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Short Communication

Fast and sensitive staining technique for catalase in polyacrylamide gel

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ABSTRACT

The described staining technique for catalase activity on polyacrylamide gel leads to sharp colourless bands on a uniform brown background. It is based on the oxidation of *o*-dianisidine with hydrogen peroxide by the catalytic action of haemin. Catalase activity can be detected down to 0.25 U. The whole staining procedure needs about 30 min and parallel detection of peroxidase activity is possible.

INTRODUCTION

Several staining techniques have been developed for visualization of the catalytic activity of catalase on gel electropherograms. A sensitive method restricted to starch gels depends upon the oxidation of iodide to iodine by hydrogen peroxide and the subsequent reaction of iodine with starch. Zones of catalase activity can be detected as colourless bands on a blue background [1–4].

Another stain uses the fact that after exposure to hydrogen peroxide the area containing the trapped gas oxygen can be stained with potassium permanganate, while the rest of the gel surface is covered with manganese dioxide. The contrast can be made more pronounced by rinsing the gels with acetic acid to remove manganese dioxide [5].

A method of distinguishing between catalase and haemoglobin is described in ref. 6 using *o*-dianisidine and "Teepol", but the mechanism of the reaction is not clear.

Catalase catalyses in a fast reaction the dismutation of hydrogen peroxide to yield water and oxygen as colourless reaction products. In contrast the peroxidase-catalysed reduction of hydrogen peroxide to water needs an external source of electrons. In the case of phenols such as *o*-dianisidine as electron donors, coloured oxidation products are formed. This different mode of hydrogen peroxide decomposition of catalase and peroxidase can be used for the simultaneous histochemical detection of catalase and peroxidase activities. Gregory and Fridovich [7] describe a method for the detection of catalase activity in polyacrylamide gels depending on the peroxidation of diphenols with hydrogen peroxide to coloured products under the catalytic influence of horseradish peroxidase (HRP). This method is still applied [8]. In the present paper we describe a more sensitive and time-saving modification of this technique using *o*-dianisidine, hydrogen peroxide and haemin as a catalytic agent.

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EXPERIMENTAL

Chemicals

o-Dianisidine (3,3′-dimethoxybenzidine), bovine haemin and bovine liver catalase (C 40) were purchased from Sigma (Munich, Germany). All other chemicals were of analytical grade (Merck, Darmstadt, Germany).

Preparation of wood and seed extracts

Twigs (diameter about 1 cm) were cut into splinters using a slow-turning lathe in order to prevent excess heating. Seed was milled by hand with a pestle at 4°C. Aliquots of 1 g of the splinters and the seed powder were extracted overnight at 4°C in 3 ml of buffer according to ref. 9. After removing the splinters by filtration the solution obtained was used for electrophoretic analysis without further concentration.

Polyacrylamide gel electrophoresis (PAGE)

Gel electrophoresis was performed in a vertical slab gel apparatus as described earlier [10]. A separation gel containing 15.0% acrylamide, pH 8.6, cross-linked 1:75 with N,N′-bismethyleneacrylamide was used. A 50-μl sample of the wood extract and variable amounts of pure catalase solutions were used. The electrophoresis buffer system consisted of Tris–glycine, pH 8.9. The separation time was 3 h with a constant voltage of 300 V and a starting current of 120 mA. After electrophoresis the gels were washed in distilled water for 30 min.

Detection of catalase

After electrophoretic separation the gel was soaked in a phosphate-buffered solution (0.06 *M*, pH 7.5) containing 0.1 m*M* *o*-dianisidine and 0.05 m*M* haemin, dissolved in a minimal volume of 0.01 *M* sodium hydroxide, for 20 min (solution A). After this the gel was rinsed with distilled water and incubated in a phosphate-buffered solution (0.06 *M*, pH 7.5) containing 0.02 m*M* *o*-dianisidine and 0.1 *M* hydrogen peroxide for 2 min (solution B). The gel was again rinsed with distilled water immediately after the zones of catalase activity appeared as transparent bands on the brown background.

RESULTS AND DISCUSSION

The haemin-catalysed oxidation of *o*-dianisidine by hydrogen peroxide in polyacrylamide gel results in a uniform brown staining of the gel within a few minutes. At those positions where the decomposition of hydrogen peroxide is performed by catalase, sharp colourless bands appear rapidly after insertion of the gel into incubation solution B. As peroxidase is more effective in oxidizing *o*-dianisidine at the expense of hydrogen peroxide than haemin, zones of peroxidatic activity appear as dark-brown strains. After a few minutes maximum contrast between the colourless zones of catalase activity, the dark-brown zones of peroxidase activity and the amber background is obtained. Fig. 1 shows the simultaneous catalase and peroxidase staining of wood and seed extracts of *larix decidua* harvested at different altitudes. At the certain time of harvest the wood samples show peroxidase and seed catalase isoenzymes only. This result will be explored in further work.

The sensitivity of the method was determined by using serial dilutions of bovine liver catalase. Using gels with 0.75 mm thickness catalase activity can be detected down to concentrations of 0.25 U. With increasing thickness of the gel the sensitivity of the method decreases drastically even with increased incubation time for haemin and *o*-dianisidine. In gels

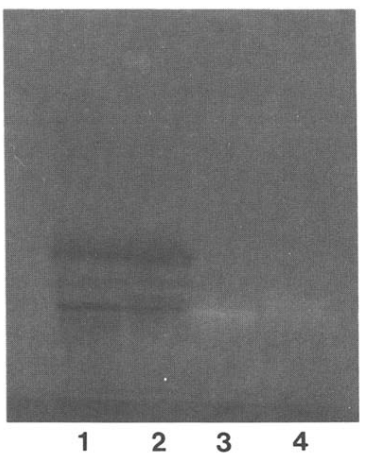

Fig. 1. Simultaneous peroxidase and catalase stain of *Larix decidua* from different altitudes. 1 = Wood of *L. decidua* from 600 m; 2 = wood of *L. decidua* from 1300 m; 3 = seed of *L. decidua* from 600 m; 4 = seed of *L. decidua* from 1300 m.

more than 4 mm thick the gel is destroyed at the positions of catalase activity by oxygen evolution.

The advantage of this technique using haemin instead of HRP is the fact that it needs less time. When gels containing concentrations of acrylamide up to 15% are used it takes several hours until HRP soaks homogeneously into the gel matrix. In contrast to HRP, with a relative molecular mass of about 40 000 daltons, haemin offers the possibility of completing the stain within 30 min because of its considerably lower molecular weight (616 daltons).

It is known that haemin displays peroxidatic function in alkaline aqueous solutions (pH >9) owing to the faster decay of the haematin–hydrogen peroxide complex compared with the decay of the haem–peroxide complex. In acrylamide gels the catalytic property of haemin is very well marked, even at neutral pH, possibly owing to the more hydrophobic character of the gel. Thus haemin is able to act as a perfect substitute for HRP in staining methods for catalase.

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