Phytochemistry, Vol. 21, No. 7, pp. 1523–1525, 1982.
Printed in Great Britain.

0031-9422/82/071523-03$03.00/0

ISOLATION OF SOYBEAN LIPOXYGENASE-2 BY AFFINITY CHROMATOGRAPHY

ANDRAWIS ANDRAWIS,* ALEX PINSKY and SHLOMO GROSSMAN

Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52 100, Israel

(*Received* 24 *July* 1981)

Key Word Index—Lipoxygenase isolation; affinity chromatography; soybean lipoxygenase; lipoxygenase-2; isoenzyme.

Abstract—Isoenzyme lipoxygenase-2 from soybean was isolated by affinity chromatography. Gel electrophoresis showed it to be a single protein. Its pH optimum of 6.5, range of 5.0–8.0 and activity which increased when Ca^{2+} was added identified the isolated enzyme as lipoxygenase-2.

INTRODUCTION

Lipoxygenase (EC 1.13.1.13) catalyses the oxidation by molecular oxygen of methylene interrupted *cis*, *cis*-pentadienes. Several conventional and non-conventional methods for the isolation of this enzyme from a variety of plant and animal tissues have been described [1–5]. The existence of different isoenzymes of lipoxygenase was revealed by several of these studies [1]. Christopher *et al.* [6] isolated and purified two isoenzymes which they named lipoxygenase-1 and -2. Lipoxygenase-1, which they characterized, exhibits a pH optimum of 9.0 and lipoxygenase-2 an optimum of 6.5.

Allen *et al.* [7] succeeded in purifying lipoxygenase by affinity chromatography. We have previously described an affinity chromatography procedure for the purification of soybean lipoxygenases after preliminary ion exchange chromatography [8]. In this study, a rapid affinity chromatography procedure for the purification of soybean lipoxygenase-2 is described.

RESULTS AND DISCUSSION

A typical chromatographic elution of the soybean preparation on a linoleyl aminoethyl Sepharose column is presented in Fig. 1. Lipoxygenase activity appeared in two peaks. The first one (A) was eluted with 50 mM NaOAc buffer and the second one (B) with 200 mM of the same buffer. The specific activity of peak B, based on diene formation from linoleate, was significantly higher than that of peak A. Similar data were obtained when linoleate oxidation was followed polarographically. The specific activities found for peaks A and B at pH 6.5 were 37.5 and 84.3 μl O_2 absorbed/mg protein, respectively. The overall purification from the starting preparation was 5.0-fold for peak A and 11.2-fold for peak B. When the activity of peak B was measured at pH 9.0, the specific activity found was 202.3 μl O_2 absorbed/mg protein and the overall purification obtained was 26.9.

Figure 2 shows the electrophoretic patterns in polyacrylamide gel after affinity chromatography. It can be seen that while peak B (200 mM) contained at least 2–3 proteins, peak A (50 mM) had only one band and is assumed to be a single protein.

Since peak A was found to be homogeneous it was further characterized and identified as lipoxygenase-2 based on its pH optimum, substrate specificity and calcium activation. This purified isoenzyme exhibited a pH optimum of 6.5 both on linoleate and methyl linoleate as substrates with an activity range from 5.0 to 8.0 while peak B exhibited a pH optimum about 9.0 on linoleate with a range from 4.5 to 10.0. Throughout

