Fluorescent Inhibitors for Functional Proteomic Analysis of Lipolytic Enzymes

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Fluorescent phosphonic acid esters have been developed as substrate analogous inhibitors for functional proteomic screening of lipases and esterases. These compounds covalently react with the nucleophilic serine in the active site of the lipolytic enzymes leading to formation of stable lipid-protein complexes (1/1, mol/mol). After protein separation by 1D-/ 2D-gel electrophoresis, single or multiple fluorescent bands/ spots are detected, depending on the active lipases and/or esterases in the sample and the specificity of the inhibitor. Fluorescence intensities of the labeled proteins can be determined in-gel using a CCD camera or a laser scanner and provide accurate information about the moles active enzyme. After tryptic in-gel digestion the fluorescent proteins can be identified by mass spectrometry. The described method is applicable to analysis of lipolytic enzymes in virtually any biological sample including purified proteins, crude industrial enzyme preparations, enzyme crystals, cultured cells, tissue homogenates and body fluids. Differential activity-based gel electrophoresis (DABGE) was developed for comparative analysis of two lipolytic proteomes in one polyacrylamide gel. For this purpose, the active lipases/esterases of two different samples are labelled with fluorescent inhibitors that possess identical substrate analogous structures but carry different cyanine dyes as reporter fluorophores. After sample mixing and protein separation by 1-D or 2-D PAGE, the enzymes carrying the sample-specific colors are detected and quantified. This technique can be used for the determination of differences in enzyme patterns, e.g. due to effects of genetic background, environment or metabolic state.