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VILVE JAASKA, VELLO JAASKA

SOLUBLE PHOSPHOHYDROLASES AND ESTERASES IN MAIZE SEEDLINGS

Plant tissue extracts as well as individual subcellular fractions are known to exhibit phosphohydrolase activity toward a variety of different phosphate compounds, such as ATP, ADP, AMP, PP_i, β-glycerophosphate, glucose 6-phosphate, *etc.* (Jaaska, 1967; Roberts, 1957, *etc.*). However, in most cases the number of separate enzymes involved in phosphohydrolase activity and their substrate specificity still remain open.

Many kinds of plant tissues are shown to contain a nonspecific phosphohydrolase system. Highly purified phosphohydrolase preparations capable of hydrolyzing different phosphomonoesters as well as phosphoanhydride substrates have been isolated from sweet potato (Ito *et al.*, 1955), etiolated white lupine seedlings (Newmark, Wenger, 1960), soybean meal (Mayer *et al.*, 1961), wheat bran (Nagai, Funahashi, 1962), bean leaves (Williams, Staples, 1964), and tobacco leaves (Shaw, 1966). The attempts at a further purification of the preparations by means of starch column chromatography (Ito *et al.*, 1955) and polyacrylamide gel electrophoresis (Williams, Staples, 1964) failed to separate specific enzymes and showed the homogeneity of the preparations. From these observations it seems that in plant tissues the ability to hydrolyze different phosphate compounds may belong to a single enzyme.

On the other hand, several authors were able, by means of ion-exchange chromatographic procedures, to separate several fractions of nonspecific phosphohydrolases from the same plant tissue. Thus, Ikawa *et al.* (1964) have separated from rice ears three fractions of nonspecific acid phosphatase differing in their activity toward individual substrates, pH optimum, and some other properties. Brouillard and Ouellet (1965) reported a separation, from wheat germ, of four nonspecific phosphohydrolases, all of which likewise exhibited nonspecific esterase activity. Eight nonspecific phosphohydrolases were shown (Schormüller *et al.*, 1965) to be present in orange peels.

In our previous paper (Jaaska, Jaaska, 1968), we applied the polyacrylamide gel electrophoresis combined with histochemical methods in the study of phosphohydrolases present in protein extracts from etiolated wheat seedlings. It has been found that wheat seedlings tissues contain a system of electrophoretically distinct phosphohydrolases differing in their substrate specificity and pH optima. In acid medium, the presence of 7 or 8 nonspecific phosphoanhydride phosphohydrolase isoenzymes hydrolyzing both ATP and thiamine pyrophosphate has been revealed. Of these

isoenzymes only one or two showed detectable activity toward phosphomonoester bonds of β-glycerophosphate.

The present paper deals with the results of investigating soluble phosphohydrolases and esterases in etiolated maize seedlings, as revealed by polyacrylamide gel electrophoresis and histochemical methods.

Materials and Methods

Plant tissue extracts. Maize seedlings (*Zea mays* L., var. 'Dneprovsky Hybrid 98') were grown in the dark in a continuously aerated solution of 2.5×10^{-4}M $CaSO_4$, essentially as described by Epstein and Hagen (1952). The coleoptiles and roots of five-day-old etiolated seedlings were excised, washed in several changes of cold distilled water, and used immediately for the preparation of protein extracts.

Protein extracts were prepared by grinding small tissue pieces in a previously chilled mortar with an addition of pure white sand and a fresh-prepared pre-cooled buffer mixture at a pH of about 7.0, consisting of 0.5M sucrose, 0.1M tris-hydroxymethyl-aminomethane (Tris) and 0.09M ascorbic acid. A volume of the buffer mixture taken (in millilitres) was equal to the weight of the tissue (in grams). The resulting mash was squeezed through a piece of thin planctonic silk and centrifuged at 18,000 *g* for 30 minutes. To one millilitre aliquots of the supernatant in small vials, sucrose was added up to a final concentration of about 30 per cent together with a small amount (about 20 mg/ml) of Sephadex G-200 as an inert protein carrier, the use of which was suggested by Broome (1963). The protein extracts were stored frozen at —10 °C until used for eletrophoresis.

Polyacrylamide gel electrophoresis. A simplified procedure of "disc" electrophoresis (Davis, 1964; Steward *et al.*, 1965), using only a small-pore layer without spacer and sample layers, as suggested by Broome (1963), has been adopted in a slightly modified form to be described below.

