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Starch–iodide as a stain for peroxidases

The methods used for locating peroxidase bands after zone electrophoresis involve the peroxidation of chromogens such as benzidine and *o*-toluidine[1,2]. Many of these substances constitute health hazards because of their carcinogenic nature[3,4].

The oxidation of iodide to iodine is known to be accelerated by peroxidases[5]. In the presence of starch, iodine immediately forms an intense blue-black complex. This report describes how these reactions are adaptable to the detection of peroxidase isoenzymes after gel electrophoresis. Bands are sharper than those obtained with conventional chromogens in many cases and, of course, the reagents are completely innocuous.

For the study of this stain, zone electrophoresis of peroxidases from various sources was performed on horizontal starch gels (14%). Tris–citrate buffer (pH 7.0; $I = 0.018$ in gel and 0.27 in electrode buffer) was used, and a 150 V potential difference was applied for 18 h. After electrophoresis the gels were sliced horizontally; one section was stained by steeping in a solution of 0.02 *M* potassium iodide in 0.1 *M* phosphate citrate buffer (pH 5.4) containing 5×10^{-4} *M* H_2O_2, the other with one of the conventional peroxidase chromogens.

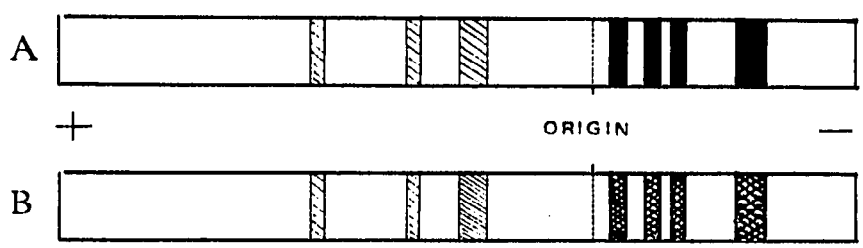

Fig. 1. Zymograms of horseradish peroxidase. (A) stained with starch–iodide; (B) stained with 3-amino-9-ethyl carbazole.

A typical result obtained with a commercial horseradish peroxidase preparation (Sigma Chemical Co., St. Louis, U.S.A.) is illustrated in Fig. 1. The four cathodic components were shown up very clearly by the starch–iodide stain which gave sharper bands than did other chromogens tested. The lack of detail from the anodic section of the gel is to be expected from a commercial preparation of horseradish peroxidase and has been reported previously[6].

Peroxidase preparations examined sometimes contained isoenzymes which were slower to react with iodide than with other chromogens, presumably a consequence of their having a relatively lower reactivity towards iodide. The starch–iodide stain proved particularly useful in studies on peroxidases from a number of algae. Some of these peroxidases (*e.g.* those from *Laminaria digitata* and *Enteromorpha linza*), which may play a role in biological iodination, displayed a much greater reactivity towards iodide than towards organic chromogens.

The iodide staining technique was also used to detect non-peroxidatic enzymes, the products of which may be coupled to a peroxidase reaction. For example, dipeptidases separated by gel electrophoresis were incubated with dipeptides and

L-amino acid oxidase, and the resultant hydrogen peroxide was coupled to horseradish peroxidase. The reactive zone was then very effectively located by the starch–iodide reaction. The technique may also prove applicable in polyacrylamide gel electrophoresis if a small percentage of soluble starch is incorporated in the polyacrylamide gel during its preparation.

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Department of Biochemistry,
University College,
Galway (Ireland)

M. J. MURPHY

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Densitometrische Bestimmung von Fe(II)–Fe(III)-Gemischen nach elektrophoretischer Trennung mittels N-(2-Hydroxyäthyl)iminodiessigsäure

Die in der Praxis bedeutsame Trennung von Eisen(II)- und Eisen(III)-ionen lässt sich u.a. auch mittels der Migrationsmethoden erreichen. Die Voraussetzungen für eine erfolgreiche Trennung sind vor allem durch die unterschiedliche Ladung sowie Tendenz zur Komplexbildung beider Valenzformen der Eisenionen gegeben. Man benutzte zu diesem Zweck Papierchromatographie[1], Ionenaustauschchromatographie[2] und in letzter Zeit auch Dünnschichtchromatographie[3]. Durch gleichzeitige Papierelektrophorese und Papierchromatographie erreichten LINGREN *et al.*[4] eine Fe(II)–Fe(III)-Trennung in verdünnter Schwefelsäure.

Mit der Einführung der N-(2-Hydroxyäthyl)iminodiessigsäure (HIDA) als einen breit anwendbaren komplexbildenden Elektrolyten für Trennung von anorganischen Ionen[5] wurde auch die Möglichkeit einer rein elektrophoretischen Trennung der Eisenionen gegeben. Tatsächlich erzielte man eine gute qualitative Separation eines äquimolaren Gemisches beider Valenzformen auf diesem Wege[6]. Weitere Untersuchungen der Möglichkeiten elektrophoretischer Ionentrennung in der Lösung von HIDA erwiesen[7], dass diese ohne jede Vortrennung bis zu einem Grenzverhältnis von etwa 1:500 durchaus erfolgreich verlaufen; die quantitative Bestimmung der ge-