the water surrounding[4] differed significantly ($H_{193} = 35.6$, $H_{225} = 27.1$ for the native enzyme and $H_{193} = 26.2$ and $H_{225} = 18.8$ for the inactivated one.) It may be supposed, therefore, that α-helical regions, buried in the hydrophobic interior of the GAPD molecule, do not get exposed to the solvent in the course of the reversible cold inactivation.

ВЫВОДЫ. Обратимая инактивация глицеральдегид-3-фосфатдегидрогеназы из скелетных мышц крысы, наступающая в результате диализа при 4° против 0,15 *M* NaCl, 5 м*M* ЭДТА, 4 м*M* ß-меркаптоэтанола pH=7,6, сопровождается изменениями дисперсии оптического вращения (исчезновение плеча на кривой в области 275–295 нм, а также значительное уменьшение величин параметров $-b_o$, A_{193}, и $-A_{225}$). Эти изменения обратимы при реактивации, наступающей при +20° в присутствии 0,15 *M* KH_2PO_4.

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Acid Phosphatases in the Tea Leaf

The occurrence of some oxidative enzymes, the polyphenol oxidases and peroxidases in the tea leaf, has been recorded in the literature[1,2]. This study describes the separation and detection of acid phosphatases in the tea leaf by horizontal starch gel electrophoresis. A modification of the method of Roussos[3] was used. 20 g of flush (clone TRI 777) 10 g of Polyclar (General Aniline and Film Corporation, New York), and 1 g of sand were homogenized in a Waring Blender for 15 min, with 100 ml af 0.2 *M* KCl in 0.05 *M* phosphate buffer (pH.7.4). A few drops of 0.005 *M* EDTA were also added. The suspension was centrifuged. All subsequent operations were carried out at 4 °C. A solution of 1 *M* acetate buffer (pH 4.2) was added to the cell-free extract in the proportion of 5:1 (v/v). After 6 h, the precipitated inactive protein was removed by centrifugation. Using 30% ethanol, the active fraction was precipitated, disssolved in phosphate buffer and subjected to horizontal starch gel electrophoresis.

Connaught hydrolysed starch and *tris*-succinic acid buffer (pH 6.0) were used for the preparation of the gel. The gel was chilled for 2 to 3 h, before the insertion of pieces of Whatman 3 MM chromatography paper, which were soaked in the enzyme extract. Acetate buffer (pH 4.0) was used for the bridge. Horizontal electrophoresis was carried out using Buchler equipment for 16 h against a potential gradient of 2 volt per cm.

The isozymes of acid phosphatases were localized on the starch gel by the azodye technique of Goldberg and Barka[4]. The incubation solution was freshly prepared from the following stock solutions. A *Tris*-succinic acid buffer (pH 6.0). B) Substrate stock solutions, 'Naphthol AS-Mx', 'Naphthol AS-BI' and 'p-nitrophenyl phosphate' (obtained from the Sigma Chemical Co.) Each substrate solution was dissolved in N, N-dimethyl formamide in 10 g per ml concentration. C) 4% sodium nitrite in distilled water. D) 2 g p-rosaniline hydrochloride (BDH) were added to 50 ml 2 *N* hydrochloric acid and gently heated. After cooling, the solution was filtered. Solutions A, B and C were stored at 4 °C. The incubation mixture was prepared as follows: 5 ml of solution A and 1 ml of solution B were mixed with 13 ml of distilled water in a beaker. A 0.8 ml aliquot of solution C was added to 0.8 ml of solution D in a test tube. This mixture was added to the solution in the beaker. After mixing, the pH was adjusted to 5.0 with N-sodium hydroxide. Each sliced gel (14×8 cm^2) was incubated in this mixture at 37 °C for 30 min. The sites of enzyme activity were localized by the formation of characteristic red azo-dyes (eg. Naphthol AS-BI. Matador colour, Naphthol AS-MX, Rose Madder Lake Colour)[5].

Six bands having acid phosphatase activity (hydrolyses mono phosphatases) were localized on the gel as shown in the Figure. 2 of these bands carried a positive charge and it was observed that in this case azo-dye formation was slow. This probably indicated that the optimum pH for these 2 isozymes was not 5.0. It is probable that the isozymes of acid phosphatase in the tea leaf have different substrate specificities. There is no evidence at present to show that the enzyme is bound to any particular fraction of the leaf extract, but it is interesting to note that Matile et al.[6] have presented considerable evidence to show the localization of some acid phosphatase activity in the spherosomes and dictyosomes of higher plant tissues.

Résumé. Les isozymes des phosphatases acides de la feuille du thé ont été séparés par électrophorèse et localisés par une méthode histochimique.

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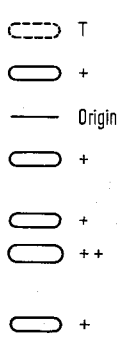

Starch-gel electrophoresis of the isozymes of acid phosphatase in the tea leaves using a discontinuous buffer system. The following abbreviations are used to denote enzyme activity at pH 5.0: T, little; +, moderate.

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