Phytochemistry, 1969, Vol. 8, pp. 2127 to 2131. Pergamon Press. Printed in England.

ALDOLASE ISOZYMES OF HIGHER PLANTS*

KAZUSUKE TAKEO

Department of Biochemistry, Yamaguchi University School of Medicine,
Ube, Yamaguchi-ken, Japan

(*Received* 11 *March* 1969, *in revised form* 9 *May* 1969)

Abstract—Distributions of aldolases in tissue extracts of three higher plants were studied by disc electrophoresis using a specific staining method for the aldolase activity. Spinach, radish and carrot all contained two aldolase isozymes, the slower-moving aldolase being present in the seeds, whereas leaves contained mainly the faster-moving aldolase fraction.

IN THE organs of terrestrial animals, such as mammals[1-5] and birds,[6] aldolase exists in various forms. In the previous paper[7] the author demonstrated the same phenomenon in fishes and other marine animals, using the disc electrophoresis and a specific staining technique. In the plant kingdom the presence of aldolase isozymes has not yet been reported. In this paper, evidence will be presented to demonstrate the occurrence of two aldolase isozymes in several higher plants.

RESULTS

General

The plants were extracted with 1 mM EDTA and the crude extracts clarified by centrifugation. Protein content[8] and aldolase activities[9] in these extracts are shown in Table 1. Disc electrophoresis[10] was carried out as described previously.[7]

Spinach, Spinacia oleracea

As previously reported,[7] when the gel was stained for aldolase activity, two non-specific bands appeared, one at the top of the gel (cathodic end) and the other in front of the tracking dye band. The former band seems to be so-called "nothing" dehydrogenase band of Dietz and Lubrano,[11] and the latter was due to thioglycolate, which was added to the sample

* Part II in the series "Studies on the Aldolase Isozymes by Gel Electrophoresis".

1 E. PENHOET, T. V. RAJKUMAR and W. J. RUTTER, *Proc. Natl Acad. Sci.* **56**, 1275 (1966).
2 E. PENHOET, M. KOCHMAN, R. VALENTINE and W. J. RUTTER, *Biochemistry* **6**, 2940 (1967).
3 U. RENSING, A. SCHMID and F. LEUTHARDT, *Hoppe-Seyler's Z. Physiol. Chem.* **348**, 921 (1967).
4 R. PIETRUSUKO and D. N. BARON, *Biochim. Biophys. Acta* **132**, 203 (1967).
5 H. B. ANSTALL, C. LAPP and J. M. TRUJILLO, *Science* **154**, 657 (1966).
6 J. J. HERSKOVITZ, C. J. MASTERS, P. M. WASSERMAN and N. O. KAPLAN, *Biochem. Biophys. Res. Commun.* **26**, 24 (1967).
7 K. TAKEO, *Comp. Biochem. Physiol.*, in preparation.
8 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).
9 A. D. SWENSON and P. D. BOYER, *J. Am. Chem. Soc.* **79**, 2174 (1957).
10 L. ORNSTEIN and B. J. DAVIS, Preprint by Distillation Product (1962).
11 A. A. DIETZ and T. LUBRANO, *Anal. Biochem.* **20**, 246 (1967).

solution. In the case of plant extracts, the tracking BPB* band was overlapped by a green pigment. In the case of spinach leaf, when the separated gel was incubated in the staining solution devoid of FDP,* NAD,* and GAPD,* a non-specific band (NS) appeared (Fig. 1B).

The electrophoretograms of proteins (A) and aldolase activity band (B) of spinach leaf extract are presented in Figs. 1A and 1B. The extracted proteins were separated into fourteen bands, the third band being the most prominent. This fraction seems to correspond to the "Fraction-1" of Wildman and Bonner.[12] Aldolase activity was present in the 7th protein band. (The non-specific bands corresponding to the tracking dye and thioglycolate were not shown in Fig. 1B.)

The electrophoretograms of spinach seed extract showed that the extracted proteins appeared in two groups, the slower group being fractionated into four sharp bands and the faster one into seven somewhat diffuse ones. Aldolase activity was associated with the fifth protein band, and had a slower mobility than the leaf enzyme (Fig. 2).

The chloroplasts were separated from spinach by sucrose fractionation according to Brooks and Criddle.[13] Aqueous extract of chloroplasts contained about 10 per cent of the total aldolase activity of the leaf extract. The chloroplast extract contained one aldolase fraction, which migrated with almost the same mobility with the aldolase fraction of the leaf extract and could not be separated from it when mixed.

