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Poster presentation

Regulation of soluble guanylate cyclase activity by direct interaction with heat shock protein Hsp90

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Background

Soluble guanylate cyclase (sGC) is the main receptor of nitric oxide (NO). NO binds to the ferrous heme moiety of sGC thus activating the enzyme and increasing cyclic GMP (cGMP) levels. sGC is inhibited by various oxidants which either convert ferrous heme to ferric suppressing NO binding or modify essential thiol groups of sGC. Certain heat shock proteins (Hsp) can protect the enzymes from this inactivation. Hence, we suggested that Hsp can block oxidant-induced inhibition of sGC.

Results

In the present study purified sGC (human and bovine) and Hsps (human and rabbit) were used. According to surface plasmon resonance binding experiments, Hsp90 can directly interact with sGC. Formation of the Hsp90-sGC complex was not inhibited by a known Hsp90 inhibitor geldanamycin. The data indicate that association is slow ($k_a = 1.08 \cdot 10^4\ M^{-1}\ s^{-1}$) and dissociation is also very slow ($k_d = 2.48 \cdot 10^{-4}\ s^{-1}$). The resulting K_D value of 23.1 nM suggests that the binding is characterized by high affinity. Cyclic GMP accumulation was measured in the presence of an NO donor. Preincubation of sGC at 37 C significantly (by 90–95%) inhibited enzyme activity. If Hsp90 (0.1 mg/ml) was present during preincubation, inhibition of sGC was completely abolished. However, other Hsps (70, 60, 40 and 25) failed to protect sGC. Hsp90 protected sGC from inhibition by various transition metal ions including Mn^{2+} and Cd^{2+} known to be potent blockers of thiol groups. On the other hand, Hsp90 had no effect on inhibition of sGC by 1*H*-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one. Thus, Hsp90 protects essential thiols of sGC (but not the heme iron) from oxidative modification.

Conclusion

The complex of Hsp90 and sGC is formed slowly but once formed, should remain relatively stable for a long period of time. Hsp90 protects essential functional groups of the enzyme but does not stimulate the enzyme activity. The interaction of Hsp90 with sGC can be important for maintenance of NO-dependent cellular cGMP synthesis in damaged tissues.