The development of an inhibitive determination method for zinc using a serine protease.

ABSTRACT

A new inhibitive heavy metals determination method using trypsin has been developed. The enzyme was assayed using the casein-Coomassie-dye-binding method. In the absence of inhibitors, casein was hydrolysed to completion and the Coomassie-dye was unable to stain the protein and the solution became brown. In the presence of metals, the hydrolysis of casein was inhibited and the solution remained blue. The bioassay was able to detect zinc and mercury with IC50 (concentration causing 50% inhibition) values of 5.78 and 16.38 mg l-1 respectively.The limits of detection (LOD), for zinc and mercury were 0.06 mg l-1 (0.05-0.07, 95% confidence interval) and 1.06 mg l-1 (1.017-1.102, 95% confidence interval), respectively. The limits of quantitation (LOQ) for zinc and mercury were 0.61 mgl-1 (0.51-0.74 at a 95% confidence interval)and 1.35 mg l-1 (1.29-1.40 at a 95% confidence interval), respectively. The IC50 value for zinc was much higher than the IC50 values for papain and Rainbow trout, but was within the range of Daphnia magna and MicrotoxTM. The IC50 value for zinc was only lower than those for immobilized urease. Other toxic heavy metals, such as lead, silver, arsenic, copper and cadmium, did not inhibit the enzyme at 20 mg l-1. Using this assay,we managed to detect elevated zinc concentrations in several environmental samples. Pesticides, such as carbaryl, flucythrinate, metolachlor, glyphosate, diuron, diazinon, endosulfan sulphate, atrazine, coumaphos, imidacloprid, dicamba and paraquat, showed no effect on the activity of trypsin relative to control (One-way ANOVA, $F_{12, 26} = 0.3527$, $p > 0.05$). Of the 17 xenobiotics tested, only (sodium dodecyl sulphate) SDS gave positive interference with 150 % activity higher than that of the control at 0.25% (v/v).

Keyword: Tryspsin; Serine protease; Inhibitive determination method.