Radish, Raphanus sativus

In Fig. 3, the aldolase zymograms of tissue extracts from leaves (A), roots (B), and seeds (C) of radish were presented diagrammatically. In the case of the leaf extract, the extracted proteins were separated into eight bands (left column of A), of which aldolase activity (right column of A) was found in two bands, the faster one being more active.

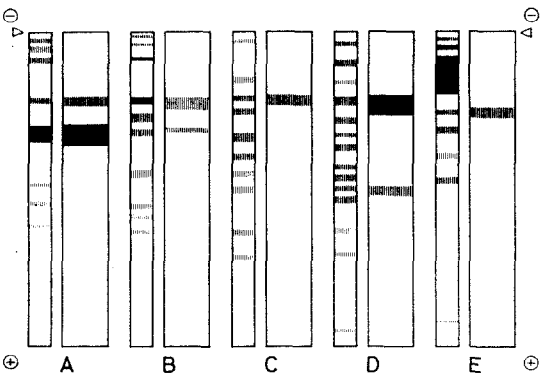

FIG. 3. DIAGRAMMATIC REPRESENTATION OF THE ALDOLASE ZYMOGRAMS OF TISSUE EXTRACTS FROM RADISH AND CARROT.

A, radish leaf; B, radish root; C, radish seed; D, carrot root; E, carrot seed. The left columns are protein fractions and the right columns are activity bands.

In roots (Fig. 3B), aldolase activity was too low to be measured according to the method of Swenson and Boyer[9] (see Table 1). However, two low active fractions were demonstrated

* Abbreviations used in this paper are: FDP, fructose 1,6-diphosphate; NAD, nicotinamide adenine dinucleotide; PMS, phenazine methosulfate; NBTZ, nitroblue tetrazolium; GAPD, glyceraldehyde 3-phosphate dehydrogenase; BPB, bromophenol blue.

[12] S. G. WILDMAN and J. BONNER, *Archs Biochem.* **14**, 381 (1949).
[13] K. BROOKS and R. S. CRIDDLE, *Archs Biochem. Biophys.* **117**, 650 (1966).

electrophoretically (right column of B), corresponding to the 4th and the 6th protein bands. The mobilities of these two root aldolases corresponded to those of the two aldolases from leaf extract. In contrast to the leaf aldolases, however, the slower-moving aldolase fraction was more active.

TABLE 1. ALDOLASE ACTIVITIES OF TISSUE EXTRACTS FROM HIGHER PLANTS

Material extracted	Protein content* (mg/ml)	Aldolase activity† (ΔE_{525} mμ/ml)	Specific activity
Spinach			
Leaves	8·2	2·3	0·28
Seeds	5·2	0·23	0·043
Radish			
Leaves	7·4	0·035	0·005
Roots	2·0	0	—
Seeds	3·9	0·22	0·055
Carrot			
Leaves	1·5	0	—
Leaves	5·0	0	—
Roots	2·2	0·12	0·052
Seeds	11·8	0·14	0·012
Seeds	15·3	0·23	0·015
Garlic			
Bulbs	4·1	0·25	0·059

* Estimated by the method of Lowry *et al.*[8]

† Estimated by the method of Swenson and Boyer,[9] using dinitrophenyl hydrazine. To 1·0 ml of enzyme solution, 0·5 ml of 0·5 M hydrazine buffer, pH 7·4, at 30°, was added 0·5 ml of 0·02 M FDP. After 10 min the reaction was stopped with 1·0 ml of 0·005 M dinitrophenyl hydrazine in 2 N HCl. Chromogen was developed by adding 7·0 ml of 0·375 N NaOH. Absorbance at 525 mμ (ΔE_{525} mμ) was measured exactly 10 min after addition of NaOH.

In the case of seed (Fig. 3C), only one fraction exhibited aldolase activity (right column of C), which corresponded to the 3rd protein band (left column of C). Its mobility was the same as that of the slower aldolase fraction from leaves or roots.

Carrot, Daucus carota, *Linné*

In Fig. 3D and E, the aldolase zymograms from carrot root (D) and seed extract (E) were presented. From the root extract, two aldolase bands were separated (right column of D), one of which migrated slower and was more active and corresponded to the 4th protein band (left column of D), and the other migrated faster, corresponding to the 10th protein band. In the case of seed (E), the aldolase activity (right column) was demonstrable only in one band, which corresponded to the 4th protein band (left column of E). Its mobility was almost the same as the slower-moving aldolase fraction from root extract.