The following stock solutions were used to prepare the gel:

Stock A: 5.0 g acrylamide, 0.1 g N,N′-methylenebisacrylamide, distilled water — 20 ml.

Stock B: 12.1 g tris-hydroxymethyl-aminomethane (Tris), 20 ml 1N nitric acid (HNO_3), 2 ml 20 per cent triethanolamine solution, distilled water up to 100 ml.

Stock C: 2 mg riboflavin in 100 ml distilled water.

Stock solutions were stored in brown botties in the cold. The gel solution was prepared immediately prior to use by mixing the stock solutions in the following proportions (by volume): 2A + 1B + 1C. Thus, the final concentrations of components in the gel solution were 10 per cent acrylamide, 0.2 per cent N,N′-methylenebisacrylamide, 0.25M Tris, 0.05M nitric acid, 0.1 per cent triethanolamine, and 0.5 mg per cent riboflavin.

Gels were prepared by photopolymerization in glass tubes, 55 mm long and 3 mm inside diameter, inserted vertically in a row on a special rubber stand. The gel solution was pipetted into each tube to a level of 45 mm, and 5 mm of distilled water was carefully layered on top of the solution, using a small glass pipette fitted with cotton-wool to avoid mixing. The tube stand was placed between two day-light fluorescent lamps at a distance of 2—3 cm for about 30—40 minutes. After photopolymerization, the water layer was replaced by cathode buffer solution (0.01M Tris, 0.08M glycine, pH 8.8). The protein solution prepared as described above was layered directly on top of the small-pore gel under the cathode buffer.

Electrophoresis was performed in a refrigerated device similar to that described in detail by Davis (1964). The tops of the tubes with rubber packings were inserted in a vertical position into the holes on the bottom of the upper electrode (cathode) vessel. The upper vessel was placed above the lower one so that the glass tubes were totally immersed in the refrigerated and magnetically stirred anode buffer solution (0.1M Tris, 0.02M nitric or acetic acid, pH 8.9). A current of 2.0—2.5 mA per tube was applied for 1—1.5

hours until the buffer front, seen as a brown-coloured disc formed by the polyphenols present in plant tissue extracts, reached the bottom of the tubes.

Enzyme staining procedures. After electrophoresis, the gels were removed from the tubes with a hypodermic needle inserted between the glass wall and the gel, washed in several changes of distilled water, and stained to localize phosphohydrolase activity, using histochemical procedures.

Modified metal-salt procedures of Gomori and azo-dye coupling methods were used to localize phosphohydrolase activity in gels. To avoid heavy background staining in Gomori's lead-sulphide method, nitrate salts were used in all electrophoretic buffer solutions and reaction mixtures instead of ordinary chloride or sulphate salts.

For the detection of acid phosphatase activity by lead-sulphide method, the gels were washed for 15—20 min in 0.1M acetate buffer at a pH of 5.2, and thereafter incubated for 20—60 min at room temperature (20—25°) in an incubation mixture containing 0.1M acetate buffer, pH 5.2, 2.5mM lead nitrate, 0.5mM magnesium nitrate, and 1mM phosphate substrate. ATP, ADP, AMP, β-glycerophosphate, and thiamine pyrophosphate (TPP) were used as substrates in parallel incubations. In control tests the gels were incubated in substrateless reaction mixtures. Following incubation, the gels were washed for 2—3 hours in several changes of distilled water, and the sites of lead phosphate precipitates corresponding to the localization of phosphohydrolase activity were stained brownish-black or black in a dilute solution of yellow ammonium polysulphide. Excess of ammonium sulphide was removed by washing the gels in a dilute solution of sodium metabisulphite.

Acid phosphohydrolase activity was also localized by using a modification of azo-dye coupling method of Barka (1960) with α-naphthyl phosphate as a substrate and hexaazotized pararosanilin as a coupler.

To 1.0 ml aliquot of pre-cooled 0.02M solution of pararosanilin (basic fuchsin) in 0.6N hydrochloric acid, 0.2 ml of cold 3.5 per cent sodium nitrite was added, and the mixture vigorously shaken. The mixture was left standing at 0—5° for at least 10 min. Just before incubation, 10.0 ml of a mixture containing sodium hydroxide (8.0 g/l) and maleic acid (11.6 g/l) were added for neutralization and buffering at a final pH of about 6, followed by 0.2 ml α-naphthyl phosphate solution (25 mg/ml) in ethanol. The gels were incubated in this reaction mixture for about 20—60 min, until distinct reddish zones indicating phosphatase activity had developed.

