SUPPLEMENTARY DATA FOR:

Human Cyclooxygenase-2 Is a Sequence Homodimer That Functions as a Conformational Heterodimer

Liang Dong*, Alex J. Vecchio[+], Narayan P. Sharma*,

Brice J. Jurban*, Michael G. Malkowski[+], and William L. Smith*[¶]

*Department of Biological Chemistry, University of Michigan Medical School,

Ann Arbor, MI 48109 USA

[+] Hauptman-Woodward Medical Research Institute and Department of Structural Biology,

The State University of New York at Buffalo, Buffalo, NY 14203 USA

Running Title: Allosteric Regulation of Cyclooxygenase-2

Key words: COX-1, COX-2, prostaglandin endoperoxide H synthase, NSAID, half-of-sites, arachidonic acid, palmitic acid, diet, fatty acids, coxib

¶ **Corresponding author**: William L. Smith, Department of Biological Chemistry, University of Michigan Medical School, 5301 MSRB III, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0606. Tel: (734) 647-6180; Fax: (734) 763-4581, E-mail: smithww@umich.edu

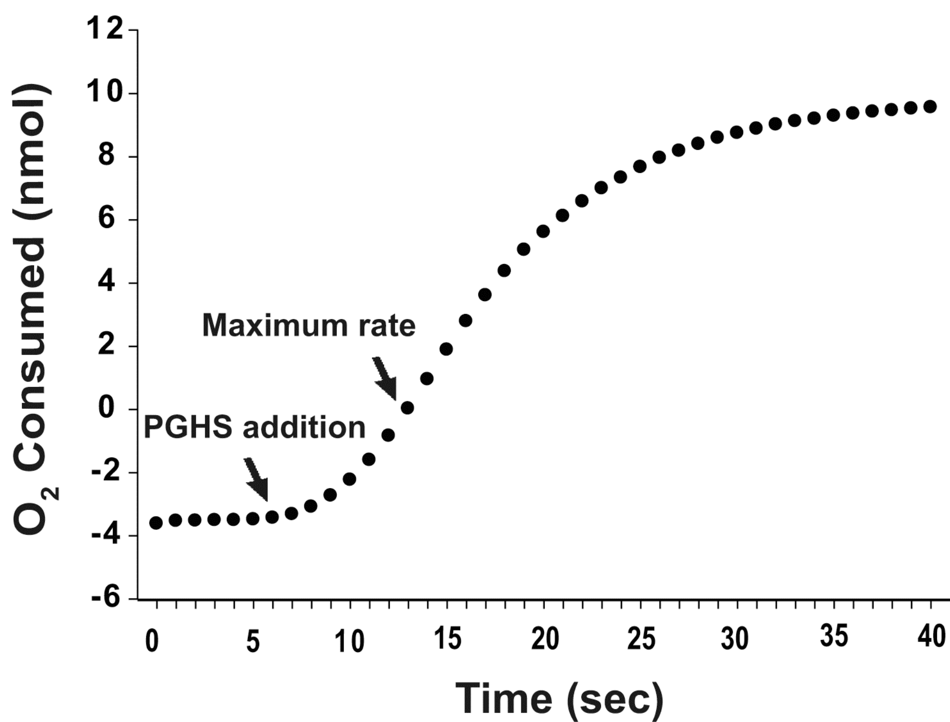

Fig. S1. O_2 electrode response in the oxygenation of AA by huPGHS-2. The assay was performed under standard assay conditions and recorded using DasyLab (DasyTec) software (1) as detailed in Materials and Methods. In this particular reaction, the volume was 3 ml, the final concentration of AA was 3 μM and the final concentration of huPGHS-2 in the assay chamber was 12 nM.

Table S1. K_d Values for heme binding to huPGHS-2 in the absence and presence of ligands that can bind to the COX active site. Spectroscopic measurements of heme binding were performed and K_d values calculated as described in Experimental Procedures and the legend to Fig. 1. Values are means $\pm$ S.E.M. from a single experiment with the same enzyme preparation except for the value with palmitic acid alone (*), which was determined using a different enzyme preparation. The concentration of each COX ligand used in the different titrations was sufficient to occupy one COX monomer. Measurements of the effects of various COX ligands on heme binding were performed using three different enzyme preparations and no statistically significant differences were seen in the K_d values determined without ligand vs. with any COX ligand or combination of ligands. A $K_d = 88 \pm 17$ nM (n = 6) for heme binding in the absence of COX ligand was determined using six different enzyme preparations. None of the values shown in this table are significantly different from one another as determined with a student t-test ($p<0.05$).

