Phytochemistry, 1971, Vol 10, pp 1171 to 1174 Pergamon Press Printed in England

POLYACRYLAMIDE-GEL ELECTROPHORESIS OF PEA AND GREEN BEAN CARBOXYLESTERASES*

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(*Received* 6 *March* 1970)

Abstract—Aqueous extracts of peas (*Pisum sativum* L) and green beans (*Phaseolus vulgaris* L) were subjected to zone electrophoresis on polyacrylamide-gel α-Naphthyl acetate and propionate were hydrolyzed in preference to α-naphthyl butyrate and naphthol AS acetate by most of the vegetable esterases. At least 14 bands of esterase activity in green beans were separated into three groups. Components of the two faster migrating groups demonstrated similar substrate and inhibitor specificities and, hence, appeared to be isozymes, whereas components of the slowest migrating group differed in substrate specificity Results with pea esterases revealed the presence of seven distinct esterase bands. The six slower migrating bands behaved as isozymes. Band 7, which may contain four components, was more resistant to organophosphorus inhibitors and showed differences in substrate specificities from bands 1–6

INTRODUCTION

ELECTROPHORESIS, followed by characterization of the separated enzymes based on reaction with various substrates and inhibitors, has been an important contribution to the study of esterases.[1,2] Introduction of starch-gel and polyacrylamide-gel as a medium for electrophoretic separations has increased the capacity to resolve proteins. Using these techniques the presence of multiple forms of esterases has been demonstrated in various plant species.[3–9] Recent reports from this laboratory[10–13] demonstrate that considerable variation exists in regard to the number and substrate and inhibitor specificities of esterases in certain species.

In the present study, esterases of the pea (*Pisum sativum* L.) and green beans (*Phaseolus vulgaris* L.) were separated by polyacrylamide-gel electrophoresis and their substrate and inhibitor specificities were examined.

* Technical Paper No 2804, Oregon Agricultural Experiment Station.

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RESULTS

Substrate Specificity

Activity of bean esterase with four substrates is shown in Fig. 1 A–D. At least 14 bands of esterolytic activity were separated into three groups. The slow-moving group, containing bands 1–5, constitutes group 1. Group 2, intermediate in mobility, appeared as a wide zone of activity, which was later shown[14] to contain 5 bands. The fast-moving components, bands 11–14, comprise group 3.

Bands 1 and 2 in group 1 were active towards α-naphthyl acetate and propionate, but did not hydrolyze α-naphthyl butyrate. In contrast, all three of these substrates were hydrolyzed by bands 3, 4 and 5. Group 2 appeared to be similar to bands 1 and 2 of group 1 in that α-naphthyl acetate and propionate were attacked, while α-naphthyl butyrate was not. The three α-naphthyl substrates were hydrolyzed by group 3 with activity decreasing as the acyl chain length increased. In the zymogram produced with naphthol AS acetate as substrate, the bands appeared purple in color and became visible after 12 hr of incubation. The third group of esterase activity was more active toward this substrate than the first and second groups.

At least seven bands of esterase activity are visible in the zymogram prepared from pea extract (Fig. 2 A–D). These bands may be divided into groups; the first group was slow in mobility and contained bands 1–6 and the second group was band 7. α-Naphthyl propionate was the preferred substrate for the first group followed by α-naphthyl acetate and butyrate, respectively. The activity of band 7 decreased as the acyl chain on the α-naphthyl substrates was increased to the point where α-naphthyl butyrate was hydrolyzed only slightly. All seven bands showed activity with naphthol AS acetate, but required 7 hr of incubation to develop.

Inhibitor Studies

Inhibition by organophosphorus compounds has been the basis for the classification of esterases.[10–12,15] In the present studies, α-naphthyl acetate was employed as substrate to determine the effect of three organophosphorus inhibitors on the esterases separable by polyacrylamide-gel electrophoresis. The effect of various concentrations of diethyl *p*-nitrophenyl phosphate (paraoxon) on the esterase activity of green beans is shown in Fig. 1 E–H. Streaking of the bands was noticed in all gels incubated in Triton X-100, so sensitivity of the individual bands to the inhibitors could not be determined. Group 1 was most sensitive to paraoxon, while groups 2 and 3 were more resistant and appeared to be of equal resistance. All the esterases were inhibited at 10^{-2} M paraoxon. In contrast to this, activity was present in both groups 2 and 3 at 10^{-2} M diethyl *p*-nitrophenyl thiophosphate (parathion) (Fig. 1 K). Hence, the substitution of the oxygen atom in paraoxon with the sulfur atom (parathion) decreases the inhibition of the green bean esterases. This observation is in agreement with previous work in this laboratory which showed that 60 per cent of the green bean esterases were inhibited at 10^{-2} M parathion.[11]

Figure 1 M–P shows that the esterases in group 1 were inhibited at 10^{-4} M diisopropylphosphorofluoridate (DFP) and that those in group 3 were inhibited at 10^{-3} M DFP. Therefore, group 3, which was resistant to paraoxon and parathion, was inhibited by DFP. This indicates that considerable variation exist in the sensitivity of these esterases to organophosphorus inhibitors.

