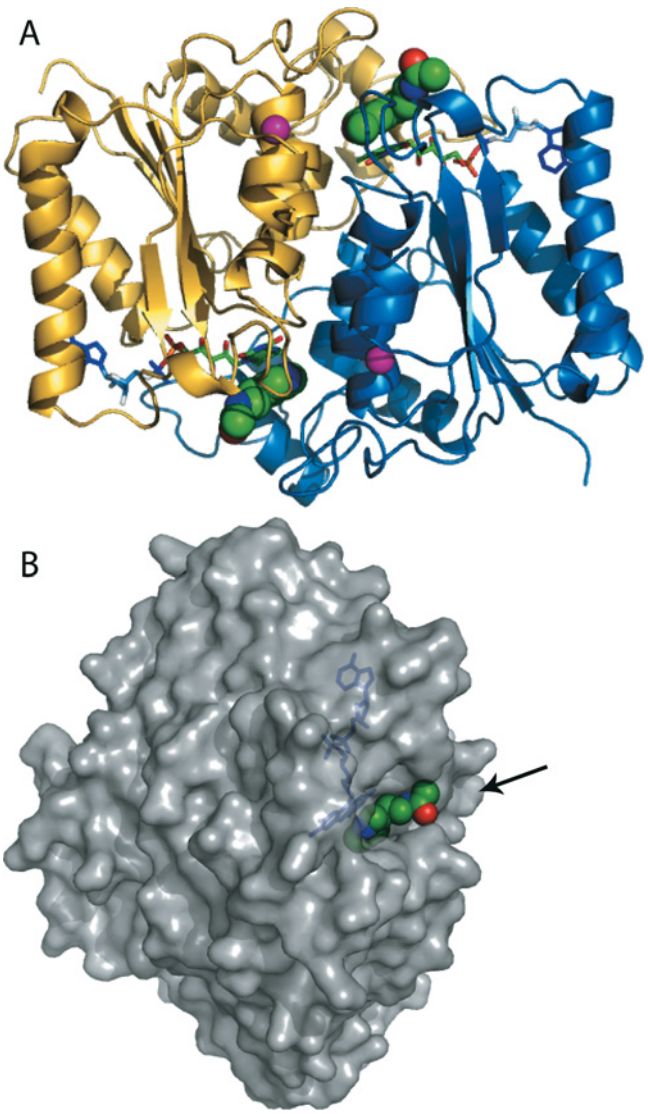

Figure S2 Structure of QR2 complexed to melatonin

(**A**) Ribbon diagram of the secondary structure of the protein; one monomer is coloured yellow, and the other is blue. FAD is shown as a stick diagram, and melatonin is shown in space-filling representation. The zinc atoms are coloured magenta. (**B**) Surface representation of QR2 (grey). The substrate-binding site shown by the arrow is exposed to the solvent. FAD (blue sticks) is shown in transparency.

[1] To whom correspondence should be addressed (email mesecar@uic.edu).

The atomic co-ordinates and structure factors have been deposited in the Protein Data Bank under codes 2QWX, 2QX4 and 2QX6 for the QR2 (quinone reductase 2)–melatonin complex, and 2QX8 and 2QX9 for the QR2–2-iodomelatonin complex.

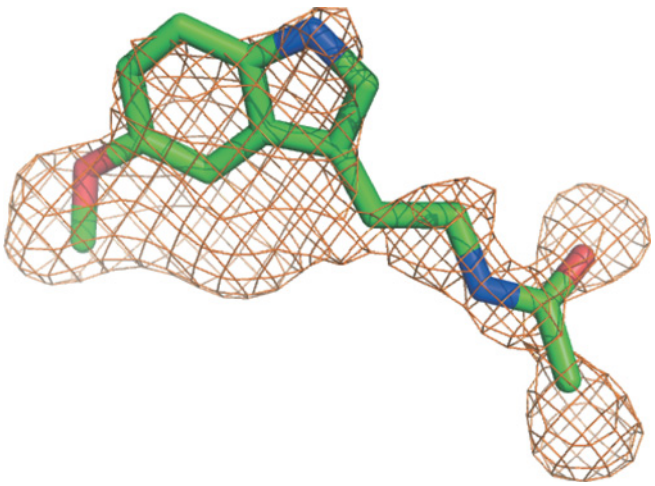

Figure S3 Representative difference electron-density map of melatonin in the active site (site 1) of one monomer

Density is contoured at the 3σ level.

