

Supporting Information

Alterio et al. 10.1073/pnas.0908301106

	SIGNAL SEQUENCE	HIS-TAG & PROTEASE SITE	PG DOMAIN
CA IX	MAPLCPSFWLPLLIIPAPAGLTVQLLLSLLLIMPVHPQRL	FRMQEDSPLGGSSGEDDPLGEEDLPSEEDSFRQEDDPGEEDLPGEEDLPGEEDLPVVKPKSEEGSLKLEDLPVEAFGDPQEPQNNHRDKEGDDQSHWR	
P1	MAPLCPSFWLPLLIIPAPAGLTVQLLLSLLLIMPVHPQRLHHHHHHHLLVPRGS	QRLPRMQEDSPLGGSSGEDDPLGEEDLPSEEDSFRQEDDPGEEDLPGEEDLPGEEDLPVVKPKSEEGSLKLEDLPVEAFGDPQEPQNNHRDKEGDDQSHWR	
P2	MAPLCPSFWLPLLIIPAPAGLTVQLLLSLLLIMPVHPQRLHHHHHHHLLVPRGS		HRDKEGDDQSHWR
P3	MAPLCPSFWLPLLIIPAPAGLTVQLLLSLLLIMPVHPQRLHHHHHHHLLVPRGS		DQSHWR
P4	MAPLCPSFWLPLLIIPAPAGLTVQLLLSLLLIMPVHPQRLHHHHHHHLLVPRGS		DQSHWR
CA DOMAIN			
CA IX	YGGDPPWPRVSPACAGRFQSPVDIRPQLAFCPALRPLELLGFQLPPLPELRRLRNNHSHVQLTLPGLMALGPGREYRALQILHWGAAGRFGSEHTVEGHRFPAEI		VHLSLTAFAFARVDEALGRPGGLAVLAAFLEEPEENSAYEQLLSRLEETAEEG
P1	YGGDPPWPRVSPACAGRFQSPVDIRPQLAFCPALRPLELLGFQLPPLPELRRLRNNHSHVQLTLPGLMALGPGREYRALQILHWGAAGRFGSEHTVEGHRFPAEI		VHLSLTAFAFARVDEALGRPGGLAVLAAFLEEPEENSAYEQLLSRLEETAEEG
P2	YGGDPPWPRVSPACAGRFQSPVDIRPQLAFCPALRPLELLGFQLPPLPELRRLRNNHSHVQLTLPGLMALGPGREYRALQILHWGAAGRFGSEHTVEGHRFPAEI		VHLSLTAFAFARVDEALGRPGGLAVLAAFLEEPEENSAYEQLLSRLEETAEEG
P3	YGGDPPWPRVSPACAGRFQSPVDIRPQLAFCPALRPLELLGFQLPPLPELRRLRNNHSHVQLTLPGLMALGPGREYRALQILHWGAAGRFGSEHTVEGHRFPAEI		VHLSLTAFAFARVDEALGRPGGLAVLAAFLEEPEENSAYEQLLSRLEETAEEG
P4	YGGDPPWPRVSPACAGRFQSPVDIRPQLAFCPALRPLELLGFQLPPLPELRRLRNNHSHVQLTLPGLMALGPGREYRALQILHWGAAGRFGSEHTVEGHRFPAEI		VHLSLTAFAFARVDEALGRPGGLAVLAAFLEEPEENSAYEQLLSRLEETAEEG
TRANSMEMBRANE AND INTRACYTOSOLIC REGIONS			
CA IX	SETQVPLDISALLPSDFSRYFYEGSLITPPCAQGVIIWVFNQIVMLSAKQLHTLSDTLWGPDSRLQINFRATQPLNGRVIEASFPAQVDSPPRAAEVQVNSCLAAGDILALVPLGLFAVTSVAVLQVRRQHRRGITKGGVSYRPAEVAETGA		
P1	SETQVPLDISALLPSDFSRYFYEGSLITPPCAQGVIIWVFNQIVMLSAKQLHTLSDTLWGPDSRLQINFRATQPLNGRVIEASFPAQVDSPPRAAEVQVNSCLAAGD		
P2	SETQVPLDISALLPSDFSRYFYEGSLITPPCAQGVIIWVFNQIVMLSAKQLHTLSDTLWGPDSRLQINFRATQPLNGRVIEASF		
P3	SETQVPLDISALLPSDFSRYFYEGSLITPPCAQGVIIWVFNQIVMLSAKQLHTLSDTLWGPDSRLQINFRATQPLNGRVIEASFPAQVDSPPRAAEVQVNSCLAAGD		
P4	SETQVPLDISALLPSDFSRYFYEGSLITPPCAQGVIIWVFNQIVMLSAKQLHTLSDTLWGPDSRLQINFRATQPLNGRVIEASF		

Fig. S1. Sequence alignment showing hCA IX and the different recombinant products P1, P2, P3, and P4. After protease treatment, the signal sequence and the His-tag (both shown in light gray) were removed from the proteins. The Cys/Ser mutation of P4 is shown in black. The catalytic His residues are highlighted in dark gray.

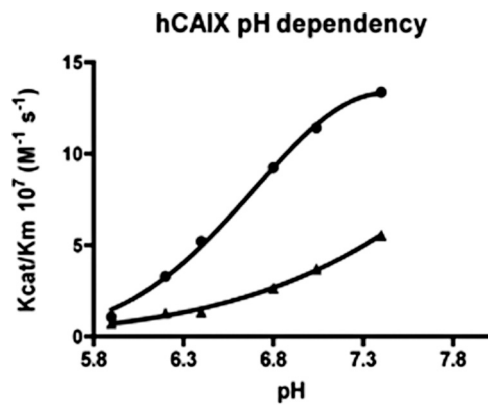


Fig. S2. Variation of k_{cat}/K_M for hCA IX recombinant forms (P1, indicated with filled circles, and P4, indicated with filled triangles) versus pH, for the CO_2 hydration reaction at 25 °C. The titration curve of the P1 form has a pK_a of 6.49; that of the P4 form has a pK_a of 7.01.

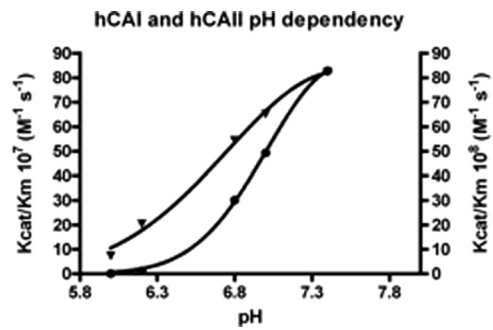


Fig. S3. Variation of k_{cat}/K_M for hCA I (inverted triangles) and hCA II (filled circles) versus pH, for the CO_2 hydration reaction at 25 °C. A pK_a of 6.90 was obtained for the hCA I curve and of 7.10 for hCA II.