

Detection of protease activities using specific aminoacyl or peptidyl p-nitroanilide after sodium dodecylsulfate polyacrylamide gel electrophoresis and their applications.

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Abstract

A general method for detecting protease activities on acrylamide or agarose gels after sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) using specific aminoacyl p-nitroanilide (NA) or peptidyl NA as substrate is described. This method is extended from the spectrophotometric assay of p-nitroaniline, which is a chromogenic product liberated by protease action on aminoacyl NA or peptidyl NA. The acrylamide gel containing protein bands was dipped directly into a solution which contained specific synthetic aminoacyl NA or peptidyl NA as a substrate or had been overlaid with an agarose gel containing the same substrate. The p-nitroaniline released on the acrylamide or agarose gel by the specific protease was diazotized with sodium nitrite and then coupled to N-(1-naphthyl)-ethylenediamine to produce distinct activity band(s). The substrates used for protease activity staining on gels were identical to those used for spectrophotometric assays. Some applications are described.