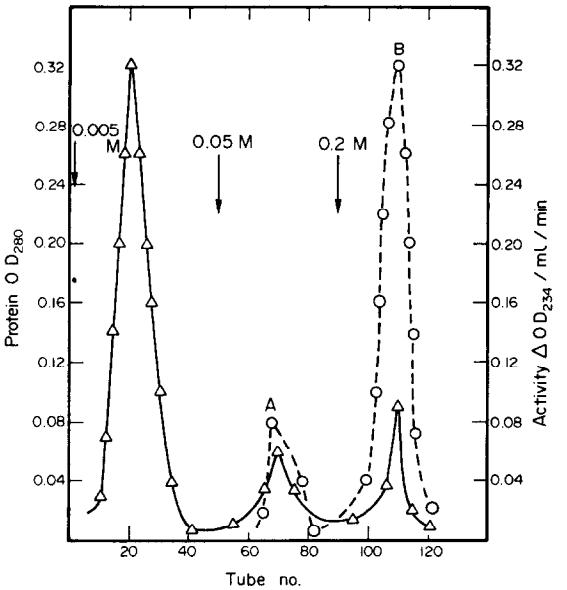

Fig. 1. Affinity chromatography elution pattern of soybean lipoxygenase. Legend: Δ, *A* at 280 nm; ○, enzyme activity as measured by *A* at 234 nm of the product. Elutent: acetate buffer, pH 5.0.

*In partial fulfilment of a thesis for the M.Sc. degree, 1980.

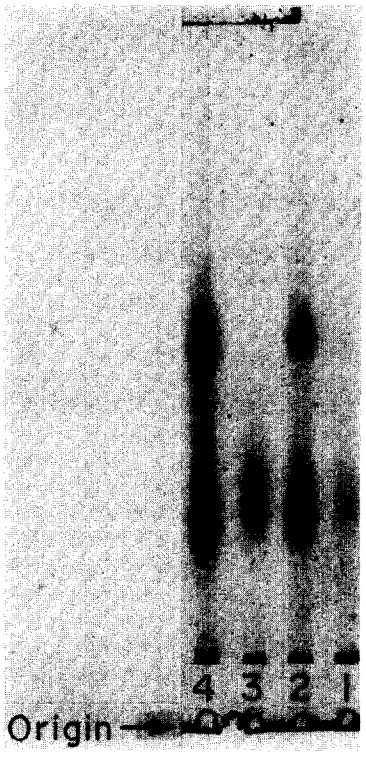

Fig. 2. Electrophoretic diagram of the eluate from affinity chromatography. The electrophoresis was on 10% polyacrylamide gel in 4 mM Tris–glycine buffer, pH 8.9. Staining was with Coomassie blue. 1—10 μl 0.05 M eluate (isoenzyme 2). 2—10 μl 0.2 M eluate (isoenzyme 1). 3—20 μl 0.05 M eluate (isoenzyme 2). 4—20 μl 0.2 M eluate (isoenzyme 1).

the whole range, peak B was more active towards linoleic acid than was lipoxygenase-2 (peak A). However, when methyl linoleate was used, the purified isoenzyme (lipoxygenase-2) proved to be more active than peak B throughout the same range. The specific activity of lipoxygenase-2 on methyl linoleate was about 1.7-fold higher than on linoleic acid. These data are in agreement with the properties of soybean lipoxygenase-2 described in the literature as to the substrate specificity and pH profile of this isoenzyme [6, 9].

It is now well established in the literature that linoleate oxidation by lipoxygenase-2 is activated by calcium ions [10]. In the present study we found that linoleate oxidation by the purified lipoxygenase-2 was increased by 208% when Ca^{2+} was added in a concentration of 3 mM and by 416% when the concentration of Ca^{2+} was elevated up to 5 mM Ca^{2+}. When methyl linoleate was the substrate, or when Tween 20 was used in preparing the substrate, no Ca^{2+} effect was noted; similarly no calcium effect was noted for peak B. The K_m of this lipoxygenase-2 on linoleic acid at pH 6.5 was 5.5×10^{-4} M.

The affinity procedure described here for the isolation of soybean lipoxygenase-2 is fairly rapid and simple when compared to the procedures described in the literature for the isolation of this isoenzyme [1]. An attempt to purify this isoenzyme by affinity chromatography under different conditions has been reported by Allen *et al.* [7], but whereas this group succeeded in purifying lipoxygenase-1, the results obtained with lipoxygenase-2 were unsatisfactory.

In this study, it was shown again that a linoleyl aminoethyl Sepharose column reveals high affinity toward lipoxygenases at pH 5.0 in low ionic strength medium. Moreover, there is an outstanding difference between the extent of affinity of lipoxygenase-1 and -2. Based on its low affinity to linoleic acid under the condition described, it is possible to elute most of this isoenzyme by 50 mM NaOAc buffer at 5.0. Due to their low K_m on linoleic acid, the other isoenzymes remain bound to the substrate. The method described here can be easily used for an intensive study of lipoxygenase-2.

