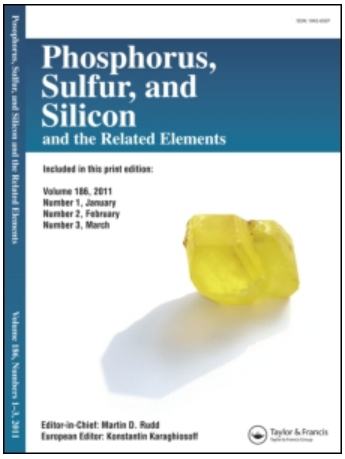

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The Influence of Inorganic Salts on the Inhibition of Acetylcholinesterase by O,O-Diethylthiophosphates

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THE INFLUENCE OF INORGANIC SALTS ON THE INHIBITION OF ACETYLCHOLINESTERASE BY O,O-DIETHYLTHIOPHOSPHATES

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The influence of various inorganic salts (NaCl, KCl, CsCl, KNO_3, Na_2SO_4, K_2SO_4, $MgCl_2$, $CaCl_2$) on the kinetics of the reactions of acetylcholinesterase with neutral and cationic O,O-diethylthiophosphates, $(C_2H_5O)_2P(O)SX$, where $X = (CH_2)_nSC_2H_5$ and $(CH_2)_nS^+(CH_3)C_2H_5$ (n=1-6), has been studied at 25°C and pH 7.5. The salt effect in the second-order rate constant can be quantitatively described by the equation $\log(k_2/K_s) = \log(k_2/K_s)^o - \Psi\log[M] + bc$ where bc is the term for the kinetic salting effect, $\Psi\log[M]$ is the electrostatic term, c and [M] are the molar concentrations of salt and salt cation. The salting effect is observed in the binding step (K_s) as well as in the enzyme phosphorylation step (k_2). Parallel increase of b with increasing n has been observed for the series of both ionic and neutral inhibitors. Electrostatic effect is observed only in the binding step for cationic inhibitors and does not depend on the distance between the onium atom and the reaction center in the inhibitor molecule. The observation of uniform Ψ is in accordance with the Manning's polyelectrolyte theory, suggesting that acetylcholinesterase can be considered as negatively charged polyelectrolyte. According to the theory the release of condensed counterions from the polyelectrolyte provides electrostatic contribution to the binding of cationic ligands, and the presence of localized anionic site on the acetylcholinesterase molecule is not required to account for the observed electrostatic effect. The obtained mean value of $\Psi = 0.49 \pm 0.08$ can be used as a quantitative parameter to characterize the effective charge density of acetylcholinesterase.