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Fluorescence labeling of thiol proteinase inhibitors extracted from urine of colorectal cancer patients

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Role of cysteine endopeptidases in carcinogenesis is ell documented fact. These enzymes are especially important in penetration processes of tumor cells which lead to metastasis. In a number of studies *in vitro* and *in vivo* it has been shown that the activity of the peptidases can be regulated by specific protein inhibitors and activators. Levels of the inhibitors and the activators in an organism are likely related to activation of self-defense mechanisms. In many cases enhanced level of the inhibitors has been observed in body fluids of cancer patients. In this work a method of fluorescence labeling of the inhibitors isolated from urine of patients with colorectal cancer was evaluated. The reaction of ophtaldialdehyde with protein amino groups was used for the labeling procedure. The reaction results in production of highly fluorescent isoindole derivatives of the proteins. Efficient energy transfer between native protein fluorophores and the incorporated probe was proved. This allows monitoring of conformational changes upon intermolecular interactions. The labeling by o-ophtaldialdehyde was compared with the labeling with fluorescamine. Fluorescence labeling of the inhibitors gives new possibilities in investigation of the mechanisms of cysteine peptidase inhibition.