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Complete inhibition of pea esterases was observed (Fig. 2 I–L), with the exception of band 7, with parathion at the highest concentrations (10^{-2} M) used in this study. Band 7 was more resistant to 10^{-3} M parathion and paraoxon (Fig. 2 F and J) than the slow moving bands (1–6). DFP at 10^{-3} M almost completely inhibited all the esterase bands (Fig. 2 N). Some inhibition was noted at 10^{-4} M with all the inhibitors. This correlates with previous work[10] which noted 80 per cent and 50 per cent inhibition of pea esterases by 10^{-4} M DFP and parathion, respectively

DISCUSSION

Seven and 14 bands of esterase activity were separated from extracts of peas and green beans, respectively, by polyacrylamide-gel electrophoresis. The difference between the esterase of these two plants was the presence of group two esterases in the green beans. Reasons for this difference are not readily apparent since both plants belong to the same family (Leguminosae) and similar tissue (seeds) were used from both plants. However, the green beans did include the pod, while the peas did not, which could account for the difference. Varying numbers of esterase bands have also been reported in extracts of cucumber, soybean, wheat seeds, and corn using starch-gel electrophoresis.[3]

Although the esterases of the green bean and pea did appear electrophoretically different, their substrate and inhibitor specificities were somewhat similar. Most esterase bands from both vegetables hydrolyzed α-naphthyl acetate and propionate in preference to α-naphthyl butyrate and naphthol AS acetate. However, substrate specificity differences appeared more pronounced in the green beans than the pea (Figs. 1 and 2). These results are in close agreement with the substrate specificity of the root nodule esterases.[7] Interestingly, all the bands in group 1 of the green beans did not have the same substrate specificity. Bands 1 and 2 did not hydrolyze α-naphthyl butyrate, while band 3, 4 and 5 did. With the pea, α-naphthyl butyrate was not hydrolyzed by band 7. It is remarkable that esterase bands showing similar substrate specificities possess striking differences in electrophoretic mobility and inhibitor specificities (Figs. 1 and 2).

All organophosphorus inhibitors used in this study inhibited the esterases of both peas and green beans. However, green bean esterases were more resistant than pea esterases, which confirms previous results from this laboratory.[10,11] Studies with purified fractions have demonstrated that bands 1–6 of the pea zymogram were isozymes.[12] The similar substrate and inhibitor specificities (Fig. 2) confirm the isozymic nature of these bands. Band 7 was originally thought to be homogeneous; however, the zymogram in Fig. 2N shows that this band contained at least three and possibly four components. The similar substrate and inhibitor specificities of these components, as well as, those components in groups 2 and 3 of the green bean zymograms (Fig. 1) suggest that the components of these groups are isozymes. On the other hand, group 1 of the beans, due to the difference in substrate specificity of bands 1 and 2 from 3, 4 and 5, did not appear to be isozymes. Isozymic forms of esterases have also been demonstrated in extracts from maize kernels,[5] plants of the Cucurbitaceae,[4] wheat seeds, cucumber, corn, soybean seedlings[3] and *Solanum* tubers.[6]

The apparent absence of choline esterases in our preparation was established in previous work.[10,11] This information, with the almost complete inhibition of esterase activity with 10^{-2} M paraoxon, indicates that the majority of esterases present in both peas and beans were carboxylesterases (E.C. 3.1.1.1, carboxylic ester hydrolases).

EXPERIMENTAL

Enzyme Extraction

Peas and green beans were lyophilized and stored as previously described [10,11] Dried peas were powdered in a Waring Blendor for 2 min and the resulting powder was slurried with 9 parts (w/v) distilled water. After centrifugation of the slurry at 27,000 *g* for 40 min, the pH of the supernatant was reduced to 5 5 with 0 1 N HCl and the precipitate was removed by centrifugation (12,000 *g* for 10 min). The clear supernatant, after readjustment of the pH to 6·5 with 0 1 N NaOH, was used as the enzyme source from peas. A similar extract was prepared from green beans with the exception that protamine sulfate was the precipitant.[12]

Substrates and Inhibitors

Substrates were prepared in 100 ml of 0 1 M NaOAc (pH 5 5) containing 70 mg of Fast Blue RR salt and 40 mg of the ester. Inhibitors were prepared in 1 per cent (w/v) Triton X-100. Parathion and paraoxon (0·1 M) required 60 sec homogenization in a microblender to form an emulsion DFP was soluble at the concentrations used

Polyacrylamide-ge Electrophoresis

Electrophoresis was performed on a 7%, continuous polyacrylamide gel as described previously.[12] The gel slab was cut into four strips and incubated at 37° for 30 and 90 min for peas and green beans, respectively. To study the effect of inhibitors, the individual gel strips were incubated in the inhibitor solutions for 60 min at 37°. Longer incubation times did not cause further inhibition After the incubation period the gel was removed from the inhibition solution and placed in the substrate solution for the prescribed time

Acknowledgements—The authors wish to acknowledge the valuable technical assistance of Mrs. William Smith. This investigation was supported, in part by U.S Public Health Service Research Grant AM 09306 from the National Institute of Arthritis and Metabolic Diseases.