Sensitivity of shark (*Squalus acanthias*) CFTR to mercury is mediated by cysteine residues located in the first membrane spanning domain

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Previous studies in our laboratory by Sirota et al.[1] have shown shark CFTR (sCFTR) to be much more sensitive to inhibition by mercury than human CFTR (hCFTR). The present study investigated the molecular site(s) responsible for this difference by constructing human/shark CFTR chimeric proteins.

sCFTR has 7 unique Cys residues. Two are located in the first membrane spanning domain (MSD1), two in the regulatory domain (RD), one in the second membrane spanning domain (MSD2) and two in the C-terminus of the protein. One or more of these Cys residues likely mediates the greater sensitivity of sCFTR to mercury. To elucidate which of these residues may be mediating this sensitivity we first constructed a chimeric protein replacing the human MSD1 with shark MSD1. Using *Xenopus laevis* oocytes and two electrode voltage clamping (TEVC), we investigated whether the unique Cys residues in the MSD1 of sCFTR would confer mercury sensitivity to hCFTR.

A chimeric cDNA construct was prepared using PCR and restriction enzyme digestion. The MSD1 of shark was amplified using a sense primer with an overlapping sequence homologous to the N-terminal of hCFTR and an antisense primer with an introduced restriction site for BsiW I (sense: AAAATCCTAAACTCATTAATGCACTTCGCCGATG, antisense: CACGTTCGTACGGCAGATG GAAATTGTCTG). The N-terminal end of hCFTR was then amplified in a separate PCR (sense: ATACGACTCACTATAGG, antisense: GCATTAATGAGTTTAGG). Both fragments were combined in a third reaction. This product was joined to the rest of hCFTR using Not I and BsiW I restriction sites, and cloned in the pBlueskript KS(-) vector (Invitrogen).

Human/shark MSD1 chimera cRNA as well as the hCFTR and sCFTR cRNAs were each injected into *Xenopus laevis* oocytes, and TEVC was performed after two to three days of incubation. After establishing baseline conductance in ND96 solution, chloride conductance was activated by adding 10 μM forskolin and 1 mM isobutylmethylxanthine (IBMX) to the bath solution. Average conductance during steady state was 199±23 μS for the MSD1 chimera 182±37 μS for sCFTR, and 411±66 μS for hCFTR. After 30 min. of activation, $HgCl_2$ (1 μM) was added to the bath solution for 30 min.

Figure 1 shows a representative experiment from each group. Human CFTR (Figure 1, panel A) showed only partial inhibition of forskolin +IBMX stimulated chloride conductance by 1 μM mercuric chloride. In contrast, sCFTR (panel B) and the human/shark MSD1 chimera (panel C) showed nearly complete inhibition under identical conditions. Panel D illustrates the mean percent inhibition by 1 μM mercuric chloride of the three protein constructs: (44% vs. 89% and 90%, $p<0.0001$) comparing hCFTR to both sCFTR and human/shark MSD1 chimera.

