TRITIUM-HYDROGEN EXCHANGE OF CROTOXIN AND ITS SUBUNITS

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Crotoxin, the main toxin of the venom of *Crotalus d. terrificus,* contains two protein subunits: phospholipase A (EC 3.1.1.4) and crotapotin. The interaction of the two units produces a complex with the full toxicity of crotoxin. The subunits purified from the crude venom have molecular weights of 8300 for crotapotin and 13400 for phospholipase A. After gel filtration on Sephadex, a stable complex is formed by the interaction of the two proteins (1:1 molar ratio) restoring the original crotoxin toxicity. In protein tritium-hydrogen exchange studies, the back exchange kinetic constants of tritium-labelled phospholipase A, crotapotin and crotoxin were measured in gel filtration columns of Sephadex G-25-C. The best fit of the experimental data for phospholipase A shows two distinct kinetic classes of exchangeable hydrogens: 68% are rapidly exchanged, but the remaining 32% characterize a very slow class. An analogous pattern was found for crotapotin, where 83% of the exchangeable hydrogens were rapidly exchanged with the solvent. In contrast to the behavior of the individual subunits, three exponential classes of exchangeable hydrogens were demonstrable for crotoxin, with about 26 protons exhibiting intermediate exchange rates. These data suggest the existence of a conformational change upon the interaction of phospholipase A with crotapotin that may be responsible for the full toxicity of the complex.