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SEPARATION OF PHOSPHORYLASE AND UDPG-PYROPHOSPHORYLASE FROM BEAN LEAVES BY POLYACRYLAMIDE GEL DISC ELECTROPHORESIS[1]

JOHANNES TSCHEN[2]

Abstract

Phosphorylase and UDPG-pyrophosphorylase from bean leaves (*Phaseolus vulgaris* L.) were separated by polyacrylamide gel disc electrophoresis.

The enzymes were separated in the gels which contained 5.5% polyacrylamide and 0.1% glycogen.

After staining with iodine solution the specific reaction of phosphorylase was localized by an intense blue zone in the middle of the separating gel, and UDPG-pyrophosphorylase coupled with UDPG-transglycosylase by an intense brown zone in the gel close to the cathode. Only one specific band of phosphorylase has been observed in the gels.

Introduction

Starch is synthesized by transferase from adenosine diphosphate glucose (ADPG) and uridine diphosphate glucose (UDPG) (Leloir *et al.* 1961, Recondo and Leloir 1961, Murata *et al.* 1964) and by plant phosphorylase from glucose–1–phosphate (G–1–P) (De Fekete 1968, Tsai and Nelson 1968).

Tschen and Fuchs (1970) were able to localized some polysaccharides-synthesizing enzymes in rusted bean leaves by histochemical techniques. Specific reactions and localizations of phosphorylase and enzymes in ADPG-pathway were demonstrated in bean leaf tessues. However, no enzyme in UDPG-pathway was detected except in the infecting fungus.

Different types of phosphorylase in plant tissues have been separated by polyacrylamide gel disc electrophoresis (Frederick 1967, Siepmann and Stegemann 1967). However, no electrophoretic study of transferase in starch synthesis from UDPG has been reported, nor have the phosphorylase and

(1) Some experiments were carried out in Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Göttingen, Germany. The author thanks the Deutsche Forschungsgemeinschaft, the National Science Council and the Joint Comission on Rural Reconstruction for supports of this study.

(2) Associate Professor, Institute of Botany, National Chung-Hsing University, Taichung, Taiwan. (Former Wissenschaftlicher Assistent, Universität Göttingen, Germany)

UDPG-pyrophosphorylase from plant tissues separated by polyacrylamide gel disc electrophoresis.

In the present study we attempted to examined UDPG-pyrophosphorylase in bean leaf by coupling with UDPG-transglycosylase and to separate this enzyme and phosphorylase by polyacrylamide gel disc electrophoresis.

Materials and Methods

Beans (*Phaseolus vulgaris* L. var. Favorit) were grown from seeds in a controlled environment chamber set at a photoperiod of 16 hours at about 6,000–8,000 Lux at plant height and 25°C. Samples of bean leaves were collected 10–22 days after sowing. Three grams of primary leaves were ground with quartz sand and 3 ml of the extraction medium containing 0.01 M *Tris*–HCl buffer at pH 8.4 and 5% sucrose in a mortar. The extracts were strained through 3 layers of cheesecloth, and centrifuged at 30,000×g for 20 minutes. The supernatant fluids (0.2 ml) which contained about 0.7 mg of soluble protein (measured by the method of Lowry *et al.* 1951) were used for electrophoretic separation.

Polyacrylamide separating gels (6×100 mm) were prepared after Davis (1964) in which 5.5% polyacrylamide and 0.1% glycogen were contained (Siepmann and Stegemann 1967). Electrophoresis was carried out for 30 minutes at a current of 0.5 mA per tube and afterward at 4 mA per tube. After electrophoresis the gels were washed with cold 0.1 M acetate buffer at pH 6 for phosphorylase assay and with 0.1 M *Tris*–HCl buffer at pH 7.4 for UDPG-pyrophosphorylase assay. The gels were respectively incubated at 37°C for 4 hours in the reaction mixtures (Tschen and Fuchs 1970) containing 0.8 ml 0.1 M acetate buffer, pH 6; 10 mg G–1–P; 1 mg ATP; 0.1 ml 0.1 M $MgCl_2$; 0.1 ml 0.05 M NaF for phosphorylase; and 0.9 ml 0.1 M *Tris*–HCl buffer, pH 7.4; 10 mg G–1–P; 5 mg UTP; 0.1 ml 0.1 M $MgCl_2$; 1 mg G–6–P; 2 mg EDTA for UDPG-pyrophosphorylase.