Esterase activity was revealed on gels in a similar way, only α-naphthyl acetate was substituted for α-naphthyl phosphate as a substrate.

For the detection of alkaline phosphatase activity, the gels were washed for 10—15 minutes in a solution containing 5mM Tris and 10mM calcium nitrate followed by incubation for about one hour in a reaction mixture consisting of 0.05M Tris-nitrate buffer, pH 9.2, 10mM calcium nitrate and 2mM phosphate substrate. ATP, ADP, AMP, thiamine pyrophosphate and β-glycerophosphate were used as substrates in parallel incubations. The sites of calcium phosphate precipitates formed by phosphohydrolase activity were visualized by different staining methods.

In the first method, the gels were washed for two hours at least in a solution containing 5mM Tris and 10mM calcium nitrate, to remove the excess of the substrate left in gels. Thereafter, calcium phosphate precipitates were converted, as suggested by Allen and Hyncik (1963), into lead phosphate by immersing the gels for 10 min in 0.1M acetate buffer at a pH of 5.2, containing 3mM lead nitrate. After washing for 2—3 hours in repeated changes of distilled water, the gels were stained in a dilute solution of yellow ammonium polysulphide.

In the second method, the gels were washed with water for a brief period of time and then stained for phosphate ions by incubating in a freshly prepared mixture of 0.2 per cent ammonium molybdate and 0.2 per cent ascorbic acid in 0.2M acetate buffer at a pH of 4.0, containing 2×10^{-3}M copper sulphate. The regions occupied by phosphohydrolases active in alkaline medium stain deep-blue.

Results

Phosphohydrolase preparations derived from different sources are known to vary significantly in respect to their pH optima. According to the pH optima they are conditionally divided into two classes, conventionally named as "acid" and "alkaline" phosphohydrolases (or phosphatases). As shown in our previous paper (Jaaska, 1967), a cytoplasmic fraction from the roots of maize seedlings exhibited phosphohydrolase activity toward different substrates in acid as well as in alkaline media. For this reason, in the present investigation we studied the electrophoretic patterns of phosphohydrolases by incubating the gels in both acid and alkaline reaction media.

Fig. 1 presents polyacrylamide gel electrophoretic patterns of soluble phosphohydrolases from maize coleoptiles revealed in acid medium by using different phosphate compounds as substrates. It is shown in Fig. 1 that a number of electrophoretically distinct phosphohydrolases differing in their substrate specificity are present in maize seedlings. The zones of enzyme activity moving to the anode are numbered 1 through 9 in the order of decreasing mobility toward the anode. In all, up to at least nine electrophoretically distinct phosphohydrolase bands can be detected in zymograms. The lowest band seen at the bottom of the zymograms is an artifact which was formed due to the reaction of lead or diazo compound with substances moving just ahead of the buffer front. This band was always seen in the control zymograms when no substrate was added to the reaction mixture.

A considerable variation in the number of bands in zymograms was observed, depending on the nature of the phosphate substrate used. The maximum number of bands in a single zymogram, seven or eight, was detected with ATP or ADP, respectively. The ATPase and ADPase zymograms were essentially identical in their electrophoretic behaviour, except the extra band 9 in the ADPase zymogram. The thiamine pyrophosphatase (TPPase) zymogram revealed six bands showing the same mobility as band 2 through 7 in the zymograms of ATPase and ADPase. Band 1, however, is absent in the TPPase zymogram. When using AMP as a substrate, four main zones of 5′-nucleotidase activity were stated. Only one zone of enzymatic activity can be seen in zymograms when phosphomonoester substrates, β-glycerophosphate or α-naphthylphosphate were used. It is not excluded, of course, that a broad zone in the zymogram of β-glycerophosphatase is due to the activity of more than one enzyme of close electrophoretic mobilities. However, the zymogram with α-naphthyl phosphate as a substrate also revealed but a single diffuse band of similar mobility as that of β-glycerophosphatase band.

When comparing the zymograms obtained by using different substrates, clear-cut differences in the substrate specificity of individual phosphohydrolases can be stated. The most mobile phosphohydrolase corresponding to band 1 was able to split phosphoanhydride bonds of ATP and ADP, but not that of TPP or the phosphomonoester bonds of AMP, β-glycerophosphate and α-naphthyl phosphate. Thus, enzyme 1 can be classified as an apyrase.