EXPERIMENTAL

Activity determination. Determination of lipoxygenase activity by use of an oxygen monitor was according to Grossman *et al.* [11]. The spectrophotometric determination of the product at 234 nm was according to Ben-Aziz *et al.* [12].

Protein determination of the fractions isolated was according to Lowry *et al.* [13] or by absorption at 280 nm.

Homogenate preparation. Phosphate buffer pH 6.5 (0.005 M, 50 ml) was added to 10 g ground soybeans. The suspension was mixed with the aid of a magnetic stirrer for 30 min at 4° and then centrifuged at 30000 *g* for 10 min. The clear supernatant was brought to pH 4.5 with 1 N HCl and the precipitated proteins were separated by centrifuging at 30 000 *g* for 10 min. The pH of the supernatant was then brought to 5.5 with 1 N NaOH and dialysed against hourly changes of 5 mM acetate buffer, pH 5.0, for 5 hr at 4°, the mixing being done with a magnetic stirrer. No precipitates appeared. The resulting clear dialysate was further purified by affinity chromatography as described below.

Affinity chromatography. Linoleyl aminoethyl Sepharose (25 × 60 mm) equilibrated with 5 mM NaOAc buffer, pH 5.0, at 4°. After 20 min of incubation the column was washed with the equilibrating buffer. Peak A (lipoxygenase 2) eluted. Peak B eluted with 200 mM acetate at pH 5.0.

Electrophoresis. Electrophoresis was on 10% polyacrylamide gel according to Davis [14] on the apparatus perfected by Avtalion [15]. The run was in 4 mM Tris–glycine buffer pH 8.9 at 100 V and 50 mA. Bromophenol blue dissolved in glycerin served as marker. The run took 4.5 hr at room temp. Staining was with Coomassie blue.

REFERENCES

1. Eskin, N. A. M., Grossman, S. and Pinsky, A. (1977) *Crit. Rev. Food Sci. Nutrit.* **9**, 1.
2. Ho, P. P. K., Walts, C. P. and Sullivan, H. R. (1977) *Biochem. Biophys. Res. Commun.* **76**, 398.
3. Halagk, W., Schewe, T., Hiebsch, Ch. and Rapoport, S. (1977) *Acta Biol. Med. Ger.* **36**, 405.
4. Nugteren, D. H. (1975) *Biochim. Biophys. Acta* **380**, 299.
5. Shahin, I., Grossman, S. and Sredni, B. (1978) *Biochim. Biophys. Acta* **529**, 300.
6. Christopher, J., Pistorius, E. and Axelrod, B. (1970) *Biochim. Biophys. Acta* **198**, 12.
7. Allen, J. C., Eriksson, C. and Galpin, R. J. (1977) *Eur. J. Biochem.* **73**, 171.
8. Grossman, S., Trop, M., Yaroni, S. and Wilchek, M. (1972) *Biochim. Biophys. Acta* **289**, 77.

9. Yamamoto, A., Yasumoto, K. and Mitsuda, H. (1970) *Agric. Biol. Chem.* **34**, 1169.
10. Zimmerman, G. L. and Snyder, H. (1974) *J. Agric. Food Chem.* **5**, 802.
11. Grossman, S., Ben-Aziz, A., Budowski, P., Ascarelli, I., Birk, Y. and Bondi, A. (1969) *Phytochemistry* **8**, 2287.
12. Ben-Aziz, A., Grossman, S., Ascarelli, I. and Budowski, P. (1970) *Analyt. Biochem.* **34**, 88.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
14. Davis, B. I. and Orstein, L. (1968) In *Methods in Immunology and Immunochemistry* (Williams, C. A. and Chase, W. H., eds.), Vol. 2, p. 48. Academic Press, New York.
15. Avtalion, R. R. Israel Pat. Nos. 34815, 31 July 1973, and No. 42829, 31 December 1976.