After incubation the polyacrylamide gels were fixed in a mixture of methanol-acetic acid-water (30:5:65, v/v). The enzyme activities of phosphorylase and UDPG-pyrophosphorylase coupled with UDPG-transglycosylase in the gels were detected by staining the reaction products, glucans (Tschen and Fuchs 1970), with an iodine solution (0.01 M iodine in 0.01 M potassium iodine). The stained gels were pressed between two sheets of Plexiglas for photography. The relative activities of phosphorylase and UDPG-pyrophosphoralse in the polyacrylamide gels were measured by a densitometer at a wavelength of 620 nm and 465 nm respectively.

The activities of phosphorylase and UDPG-transglycosylase coupled with UDPG-pyrophosphorylase in the gels were determined with 5×10^{-3} M phloridzin,

uridine triphosphate (5 mg/ml), at different pH-value and by incubation in acetate, citrate, phosphate and triethanolamine buffer systems.

Results

Phosphorylase. After staining with iodine solution the specific reaction of phosphorylase activity was shown in an intense blue zone in the middle of the gel (Fig. 1). When the gel was incubated in a reaction mixture of phosphorylase containing 0.1 M citrate buffer at pH 6.6 instead of 0.1 M acetate buffer at pH 6, both an intense blue zone in the middle of the gel and a slight brown zone close to the anode could be observed. The latter was not a result of phosphorylase isoenzyme, but rather UTP-dependent pyrophosphosphorylase caupled with transferase, because its activity could be enhanced by adding uridine triphosphate (UTP) and incubating at basic pH level. Moreover, the intensity of this band was inhibited by a concentration of 5×10^{-3} M phloridzin, an inhibitor of UDPG-pyrophosphorylase (Fig. 2).

UDPG-pyrophosphorylase. The assay of UDPG-pyrophosphorylase activity was by means of a coupling system of UDPG-transglycosylase (Tschen and Fuchs 1970) in the polyacrylamide gel, and glucans as their products were stained with iodine solution in the present study. The specific reaction of UDPG-pyrophosphorylase was shown in an intense brown zone in the gel close to the cathode after staining with iodine solution (Fig. 1 and 2). The activity of the enzyme was dependent on UTP and was inhibited by phloridzin. The appearance of phosphorylase activity in the stained gel was because the reaction mixture of UDPG-pyrophosphorylase contained glucose–1–phosphate which could be polymerized by phosphorylase during the incubation. However, phosphorylase activity was significantly below that observed in the gel incubated in the reaction mixture of phosphorylase. The enzyme was not dependent on UTP and neither could it be inhibited by phloridzin (Fig. 1 and 2).

The densitometric diagrams. The relative activities of phosphorylase and UDPG-pyrophosphorylase are shown in the Fig. 3 and 4. Phosphorylase (peak III) has a higher relative activity than UDPG-pyrophosphorylase (peak I) when the measurement of the gel was made at wavelength of 620 nm, while the relative activity of phosphorylase was lower than UDPG-pyrophosphorylase measured at 465 nm. Peak II was a nonspecific absorption of iodine by the protein in the gel.

Discussion

The specific reaction and localization of phosphorylase and starch synthesizing enzyme of ADPG-pathway have been demonstrated in bean leaves by

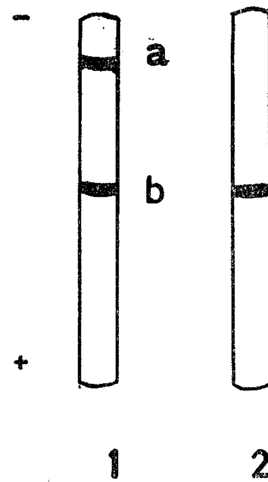

Fig. 1. Polyacrylamide gel disc electrophoresis of bean leaf extract showing phosphorylase and UDPG-pyrophosphorylase. 1. Polyacrylamide gel was incubated in a reaction mixture of UDPG-pyrophosphorylase; 2. Polyacrylamide gel was incubated in a reaction mixture of phosphorylase (or the gel was incubated in a reaction mixture of UDPG-pyrophorylase without UTP); *a.* UDPG-pyrophosphorylase; *b.* Phosphorylase.

