TAMRA/TAMRA　複合体形成を利用した絵案酵素阻害剤の親和性解析

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A simple method for determining the ligand affinity toward a zinc-enzyme model by using a TAMRA/TAMRA interaction

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ABSTRACT: Thiolate coordination to zinc(II) ions occurs widely in such functional biomolecules as zinc enzymes or zinc finger proteins. Here, we introduce a simple method for determining the affinity of ligands toward the zinc-enzyme active-center model tetramethylrhodamine(TAMRA)-labeled 1,4,7,10-tetraazacyclodecane (cyclen-zinc(II) complex (TAMRA-ZnL). The 1:1 complexation of TAMRA-labeled cysteine (TAMRA-Cys) with TAMRA-Zn(each at 2.2 μM), in which the TAMRA moieties approach one another closely, induces remarkable changes in the visible absorption and fluorescence spectra at pH 7.4 and 25℃. The 1:1 complex formation constant (K = [thiolate-bond zinc(II) complex]/[uncomplexed TAMRA-ZnL][uncomplexed TAMRA-Cys]. M-1) was determined to be 106.7 M-1 from a Job's plot of the absorbances at 552 nm. By a ligand-competition method with the 1:1 complexation equilibrium, analogous K values for thio-containing ligands, such as N-acethyl-L-cysteine, L-glutathione, and N-acethyl-L-cysteinamide, were evaluated to have similar values of about 104 M-1. As a result of the ligand affinities to TAMRA-ZnL, nonlabelled zinc(II)-cyclen induced remarkable stabilization of the reduced form of L-glutathion and a cysteine-containing enolase peptide to aerial oxidation in aqueous solution at ph 7.4 and 25℃.

抄録　TAMRA-ZnL(TAMRA- 亜鉛サイクレン) と TAMRA-Cys の 1:1 複合錯体の形成反応を利用して、配位性官能基の亜鉛酵素親和性を推定することができる簡便な可視吸収分析法を開発した。TAMRA-Z n L と TAMRA-C y s の 1:1 複合体を利用して、配位性官能基の亜鉛酵素親和性を推測することができた。そして、亜鉛サイクレンはチオール含有分子の酸化防止剤として有用であることも判った。

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