Application of Affinity Chromatography to Lipoxygenase†

Jiro SEKIYA, Tadahiko KAJIWARA
and Akikazu HATANAKA

Department of Agricultural Chemistry, Faculty of
Agriculture, Yamaguchi University,
Yamaguchi 753

Received October 31, 1977

Lipoxygenase (linoleate: C_2 oxidoreductase, E. C. 1.13.1.13) catalyzes the oxidation of C_{18} -unsaturated fatty acids with *cis*-1, *cis*-4-pentadiene system in the presence of molecular oxygen to give hydroperoxide.¹⁾ Rapid methods for purification of lipoxygenase are desired to investigate properties and physiological role of the enzyme. Grossman *et al.* reported affinity chromatography of soybean lipoxygenase.²⁾ Recently, Allen *et al.* reported a practical method for affinity chromatography of lipoxygenases.³⁾ The present note describes conditions for affinity chromatography of lipoxygenase.

Soybean lipoxygenase commercially obtained from Sigma Chemical Co. (type I) was used as an enzyme. CN-agarose was prepared from Sepharose 4B (Pharmacia Fine Chemicals) and CNBr as described.⁴⁾ 6-Hydroxyhexyl-agarose was prepared from CN-agarose and 6-aminohexanol, and aminoethyl-agarose from CN-agarose and ethylenediamine.⁴⁾ Oleyl-agarose and linoleyl-agarose were obtained by the coupling of aminoethyl-agarose with oleic acid and linoleic acid, respectively, in the presence of 1-ethyl-3 (3-dimethylaminopropyl)-carbodiimide.^{2,4)} Lipoxygenase activity was determined at 25°C polarographically with a Clark oxygen electrode as described previously.⁵⁾ Standard reaction mixture (3 ml) contained linoleic acid (1 μ mole) enzyme solution (0.1 ml) and 50 mM pyrophosphate buffer, pH 8.5. Protein was determined from the absorbance at 280 nm ($E_{1\text{cm}}^{1\%}$: 13.0). Analytical disc electrophoresis was the method described by Davis.⁶⁾

Hexanol⁷⁾ or oleic acid⁸⁾ used as a ligand has been reported to be an inhibitor of lipoxygenase and linoleic acid is a substrate. More than 90% of soybean lipoxygenase was adsorbed on 6-hydroxyhexyl-agarose or on oleyl-agarose in 10 mM Tris-HCl buffer (pH 8.5) and completely eluted with 0.1 M NaCl in the same buffer. On linoleyl-agarose, about 30% of lipoxygenase was found in passed fraction and 50~60% of the activity was eluted with 0.1 M NaCl in 10 mM Tris-HCl buffer,

† This paper has been presented at the Annual Meeting of Agricultural Chemical Society of Japan, Yokohama, April 2, 1977.

FIG. 1. Affinity Chromatography of Soybean Lipoxygenase on Linoleyl-agarose.

Five mg of soybean lipoxygenase (specific activity 7.2 units/mg protein) was applied on a linoleyl-agarose column (bed volume 5 ml), washed with 10 mM Tris-HCl buffer and then eluted with 0.1 M NaCl in the same buffer (indicated by allow). (●—●) shows enzyme activity and (-----), OD_{280} .

FIG. 2. Disc Electrophoresis of Soybean Lipoxygenase Purified by Affinity Chromatography.

(a) shows soybean lipoxygenase before affinity chromatography. (b), (c) and (d) indicate lipoxygenase eluted with 0.1 M NaCl in 10 mM Tris-HCl buffer (pH 8.5) from 6-hydroxyhexyl-, oleyl- and linoleyl-agarose, respectively.

pH 8.5 (Fig. 1). No further lipoxygenase was eluted with NaCl in the concentration higher than 0.1 M in each case. In the buffer system such as 10 mM acetate buffer (pH 5.0) and 10 mM phosphate buffer (pH 6.8) lipoxygenase could not bind to a ligand and enzyme activity was found in passed fraction. Thus, soybean lipoxygenase (type 1) is adsorbed and eluted in the buffer system at pH 8.5 for each substituted-agarose. These results are agreeable with that obtained by Allen *et al.*³⁾ but not by Grossman *et al.*²⁾ For potato lipoxygenase (optimal pH 6.0~6.3),⁵⁾ buffer system at pH 6.3 gave a good result (unpublished data). It appears that the buffer system around optimal pH is favorable. Figure 2 shows the result of disc electrophoresis of lipoxygenase eluted from substituted-agarose column.

TABLE I. EFFECT OF REPEATED RUNS OF LINOLEYL-AGAROSE ON THE RECOVERY AND PURIFICATION OF SOYBEAN LIPOXYGENASE

The same linoleyl-agarose column was used through run 1 to 6. The total recovery means the protein and enzyme activity recovered in unadsorbed and 0.1 M NaCl-eluted fraction.

Run	Total recovery (%)		0.1 M NaCl fraction			
	Protein	Enzyme activity	Recovery (%)		Specific activity (U/mg protein)	Enrichment (Fold)
			Protein	Enzyme activity		
1	70	115	23	57	21.6	3.3
2	84	119	31	64	25.9	5.1
3	59	83	20	48	29.9	3.6
4	46	97	14	47	29.9	3.7
5	60	111	18	59	26.0	3.8
6	49	108	19	63	29.3	3.4

Lipoxygenase before affinity chromatography was composed of 3 major bands and, at least, 3 minor bands. However, lipoxygenase eluted from linoleyl-agarose was purified to a single protein electrophoretically. Oleyl-agarose and 6-hydroxyhexyl-agarose did not give sufficient results electrophoretically, though the value of 2.7 for enrichment was obtained. When linoleyl-agarose is used under the above condition, there is a possibility that the conversion of linoleyl moiety as a ligand to its hydroperoxide causes the reduction of adsorption capacity. But Table I shows no reduction of adsorption capacity and purification occurred during several repeated runs. Each lipoxygenase purified in run 1 to 6 was composed of a single protein electrophoretically. These indicate either no conversion occurs or hydroperoxide, if formed, acts as a ligand as well as linoleyl moiety. From the above results, linoleyl-agarose with 10 mM Tris-HCl buffer system at pH 8.5 resulted in the best purification of soybean lipoxygenase.

We thank Mr. Akira Kitamura for his skilled technical assistance.

REFERENCES

- 1) G. A. Veldink, J. F. G. Vliegenthart and J. Boldingh, *Prog. Chem. Fats Other. Lipids*, **15**, 131 (1977).
- 2) S. Grossman, M. Trop, S. Yaroni and M. Wilchek, *Biochim. Biophys. Acta*, **289**, 77 (1972).
- 3) J. C. Allen, C. Eriksson and J. R. Galpin, *Eur. J. Biochem.*, **73**, 171 (1977).
- 4) P. Cuatrecasas, *J. Biol. Chem.*, **245**, 3059 (1970).
- 5) J. Sekiya, H. Aoshima, T. Kajiwarra, T. Togo and A. Hatanaka, *Agric. Biol. Chem.*, **41**, 827 (1977).
- 6) B. J. Davis, *Ann. N. Y. Acad. Sci.*, **121**, 404 (1964).
- 7) H. Mitsuda, K. Yasumoto and A. Yamamoto, *Arch. Biochem. Biophys.*, **118**, 664 (1967).
- 8) D. T. Downing, D. G. Ahren and M. Bachta, *Biochem. Biophys. Res. Commun.*, **40**, 218 (1970).