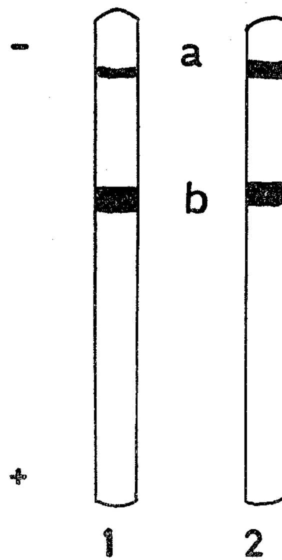

Fig. 2. Polyacrylamide gel disc electrophoresis of bean leaf extract showing phosphorylase and UDPG-pyrophosphorylase. 1. The gel was incubated in a reaction mixture of UDPG-pyrophosphorylase and with a concentration of 5×10^{-3} M phloridzin was added; 2. Polyacrylamide gel was incubated in a reaction mixture of UDPG-pyrophosphorylase; *a.* UDPG-pyrophosphorylase; *b.* Phosphorylase.

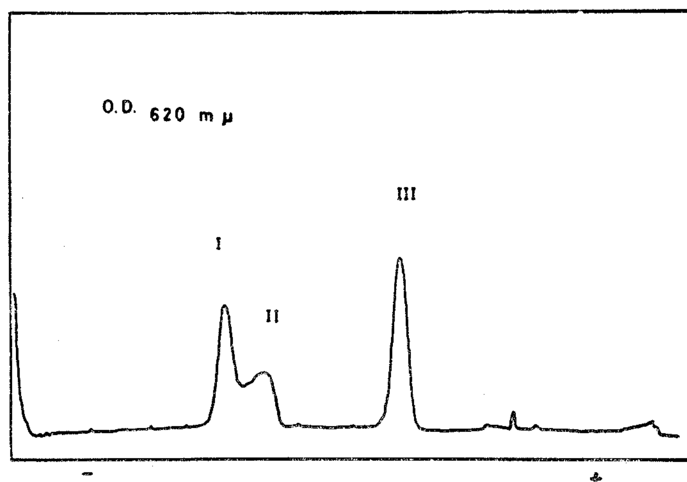

Fig. 3. Densitometric diagram of polyacrylamide gel measured at 620 nm. I. UDPG-pyrophosphorylase; II. Nonspecific absorption of iodine by protein; III. Phosphorylase.

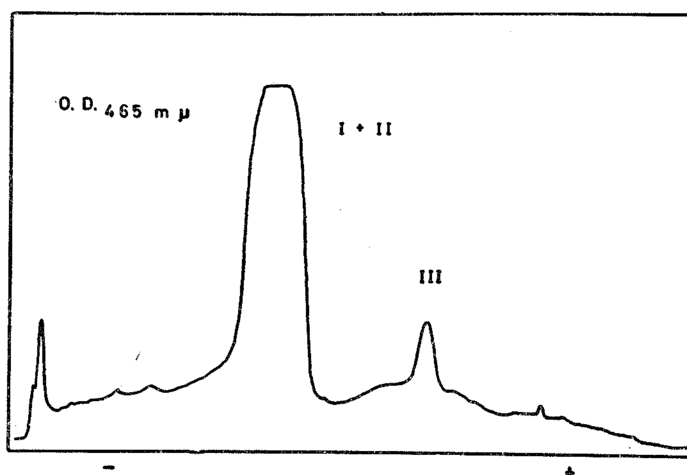

Fig. 4. Densitometric diagram of polyacrylamide gel measured at 465 nm. I. UDPG-pyrophosphorylase; II. Nonspecific absorption of iodine by protein; III. Phosphorylase.