Bands 2, 3, 4, and 7 revealed high activity toward phosphoanhydride bonds of ATP, ADP, and TPP, and showed no or only a weak activity toward phosphomonoester substrates, depending on the length of the incubation period. If much longer incubation times were used than ordinarily, bands 2, 3, 4, and 6 revealed some activity with phosphomonoester substrates. Band 6 seems to possess some 5′-nucleotidase activity splitting

phosphomonoester bonds of AMP. Therefore, enzymes 2, 3, 4, 6, and 7 mainly act as nonspecific phosphoanhydride phosphohydrolases which probably exhibit a slight phosphomonoester phosphohydrolase activity as well.

Enzyme 5 showed the most wide-ranged substrate specificity, hydrolyzing all the substrates used. It can probably be classified as a nonspecific acid phosphohydrolase capable of splitting both phosphomonoester and phosphoanhydride bonds.

The double band 8 can be characterized as 5′-nucleotidase since it shows no detectable activity toward phosphoanhydride bonds of ATP, ADP, and TPP, or phosphomonoester bonds of β-glycerophosphate and α-naphthyl phosphate. The zymograms of ADPase and 5′-nucleotidase revealed a band designated 9 that did not move far from the site of the application of the protein sample. Since band 9 revealed no activity toward ATP and TPP, its appearance in the ADPase zymogram may be due to some contamination of our ADP preparation by its degradation product AMP.

Fig. 1 also presents the zymogram of esterase obtained with α-naphthyl acetate as a substrate, which revealed two intensely stained bands and three additional less distinct bands. Two esterase bands had mobilities which roughly coincided with bands 1 and 5 on the ATPase zymogram.

In alkaline medium at pH 9.2, as seen in Fig. 2, the number of bands with phosphohydrolase activity is much reduced as compared with that observed in an acid medium. The zymograms of "alkaline" ATPase and ADPase revealed two intensely stained zones called *A* and *B*, the *A* zone being the faster moving band. The zymogram of TPPase revealed only one zone of enzymatic activity which coincided in its mobility to band *B*. No band developed in zymograms when phosphomonoester substrates AMP and β-glycerophosphate were used.

The two "alkaline" phosphoanhydride phosphohydrolases found in maize coleoptiles distinctly differed in the degree of their substrate specificity. The most mobile enzyme *A* possesses phosphohydrolase activity toward both ATP and ADP. However, it exhibited no activity toward TPP and AMP, and can thus be classified as an apyrase. Enzyme *B* hydrolyzed all the three phosphoanhydride substrates used and can be classified as a nonspecific phosphoanhydride phosphohydrolase.

The zymograms of ATPase and ADPase in Fig. 2 clearly differed in the staining intensity of the bands. In the ATPase zymogram band *B* stained more intensely as compared with band *A*, while the reverse is true for the staining intensity of bands *A* and *B* in the zymogram of ADPase. Furthermore, in alkaline medium in the presence of calcium ions, enzyme *A* probably possesses somewhat higher ADPase activity than ATPase activity judging by the relative staining intensity of the zone in the two zymograms.

The two staining methods used to localize phosphohydrolase activity in alkaline medium yielded essentially the same results, as it will be seen from the comparison of zymograms *a*, *b*, *g*, and *h* in Fig. 2. However, the method which is based on direct staining of calcium phosphate as molybdate blue is much more rapid than the lead conversion method, and is to be preferred. Furthermore, when using the lead conversion method, care must be taken to wash out the excess of substrate left in gels. Otherwise additional artifact zones will develop due to the activity of acid phosphatases, as can be seen in zymogram *h* in Fig. 2.

The electrophoretic patterns of ATPase, thiamine pyrophosphatase and

β-glycerophosphatase activities in the protein extracts from the roots of maize seedlings were also studied and were found to be essentially the same as those from maize coleoptiles.

Discussion

It is evident from the results presented here and in our previous paper (Jaaska, Jaaska, 1968) that plant tissues contain a system of electrophoretically separable phosphohydrolases considerably differing in their substrate specificity and pH optima. Polyacrylamide gel electrophoresis demonstrated the presence, in etiolated maize coleoptiles, of up to at least nine distinct fractions of phosphohydrolases active in acid medium and two fractions active in alkaline medium. By the same method, up to eight "acid" and several "alkaline" phosphohydrolases were found in wheat coleoptiles (Jaaska, Jaaska, 1968). The appearance of phosphohydrolase patterns in maize and wheat seedlings, however, was qualitatively different, indicating the species-specific nature of phosphohydrolases.

