Proteins as targets for high dilutions of drugs: Interaction between α-amylase and mercuric chloride.

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Abstract

Background: High dilutions of drugs, used in homeopathy, are usually applied by oral route or foliar spray. These dilutions first come in contact with membrane or circulating proteins. Ultra low doses of mercuric chloride, called potencies, promote activity of diastase or α-amylase in terms of breakdown of starch, a polysaccharide into a disaccharide maltose in a cell-free medium in test tubes. Mercuric chloride (HgCl\textsubscript{2}) in high doses inhibits the enzyme activity.

Aims: To see (i) whether the high and ultra low dose effects of HgCl\textsubscript{2} involve different binding sites of the enzyme and (ii) to find an explanation for the low dose effect of HgCl\textsubscript{2} in spite of absence of its original molecules.

Methodology: Merc cor mother tincture (147 mM HgCl\textsubscript{2}) in distilled water was used undiluted in this experiment. Merc cor 200c and 1000c were prepared from the mother tincture (MT) by successive dilution with water 1:100 followed by succussion in 200 and 1000 steps, respectively, and finally preserved in 90% EtOH. These potencies and blank 90% ethanol, were diluted with deionized, distilled (DD) water 1:1000 to minimize ethanol content in test solutions. Each test solution or control was mixed with the enzyme 1:10 just before experiment. The control consisted of DD water. An isothermal calorimetry (ITC) instrument was used to measure the interaction between soluble starch and α-amylase mixed with each potency (200c/1000c) of Merc cor, its mother tincture, ethanol and control. ITC is a thermodynamic technique which helps in measuring directly very small amount of heat evolved during chemical reaction. Soluble starch 90 µM was injected into 300 µl of 15µM α-amylase at 2 µl / injection. Twenty injections, one every 2 min, were given. The enzyme substrate interaction in terms of heat released (exothermic) or absorbed (endothermic) were monitored by the ITC instrument. All ITC measurements were calculated and analyzed statistically by an in-built software Origin 7.

Results and discussion: The data are presented in figures. While Merc cor MT shows endothermic reaction, all its potencies, ethanol and water control show exothermic reactions. There is wide variation in enthalpy (∆H), entropy (∆S), binding constant (K) and Gibbs free energy change (∆G) among the treatments with Merc cor MT, potencies, ethanol and also control. The results indicate that Merc cor MT and its potencies act on different binding sites of the enzyme. The variation in thermodynamic parameters suggest difference in binding interaction between the drug solutions and the enzyme. This in turn influences the enzyme substrate interaction as reported in earlier studies. The potencies are virtually water modified by the starting substance HgCl\textsubscript{2}.

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Conclusion: The mother tincture and potencies of mercuric chloride produce different effects on the enzyme substrate interaction. Potencies show wide variation in $\Delta H$, $\Delta S$, $K$ and $\Delta G$ values. It appears from the results that the drugs used in homeopathy produce dual action on proteins. At high doses they act on a binding site(s) but at ultra low doses they act on a different binding site(s). Proteins in an organism may serve as targets for initiation of action of homeopathic potencies.

Keywords: $\alpha$-amylase, mercuric chloride, high dilution, ITC, enthalpy, entropy, binding site.

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