The effects of eriochrome cyanine R on the mechanosensitive channels of *E. coli*

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The existence and characteristics of the mechanosensitive channel of large conductance (MscL) and small conductance (MscS) of *E.coli* have been well documented and extensively studied (Sukharev *et al.*, 1997). MscL and MscS have been found to play an important role in osmoregulation and the two channels are capable of compensating the absence of each other (Berrier *et al.*, 1992; Levina *et al.*, 1999). Though similar in physiological function, the channels differ somewhat in structure and characteristic. The *MscL* gene comprises of 136 amino acid residues amounting to a 15-kDa protein (Sukharev *et al.*, 1994) to form a homopentamer (Chang *et al.*, 1998). Whereas, MscS, is a 286-residue membrane protein with its 3D structure revealing a homoheptamer (Bass *et al.*, 2002). MscS is gated at pressures approximately half of which MscL is activated and has a conductance of ~1nS (Martinac *et al.*, 1987; Sukharev *et al.*, 1993) compared to MscL conductance of ~3nS (Sukharev *et al.*, 1994).

We have previously shown that parabens, which are food and cosmetic preservatives, were able to spontaneously activate MscL reconstituted in liposomes and MscS in giant spheroplasts, and also increased the mechanosensitivity of MscL (Nguyen *et al.*, 2005). Our studies therefore, broaden to other compounds which would bind to the MscL gate with greater affinity than parabens. Based on our *in-silico* data, eriochrome cyanine R bound to the MscL channel gate with a Gibbs free energy of -47.03kJ mol^{-1} which is a much lower value than that of parabens, indicating a greater affinity to the channel.

The patch-clamp studies with eriochrome cyanine R were shown to complement *in-silico* results with spontaneous MscL activity observed in 78% of the patches. The Boltzmann distribution curve of MscL in the presence of eriochrome cyanine R was markedly shifted to the left of the control curve and the Boltzmann parameters namely, α, $p_{½}$ and ΔG_o were also significantly lowered ($p < 0.05$) in the presence of eriochrome cyanine R compared to control values. That is, the mechanosensitivity of MscL was greatly increased in the presence of eriochrome.

Based on our study, it is possible that eriochrome cyanine R could be used as a lead compound for the development of a novel type of antibiotic. An antibiotic which would act by gating the mechanosensitive (MS) channels resulting in the leakage of essential ions and cell osmoticants out of the bacterium and thus, prevent its growth and survival.

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