The classification of phosphohydrolases accepted at present (Dixon, Webb, 1964) is based on their substrate specificity. Depending on the type of the phosphate bond attacked, phosphohydrolases are divided into two large groups: phosphomonoester phosphohydrolases (EC 3.1.3) and phosphoanhydride phosphohydrolases (EC 3.6.1). Phosphohydrolases of both groups are further subclassified mainly on the basis of their specificity toward a single substrate or a group of closely related substrates.

In the present study, soluble phosphohydrolases found in maize seedlings were classified on the basis of their substrate specificity as nonspecific acid phosphohydrolases, nonspecific phosphoanhydride phosphohydrolases, apyrases and 5′-nucleotidases.

One of the fractions was found to exhibit a broad substrate specificity, hydrolyzing in acid medium all the phosphomonoester and phosphoanhydride substrates studied and was classified as a nonspecific acid phosphohydrolase. It revealed no detectable activity in alkaline medium. Thus, the results of our study support the view suggested by several authors (Ito *et al.*, 1955; Mayer *et al.*, 1961; Shaw, 1966) on the basis of their studies on substrate specificity of highly purified preparations of plant phosphatases that the plant tissues contain a nonspecific enzyme capable of splitting both phosphomonoester and phosphoanhydride bonds.

Several fractions of maize phosphohydrolases were found to exhibit high activity toward all the three phosphoanhydride substrates studied, and no or only a slight activity toward phosphomonoesters. These enzymes were described as nonspecific phosphoanhydride phosphohydrolases.

Besides the nonspecific enzymes described above, the zymograms revealed the presence, in maize seedlings, of several more specific phosphohydrolases. Some of the fractions possessed distinct 5′-nucleotidase activity (EC 3.1.3.5), but revealed no or only a slight activity toward other substrates. One of the fractions was found to hydrolyze ATP and ADP, but not thiamine pyrophosphate and phosphomonoester substrates, and was therefore classified as an apyrase (EC 3.6.1.5). When comparing ATPase and ADPase zymograms obtained in acid (Fig. 1) and alkaline (Fig. 2) media, it can be seen that the apyrase bands designated *A* and 1 roughly coincide in their electrophoretic mobility. Of cource, on the basis of similar electrophoretic mobility alone, we cannot suggest with full certainty that the activity belongs to a single enzyme. Nevertheless, such a possibility must be considered.

One of the most outstanding recent approaches in modern enzymology appears to be the introduction and the further development of the concept of isoenzymes. According to this concept, most of the enzymes exhibiting similar substrate specificity can exist in physically distinct multiple molecular forms not only within a single organism, but even within a single tissue or cell. These multiple molecular forms originally called isozymes (Markert, Møller, 1959) or, more recently, isoenzymes (Webb, 1964), can be separated by means of different ion-exchange chromatographic and gel electrophoretic fractionation methods.

In the case of plant tissues, the multiple nature of enzymes has been demonstrated for phosphohydrolases (Ikawa *et al.*, 1964; Brouillard, Ouellet, 1965; Schormüller *et al.*, 1965; Jaaska, Jaaska, 1968), for peroxidases (Jermyn, Thomas, 1954; McCune, 1961; Macko, Novacký, 1966, *etc.*), for esterases (Schwartz, 1960; Schwartz *et al.*, 1964), as well as for some other enzymes.

Originally the term isozyme was introduced by Markert and Møller (1959) to describe different molecular forms of enzymes exhibiting similar substrate specificity. However, difficulties arise when we try to apply the term "isoenzyme" in this sense to physically distinct forms of nonspecific enzymes, such as esterases and phosphohydrolases. The results of the present study clearly showed that electrophoretically distinct phosphohydrolases in many cases markedly differed in their capability of hydrolyzing different phosphate substrates. Thus, the isoenzymes of maize ATPase (Fig. 1) differed by their activity toward other phosphate substrates. When proceeding from the original isoenzyme concept, we cannot define electrophoretically distinct ATPase forms as isoenzymes, since they substantially differ by their substrate specificity.