histochemical methods, whereas the activity of transferase from UDPG in starch synthesis was so low that the specific reaction was not detected with certainty in the leaf tissues (Tschen and Fuchs 1970). By using plant extract in which there was a high concentration of soluble protein (0.7 mg per gel), the specific reaction of the UDPG-pyrophosphorylase was demonstrated by coupling with UDPG-transglycosylase in the polyacrylamide gel in this study. The activity of ATP-dependent enzymes (ADPG-transferase) is more effective than UTP-dependent enzymes (UDPG-transferase) in starch synthesis (Hassid

1969). It was reported (Recondo and Leloir 1961, Murata *et al.* 1964) that with native starch granules as a source of enzyme, the gluco e residues were found to be incorporated ten times faster from ADPG than from UDPG.

It has long been known that in the animal tissues there are phosphorylase *a* and phosphorylase *b*. Phosphorylase isozymes have been reported in leaf extract of spinach, immature cotyledon of *Vicia faba* (De Fekete 1968), in maize endosperm and seed (Tsai and Nelson 1968, 1969) and in algae (Frederick 1963, 1967). In the present study, however, with the crude extracts of bean leaves which contained a high concentration of soluble protein, only one specific zone of phosphorylase could be observed.

The synthetic procucts of the enzymes in the gels were not analyzed. However, according to the typical colour reactions of iodine on polysaccharides and to the histochemical studies (Tschen and Fuchs 1970, Tschen 1971) they could be amyloses (Porter 1953, Panmüller 1968) and synthetic products of UDPG-pyrophosphorylase coupled to UDPG-transglycosylase are supposed to be amylopectins. Frydman and Cardini (1964) reported a soluble enzyme in sweet corn that synthesized phytoglycogen from UDPG and ADPG which has the chemical structure similar to that of amylopectin.

Phosphorylase and UDPG-pyrophosphorylase were distinguished in the gel by the use of phloridzin, UTP and by incubation in various buffer systems at different pH-values (Siepmann and Stegemann 1967). The distinguishing of phosphorylase from UDPG-pyrophosphorylase in the gels agreed with our histochemical evidence (Tschen and Fuchs 1970, Tschen 1971). Siepmann and Stegemann (1967) studied phosphorylase in potato by polyacrylamide disc electroporesis, they obtained only one specific zone of phosphorylase from the leaves in the gel, but the isozymes could be observed from the tubers. Phosphorylase exists as different types in plant tissues, and quantitative changes related with development of cells and organs and with season have been shown to occur (Shivaram *et al.* 1971).

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四季豆澱粉合成酵素的電泳分離

陳 昇 明

使用聚丙烯醯胺酸膠體電氣泳動法 (Polyacrylamide gel disc electrophoresis)，將四季豆 (*Phaseolus rulgaris* L.) 葉組織裏的兩種澱粉合成酵素——Phosphorylase 和 UDPG-pyrophosphorylase 分離，並加以鑑別。

上述兩種酵素以含有 5.5% 濃度的聚丙烯醯胺和 0.1% 肝醣之膠體，並通以 1.5 mA 和 4 mA 的電流加以分離。膠體分離之後侵入含有葡萄醣一磷酸 (glucose-1-phosphate) 的酵素反應試劑 (reaction mixture)，藉此觀察酵素對葡萄醣一磷酸的轉化作用以及它們在膠體內進行澱粉的合成。

膠體在完成澱粉合成之後以碘溶液處理，Phosphorylase 呈一深藍色的色帶在膠體的中間部位，而 UDPG-pyrophosphorylase 呈一深棕色的色帶在靠近膠體的陰極部位。

於鑑別 Phosphorylase 和 UDPG-pyrophosphorylase 的反應時，除了使用不同的酵素反應試劑進行實驗外，又分別使用抑制劑，輔因子以及各種緩衝劑在不同 pH 值之下進行實驗。