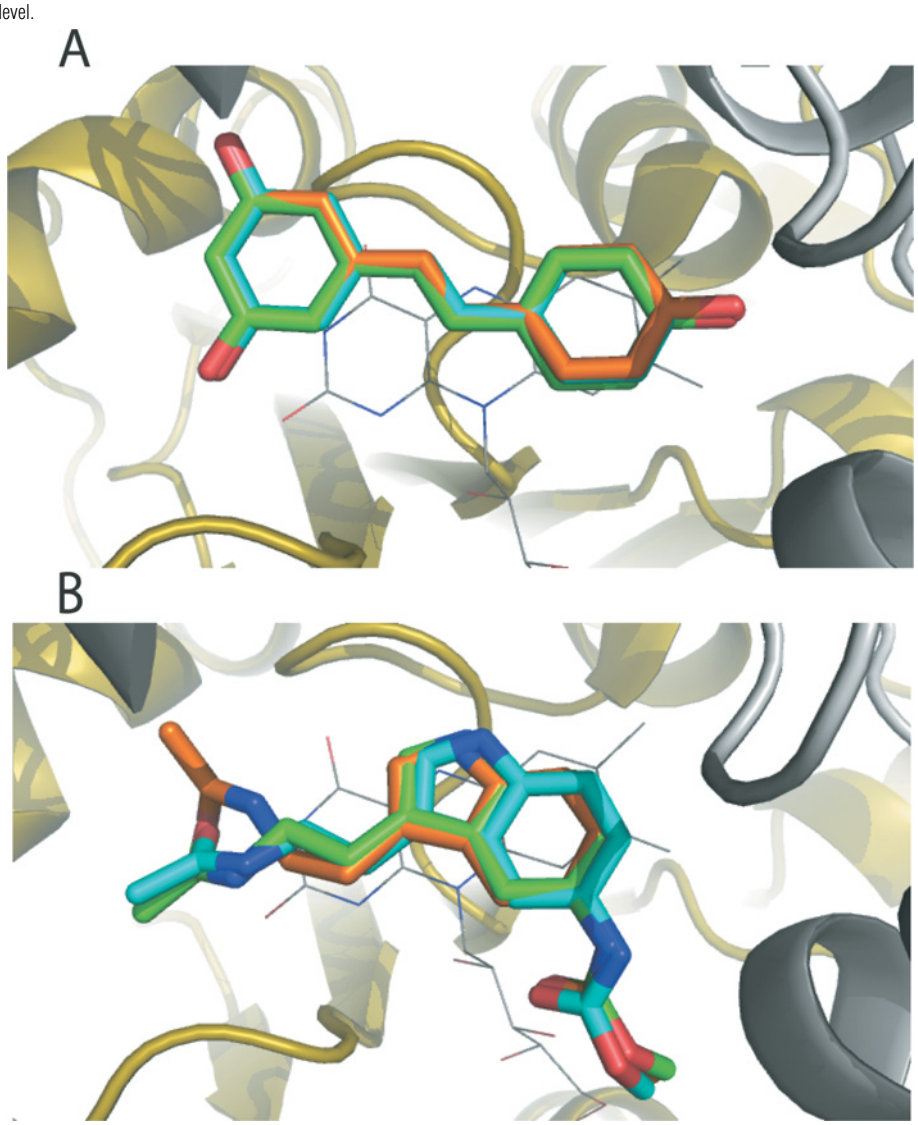

Figure S4 Results of the docking of resveratrol and MCA-NAT inside the QR2 active site

Docking solutions were generated using GOLD for resveratrol (**A**) and MCA-NAT (**B**).