Differences in the substrate specificity of electrophoretically separated forms have also been reported for peroxidases (Macko, Novacký, 1966), esterases (Augustinsson, 1961) and others. Even the isoenzymes of such a relatively specific enzyme as lactate dehydrogenase have been shown (Allen, 1961) to exhibit clear differences in their substrate specificity.

To overcome the difficulties which arise when applying the isoenzyme concept to nonspecific enzymes, it seems to us to be more reasonable to define isoenzymes as structurally distinct molecular forms of enzymes catalyzing the same chemical reaction, or, even more broadly, the same type of chemical reactions. In accordance with this definition, all electrophoretically distinct enzymes capable of hydrolyzing ATP might be termed as ATPase isoenzymes, in spite of the differences in the substrate specificity of individual isoenzymes toward other substrates.

If we accept this broader definition of isoenzymes, a further sub-classification of isoenzymes on the basis of some other properties seems to be desirable. One of the approaches is to distinguish homologous and nonhomologous isoenzymes on the basis of the genetic determination of their structure. The isoenzymes whose structure is genetically determined by the same locus or loci and which are thus structurally related can be regarded as homologous. Thus, all five vertebrate lactate dehydrogenase isoenzymes might be considered homologous since their structure is controlled by the activity of the same two gene loci (Cahn *et al.*, 1962). In addition, homologous isoenzymes can arise due to the action of autosomal allelic genes in heterozygotes or hybrids in the case of diploid and polyploid organisms (Schwartz, 1960; Mac Intyre, 1966, *etc.*).

The genetic and structural nature of the multiple forms of phosphohydrolases found in maize seedlings as well as their physiological significance remains to be studied.

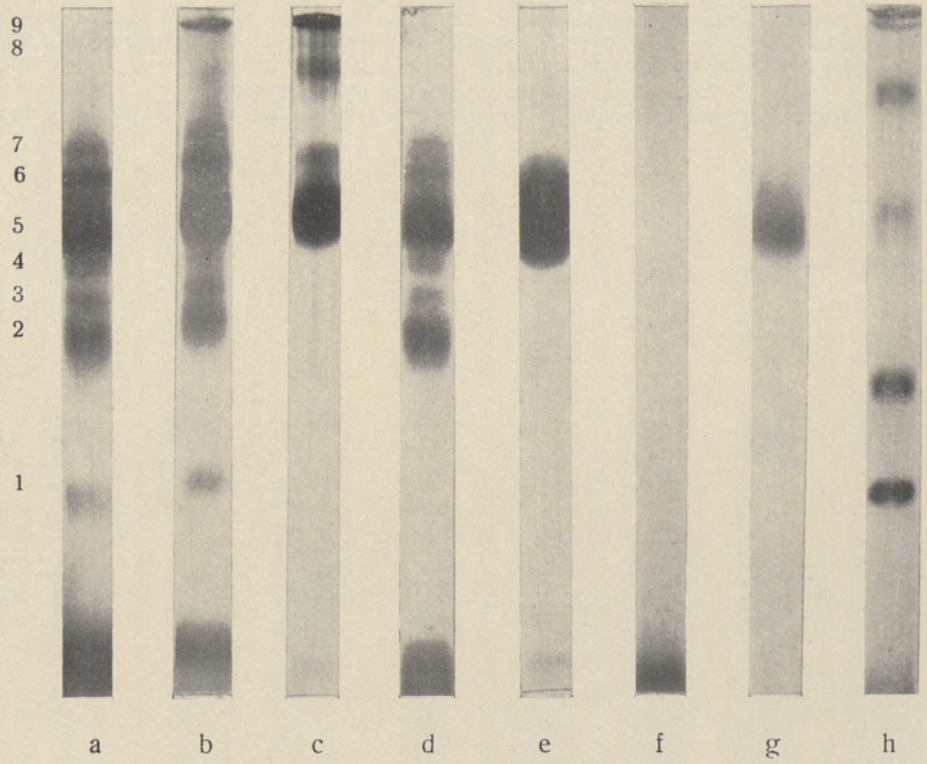

Fig. 1. Polyacrylamide gel zymograms of soluble phosphohydrolases and esterases from maize coleoptiles active in an acid medium at pH 5.2.