DISCUSSION

In contrast to animal aldolases only scattered reports were made regarding the aldolases of higher plants. Twefik and Stumpf[14] studied the distribution of aldolase activity in plants

[14] S. TEWFIK and P. K. STUMPF, *Am. J. Botany* **36**, 567 (1949).

and Hatz and Leuthardt[15] reported partial purification of plant aldolases. However, the presence of aldolase isozymes has not been reported.

Recently, Brooks and Criddle[13] and Fluri *et al.*,[16] reported an extensive purification of spinach leaf aldolase. The former group started from spinach leaf chloroplast, which were separated by fractionation in sucrose solution, the latter group by extraction with 1 mM EDTA solution. Both groups obtained an aldolase preparation, which was fairly homogeneous on disc electrophoresis and by ultracentrifugal analysis.

Latzko and Gibbs[17] reported that 60–65 per cent of aldolase activity in spinach leaf was associated with the chloroplasts, which were separated in non-aqueous media. According to Fluri *et al.*,[16] spinach chloroplasts prepared by isotonic NaCl fractionation[18] were found to be devoid of aldolase activity. It might be thought, therefore, the chloroplast aldolase was easily eluted from chloroplast in aqueous media. Zymograms of spinach leaf extracted with 1 mM EDTA showed only one aldolase fraction (Fig. 1B). Hence, the leaf aldolase might be identical with chloroplast aldolase. However, since there seem to be some discrepancies of amino acid compositions between leaf aldolase of Fluri *et al.*,[16] and chloroplast aldolase of Brooks and Criddle,[13] the possibility that the two enzymes might be different cannot be excluded.

In this paper, we have demonstrated the presence of two aldolases in 1 mM EDTA extracts from three higher plants, spinach, radish, and carrot. In all cases the seeds contained only one slowly moving aldolase fraction, whereas leaves and roots contained a second faster-moving aldolase fraction.

In the case of spinach, the seed extract contained one slowly moving aldolase, while the leaf extract had a single faster-moving aldolase fraction. In the case of radish, seeds again contained only one slowly moving aldolase, but the leaf and root contained two aldolases, the slower one of which corresponding to the seed aldolase. In the leaf, the faster-moving aldolase was predominant, while in the root the slower-moving aldolase was predominant.

Carrot also contained two aldolase isozymes. In the seed, only one slowly moving aldolase fraction was demonstrated, while the root contained two aldolases, the predominant enzyme having the same mobility as the seed aldolase.

From these results, it may be inferred that there are two aldolase isozymes in higher plants, the slower of which is found mainly in seeds.

EXPERIMENTAL

Disc electrophoresis,[10] aldolase activity estimation,[9] and protein concentration determination[8] were performed as described in the previous paper.[7]

In order to identify aldolase fractions with the corresponding protein fractions, the separated gels were cut lengthwise into two equal pieces, one of which was stained with amido black 10 B and the other half with the specific aldolase stain.[7] This was composed of 10 ml of 0·01 M arsenate buffer, pH 7·4, 1 ml of 0·01 M FDP, 1 ml of 0·01 M NAD, 0·24 mg of PMS* in 0·1 ml, 4 mg of NBTZ* in 1·0 ml, and 1·5 mg of GAPD.

Preparation of Tissue Extracts

All experiments were carried out in a cold room at 0–4°. Leaves and roots were washed with tap water and finally with distilled water and homogenized for 1 min in a blendor in 1 mM EDTA adjusted to pH 7·4 (2 ml/1g). The homogenates were gently stirred for 30 min, and centrifuged at 3000 rev/min for 60 min and then at 12,000 rev/min for 40 min. The supernatants were stored frozen at −18°.

[15] C. HATZ and F. LEUTHARDT, *Hoppe-Seyler's Z. Physiol. Chem.* **348**, 354 (1967).
[16] R. FLURI, T. RAMASARMA and B. L. HORECKER, *European J. Biochem.* **1**, 117 (1967).
[17] E. LATZKO and M. GIBBS, *Z. Pflanzenphysiol.* **59**, 184 (1968).
[18] D. L. ARNON, M. B. ALLEN and F. R. WHATLEY, *Biochim. Biophys. Acta* **20**, 449 (1956).

Seeds were powdered by stone mill and stirred for 60 min in 1 mM EDTA solution (4 ml/1g). The supernatants of 12,000 rev/min centrifugation were slightly turbid; the turbidity could not be eliminated by further centrifugation.

Acknowledgement—The author is indebted to Prof. Dr. S. Nakamura for his helpful discussion and encouragement.