Table S1 X-ray data collection and refinement statistics

$R_{merge} = \Sigma(I - \langle I \rangle)/\Sigma\langle I \rangle$. $R_{cryst} = \Sigma_{hkl}||F_{obs}| - k|F_{calc}||/\Sigma_{hkl}|F_{obs}|$. R_{free} is the R_{cryst} value for 5% of the reflections excluded from the refinement. RMSD (root mean square deviation) was calculated with Procheck (http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html). Values in parentheses indicate the highest-resolution shell.

	QR2–melatonin 1	QR2–melatonin 2	QR2–melatonin 3	QR2–iodomelatonin 1	QR2–iodomelatonin 2
Data collection					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (Å):					
a	56.635	56.576	56.530	56.595	56.595
b	83.549	83.621	83.617	83.323	81.528
c	106.352	106.492	106.274	106.213	106.178
α, β, γ (°)	90	90	90	90	90
Resolution (Å)	20–1.47	20–1.65	20–1.75	20–1.6	20–2.3
Total no. of observations	393693	112803	185420	960426	118827
No. of averaged observations	84189	59983	91839	136944	22987
R_{merge} (%)	6.6 (57.4)	7.3 (83.7)	7.2 (63.2)	9.1 (78.7)	20.3 (75.2)
χ^2	1.56	1.80	1.00	1.02	1.01
$I/\sigma I$	29.8 (2.3)	31.3 (1.71)	8.62 (1.2)	13.9 (1.6)	7.10 (2.91)
Completeness (%)	97.9 (91.9)	97.8 (99.1)	93.5 (71.5)	98.3 (92.1)	99.0 (99.9)
Refinement					
Resolution range (Å)	20–1.50	20–1.65	20–1.75	20–1.6	20–2.3
No. of reflections in working set	76077	56904	47614	63699	18872
No. of reflections in test set	4059	3017	2506	2690	959
R_{cryst} (%)	19.2	18.6	19.2	21.1	21.4
R_{free} (%)	21.3	21.8	22.0	23.5	28.5
Figure of merit	0.865	0.859	0.862	0.868	0.770
B factor (Å^2)	25.4	33.8	27.1	24.8	54.9
RMSD					
Bond length (Å)	0.013	0.012	0.019	0.014	0.009
Bond angle (°)	1.61	1.52	1.40	1.61	1.40
Dihedral angle (°)	22.0	21.9	22.3	21.8	21.5
Improper angle (°)	1.13	1.04	0.99	1.14	0.90
Ramachandran plot (%)					
Most favoured	96.1	91.2	90.1	96.7	87.2
Allowed	3.9	8.8	9.9	3.3	12.3
Disallowed	0	0	0	0	0.5

Table S2 Comparison of the steady-state inhibition kinetic parameters of QR2 using SigmaPlot 9.0

AICc, corrected Akaike information criterion.

(a) Inhibition of QR2 by melatonin with constant menadione and various NMeH concentrations

Parameter	Competitive	Non-competitive	Uncompetitive
V_{max} (μM/min)	41.8 ± 2.2	47.7 ± 3.4	47.0 ± 4.3
K_m (μM)	6.2 ± 2.3	14.6 ± 4.1	14.4 ± 5.4
K_i (μM)	7.2 ± 2.4	85.0 ± 11.9	68.5 ± 12.3
R^2	0.93	0.87	0.83
Test	Pass	Pass	Pass
Convergence	Yes	Yes	Yes
AICc	108.5	128.6	140.8

(b) Inhibition of QR2 by melatonin with constant NMeH and various menadione concentrations

Parameter	Competitive	Non-competitive	Uncompetitive
V_{max} (μM/min)	49.9 ± 2.8	52.8 ± 1.5	57.5 ± 1.6
K_m (μM)	15.7 ± 3.3	17.6 ± 1.6	23.2 ± 1.9
K_i (μM)	34.0 ± 6.3	142.6 ± 7.5	91.6 ± 4.6
r^2	0.86	0.96	0.97
Test	Fail	Pass	Pass
Convergence	Yes	Yes	Yes
AICc	190.2	116.3	97.3

Received 8 October 2007/29 January 2008; accepted 7 February 2008
Published as BJ Immediate Publication 7 February 2008, doi:10.1042/BJ20071373