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Article

A Rapid Screening of Ligand Binding by Measuring Intrinsic Fluorescence Changes of Proteins

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Abstract

HighÃƒÂ¢Ã‚Â€Ã‚Â•throughput analysis of genomic and proteomic information lead to large numbers of target molecules. The bottlenecks are generally subsequent verification steps, which require development of efficient screening tools. ProteinÃƒÂ¢Ã‚Â€Ã‚Â“protein and proteinÃƒÂ¢Ã‚Â€Ã‚Â“ligand interactions constitute the basis of most biological processes in living organisms. In general, proteins are labeled with fluorescent dyes to enhance sensitivities and the fluorescence changes are widely utilized for determining proteinÃƒÂ¢Ã‚Â€Ã‚Â“protein and proteinÃƒÂ¢Ã‚Â€Ã‚Â“ligand bindings. This study is to demonstrate that the intrinsic fluorescence of proteins by tryptophan and tyrosine and its change accompanied by ligand binding is sufficiently sensitive for studying proteinÃƒÂ¢Ã‚Â€Ã‚Â“ligand interactions. Malate dehydrogenase catalyzes interÃƒÂ¢Ã‚Â€Ã‚Â•conversion of oxaloacetate and NADH to malate and NAD^+, respectively. Malate did not change the fluorescence of malate dehydrogenase. But, the dissociation constants (K_D) for NADH and NAD^+ could be measured by fluorescence changes and they are comparable to K_M determined by enzyme kinetics. The high K_D (1.5 mM) for oxaloacetate agreed well with the ordered Bi Bi mechanism of malate dehydrogenase, in which NADH bound first and generated a binding site for oxaloacetate. The dissociation constant for cyclic diÃƒÂ¢Ã‚Â€Ã‚Â•GMP to malate dehydrogenase was also determined by fluorescence changes and it was comparable to that collected from computational molecular docking analysis. The K_D for cyclic diÃƒÂ¢Ã‚Â€Ã‚Â•GMP to methionine aminopeptidase, glycerol dehydrogenase, and peptidase B could also be determined by intrinsic fluorescence changes as 58, 58, 52 ÃƒÂŽÃ‚Â¼M, respectively. These results suggest that the intrinsic fluorescence of proteins by tryptophan and tyrosine is sensitive enough to determine proteinÃƒÂ¢Ã‚Â€Ã‚Â“ligand interactions. It provides a labelÃƒÂ¢Ã‚Â€Ã‚Â•free, quickÃƒÂ¢Ã‚Â€Ã‚Â•andÃƒÂ¢Ã‚Â€Ã‚Â•easy tool for screening the ligands to proteins.

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