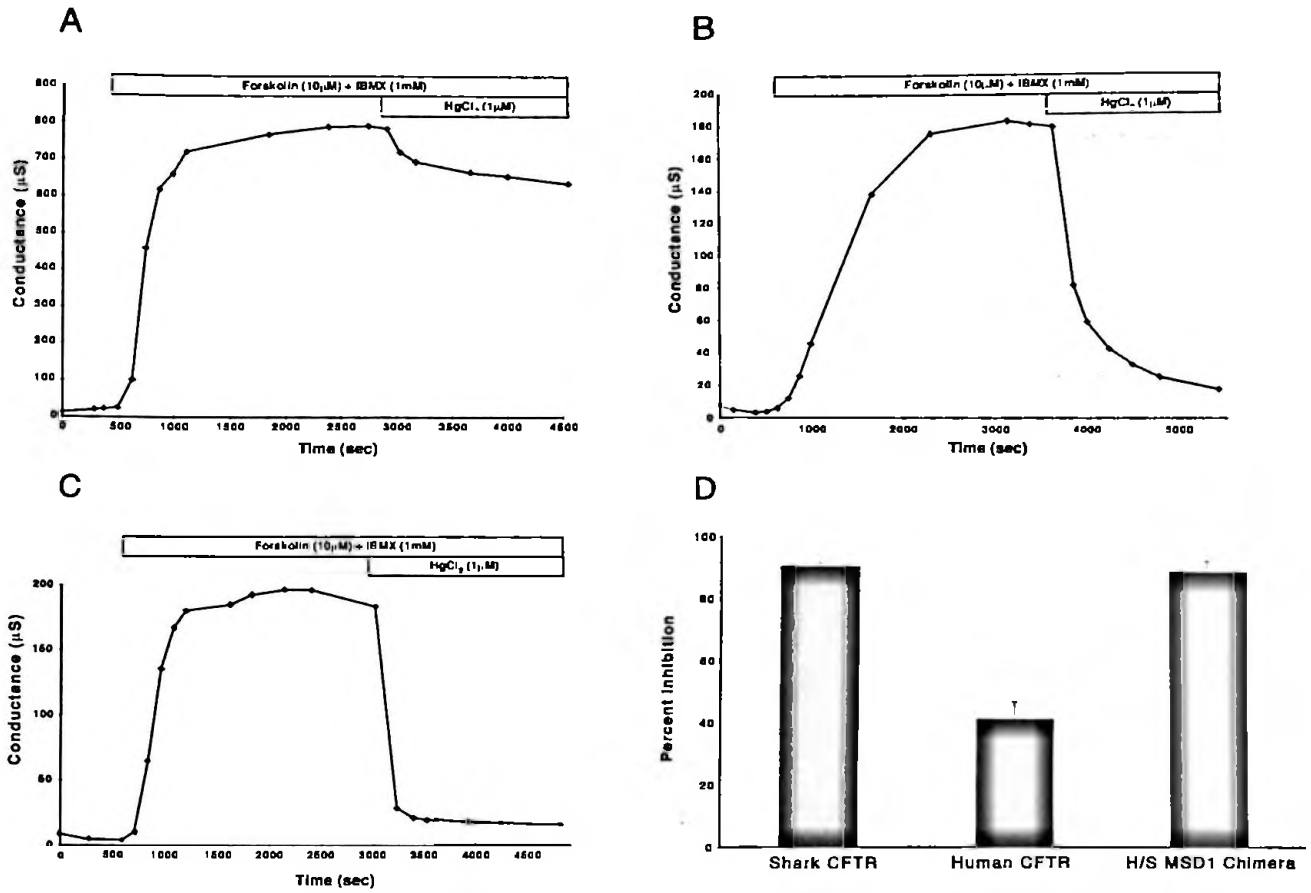

Figure 1. Differential effects of Hg on chloride conductance in hCFTR, sCFTR and human/shark MSD1chimera. Panel A: representative experiment demonstrating modest inhibition of hCFTR by 1 μM Mercuric chloride. Panel B: representative experiment demonstrating complete inhibition of sCFTR by 1 μM mercuric chloride. Panel C: representative experiment demonstrating complete inhibition of human/shark MSD1 chimera by 1 μM mercuric chloride. Panel D: percent inhibition of the three channels in 23 experiments (n= 9 for hCFTR, n=8 for sCFTR, n= 6 for human/shark MSD1 chimera. $P<0.0001$) comparing both wild type shark and chimeric construct to human CFTR.

These results demonstrate that the 2 unique Cys residues in sCFTR MSD1 confer sensitivity to mercury in the human CFTR channel and suggest that these residues are responsible for the greater sensitivity of shark CFTR to mercury. Our findings do not exclude the possibility that other shark specific cysteines may play a role in the differential sensitivities of shark and human CFTR to mercury.

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1. **Sirota, J.C., G.H. Weber, S.G. Aller, D.C. Dawson, and J.N. Forrest, Jr.** Shark and human CFTR expressed in *Xenopus* oocytes have different sensitivities to inhibition by the thiol-reactive metals mercury and zinc. *Bull. Mt. Desert Isl. Biol. Lab.* 38: 105-106, 1999.