Substrates: *a* — ATP; *b* — ADP; *c* — AMP; *d* — thiamine pyrophosphate; *e* — β-glycerophosphate; *f* — control with no substrate; *g* — α-naphthyl phosphate; *h* — α-naphthyl acetate. Zymograms *a* through *f* were obtained by means of Gomori's lead sulphide method, and zymograms *g* and *h* — by means of azo-dye staining method.

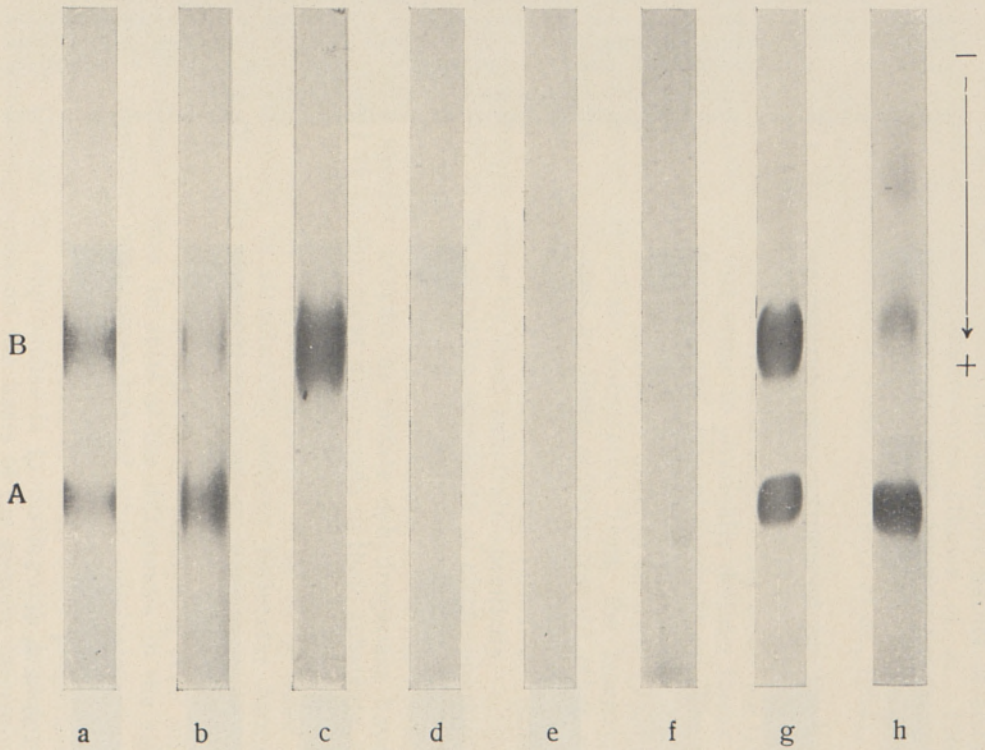

Fig. 2. Polyacrylamide gel zymograms of soluble phosphohydrolases from maize coleoptiles active in an alkaline medium at pH 9.2.

Substrates: *a* — ATP; *b* — ADP; *c* — thiamine pyrophosphate; *d* — AMP; *e* — β-glycerophosphate; *f* — control with no substrate; *g* — ATP; *h* — ADP. Zymograms *a*, *b* and *c* were obtained with the molybdate blue method, and zymograms *d* through *h* — with the lead conversion method.

Summary

Soluble phosphohydrolases and esterases of etiolated maize seedlings were studied by means of vertical polyacrylamide gel electrophoresis combined with histochemical methods for the location of enzymatic activity.

In acid medium, at least 9 electrophoretically distinct phosphohydrolase bands were detected by using different phosphate substrates. The ATPase zymogram revealed up to 7 electrophoretically distinct fractions which differed in respect of their substrate specificity toward other phosphate compounds. One of the enzymes, classified as an apyrase, hydrolyzed ATP and ADP, but showed no activity toward thiamine pyrophosphate and phosphomonoester substrates. Another fraction specified as a nonspecific acid phosphohydrolase hydrolyzed all the phosphoanhydride and phosphomonoester substrates. Several fractions showed high activity toward phosphoanhydride substrates with no or only a slight activity toward phosphomonoester substrates, and were classified as nonspecific phosphoanhydride phosphohydrolases. Some fractions revealed distinct 5′-nucleotidase activity.