COX Ligands Present in the Titration Mixture in Addition to Heme	K_d for Heme Binding (nM)
None	41 ± 13
Celecoxib (25 μM)	29 ± 13
Palmitic acid (20 μM)	53 ± 34*
Celecoxib (25 μM) plus Palmitic acid (20 μM)	34 ± 12
Naproxen (50 μM)	24 ± 4.0

Table S2. K_d Values for heme binding to huPGHS-2 following treatment with aspirin. Spectroscopic measurements of heme binding were performed and K_d values calculated as described in Experimental Procedures and the legend to Fig. 1. Values are means $\pm$ S.E.M. from a single experiment with the same enzyme preparation. Purified huPGHS-2 (5 μM) was incubated with 0, 1.0 or 2.5 mM aspirin for 60 min at 37° C (2) prior to the titration with heme. None of the values shown in this table are significantly different from one another as determined with a student t-test ($p<0.05$).

COX Ligands Present in the Titration Mixture in Addition to Heme	**K_d for Heme Binding (nM)**
None	48 $\pm$ 14
Aspirin (1.0 mM)	56 $\pm$ 20
Aspirin (2.5 mM)	74 $\pm$ 27

Table S3. **Effects of various FAs and combinations of FAs on AA oxygenation by huPGHS-2**. Purified huPGHS-2 was incubated with the indicated FAs and combinations of FAs and initial rates of O_2 consumption determined as detailed in the Experimental Procedures. The results are shown as percentages of COX rates with non-substrate FAs (12.5 µM) plus AA (5 µM) vs. AA alone (5 µM). When combinations of non-substrate FAs were tested, each FA was present at the same concentration. The average specific activity of huPGHS-2 preparations used in the experiments was 40 units/mg. Values are from at least triplicate assays and are shown as the means ± S.D. All of the values in the table were significantly different than the value with 5 µM AA alone in a student t-test ($p<0.05$).

Fatty acid	**O_2 consumption with 12.5 µM FAs + 5 µM AA relative to 5 µM AA alone (%)**	**Fatty acid**	**O_2 consumption with 12.5 µM FAs + 5 µM AA relative to 5 µM AA alone (%)**
Palmitic acid	171 ± 5.8	20:1ω9 + stearic acid + oleic acid	127 ± 16
11-*cis*-eicosaenoic acid (20:1ω9)	135 ± 3.3	Palmitic acid + 20:1ω9	158 ± 11
Oleic acid	126 ± 0.5	Palmitic acid + oleic acid	139 ± 10
Stearic acid	110 ± 0.94	Palmitic acid + stearic acid	150 ± 15
Palmitic acid + 20:1ω9 + stearic acid + oleic acid	146 ± 5.4	20:1ω9 + oleic acid	118 ± 10
Palmitic acid + 20:1ω9 + stearic acid	160 ± 10	20:1ω9 + stearic acid	113 ± 5.9
Palmitic acid + 20:1ω9 + oleic acid	144 ± 9.5	Oleic acid + stearic acid	124 ± 4.8

Table S4. The contacts made between palmitic acid and cyclooxygenase active site residues in the muPGHS-2/PA Monomer A. PA makes a total of 36 contacts with COX active site residues. Five are hydrophilic in nature (depicted in red). The contacts were generated using COOT (3) with a minimum and maximum distance of 2.4 Å and 4.0 Å, respectively, used to define a van der Waals contact.

PA bound in muPGHS-2:PA Monomer A							
COX Residue	**COX Atom**	**PA Atom**	**Distance (Å)**	**COX Residue**	**COX Atom**	**PA Atom**	**Distance (Å)**
Arg-120	NE	O1	2.8	Tyr-385	CE1	C11	3.6
	NE	O2	3.5		CE1	C13	3.6
	CZ	C1	3.9		CE1	C15	3.8
	NH2	O1	3.6		CZ	C11	4.0
	NH2	O2	2.7		CZ	C13	3.4
Phe-205	CE2	C15	3.7		CZ	C15	3.9
	CE2	C16	3.6	Trp-387	CZ2	C11	4.0
Tyr-348	CE2	C13	3.6	Val-523	CG1	C6	3.9
	CE2	C14	3.6	Gly-526	CA	C10	3.7
Val-349	CG1	C3	3.6		C	C8	3.8
	CG2	C14	3.9		C	C10	3.9
Ser-353	CA	C5	3.6	Ala-527	CA	C8	3.6
	CB	C4	3.8		CB	C1	3.8
	CB	C5	3.7		CB	C8	3.9
Tyr-355	CE2	C2	3.8	Ser-530	CA	C16	3.9
	CE2	C4	3.8		CB	C12	3.8
	CZ	C4	3.7		CB	C14	3.6
	OH	O2	2.8		CB	C16	3.5

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