In alkaline medium only two electrophoretically distinct phosphohydrolases were found. One of them hydrolyzed all the three phosphoanhydride substrates tested and was classified as a nonspecific phosphoanhydride phosphohydrolase. The second enzyme acted as an apyrase hydrolyzing ATP and ADP and showing no activity toward thiamine pyrophosphate. None of the enzymes showed phosphomonoester phosphohydrolase activity in alkaline medium.

The zymogram of esterase revealed two intensely stained bands and three additional, less distinct bands.

The applicability of the term "isoenzyme" for describing electrophoretically distinct phosphohydrolases has been discussed.

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Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

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VILVE JAASKA, VELLO JAASKA

MAISIIDANDITE LAHUSTUVAD FOSFOHÜDROLAASID JA ESTERAASID

Resümee

Uuriti etioleeritud maisiidandite koleoptiilide ja juurte lahustuvate fosfohüdrolaaside ja esteraaside fraktsioonilist koostist ning substraatset spetsiifilisust, rakendades elektroforeesi polüakrüülamiidgeelis ja sellele järgnevaid histokeemilisi värvimismeetodeid ensüümide aktiivsuse avastamiseks.

Mitmesuguste fosfaatsete substraatide kasutamisega täheldati happelises keskkonnas vähemalt üheksat erineva elektroforeetilise liikuvusega fosfohüdrolaasi fraktsiooni. ATF-aasi ensümogrammil esines seitse fraktsiooni, millest mõned erinesid substraatse septsiifilisuse poolest teiste fosfaatsete ühendite suhtes. Leeliselises keskkonnas ilmnes ainult kaks erineva elektroforeetilise liikuvusega fosfohüdrolaasi, mis hüdrolüüsisid fosfoanhüdriide, mitte fosfomonoestreid. Esteraasi ensümogrammil oli kaks kõrge ja kolm madalama aktiivsusega tsooni.

Substraatse spetsiifilisuse alusel jaotati maisiidandite lahustuvad fosfohüdrolaasid nelja rühma: mittespetsiifilised happelised fosfohüdrolaasid, mittespetsiifilised fosfoanhüdriidide fosfohüdrolaasid, apüraasid ja 5′-nukleotidaasid.

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

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ВИЛЬВЕ ЯАСКА, ВЕЛЛО ЯАСКА

РАСТВОРИМЫЕ ФОСФОГИДРОЛАЗЫ И ЭСТЕРАЗЫ В ПРОРОСТКАХ КУКУРУЗЫ

Резюме

С помощью вертикального электрофореза в полиакриламидном геле в сочетании с гистохимическими методами изучали растворимые фосфогидролазы и эстеразы в белковом экстракте из колеоптилей и корней этиолированных проростков кукурузы.

Применяя отдельные фосфатные субстраты (АТФ, АДФ, АМФ, тиаминпирофосфат, β-глицерофосфат и α- нафтилфосфат), в кислой среде при pH 5,2 удалось выявить не менее девяти электрофоретически разделимых фракций фосфогидролаз. На зимограмме АТФ-азы было выявлено до семи фракций, различающихся по электрофоретической подвижности и специфичности к другим фосфатным соединениям. Один из обнаруженных ферментов, расщепляющий АТФ и АДФ, но не тиаминпирофосфат и фосфомоноэфирные субстраты, был классифицирован как апираза. Другую фракцию, гидролизующую как фосфоангидридные, так и фосфомоноэфирные связи, следует рассматривать как неспецифическую кислую фосфогидролазу. Несколько ферментов, которые обнаруживали высокую активность в отношении фосфоангидридных субстратов и не расщепляли фосфомоноэфирные субстраты, были отнесены к группе неспецифических фосфогидролаз фосфоангидридов. Некоторые фракции обладали 5′-нуклеотидазной активностью.

В щелочной среде при pH 9,2 были выявлены лишь две фракции фосфогидролаз. Одна из них гидролизовала все три испытанных фосфоангидридных субстрата и поэтому была классифицирована как неспецифическая фосфогидролаза фосфоангидридов. Вторая действовала как апираза, расщепляя АТФ и АДФ, но не тиаминпирофосфат. Ни один из рассматриваемых ферментов не имел в щелочной среде фосфомоноэстеразной активности.

На зимограмме эстеразы было обнаружено две интенсивно окрашенные и три менее отличимые фракции.

Обсуждается вопрос о применимости понятия «изоферменты» для обозначения электрофоретически разделяемых форм фосфогидролаз.

Институт зоологии и ботаники
Академии наук Эстонской ССР

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