Preliminary Kinetic Studies on the Esteratic and Lipolytic Components of a Commercial Wheat Germ Lipase

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Commercial wheat germ lipase was fractionated by gel filtration and ion-exchange chromatography. The activity of isolated fractions was tested with *p*-nitrophenyl acetate (PNPA) and dimercaptopropanol (DMP) tributyrate as substrate. Three active components were obtained: an esterase catalyzing only the hydrolysis of PNPA, a lipase catalyzing the hydrolysis of DMP tributyrate and a nonspecific esterase catalyzing the hydrolysis of both PNPA and DMP tributyrate. PNPA hydrolysis by the esterase fraction obeyed Michaelis−Menten kinetics. The nonspecific esterase fraction and the commercial enzyme preparation yielded nonlinear Lineweaver−Burk plots. The commercial preparation yielded a linear Lineweaver−Burk plot with DMP tributyrate as substrate, indicating that nonspecific esterase and lipase components had similar K_m values for the lipid substrate. However, the components had different heat stabilities as evidenced by nonlinear heat inactivation curves.

Keywords: *Wheat germ; lipase; fractionation; kinetics*

INTRODUCTION

Wheat germ lipase catalyzes the hydrolysis of simple esters, triglycerides, and water-soluble long-chain fatty acid esters of sorbitan (Singer and Hofstee, 1948a,b; Fink and Hay, 1969). Stauffer and Glass (1966) described the presence of three distinct esterolytic activities in aqueous extracts of wheat germ: a lipase, a tributyrinase, and an esterase.

Although wheat germ lipases are commercially available, none of the preparations have been purified to homogeneity. Therefore, the molecular and catalytic properties of the component enzymes are not known (Mounter and Mounter, 1962; Pancholy and Lynd, 1972).

In this paper, the substrate-dependent kinetic behavior of a commercial preparation of wheat germ lipase was determined. The preparation was then fractionated by ion-exchange chromatography (IEC), and kinetic studies were done on the fractions to define the distinguishing features of the active components.

MATERIALS AND METHODS

Lipase [triacylglycerol acyl-hydrolase (EC.3.1.1.3) from wheat germ, type I; specific activity, 7 units/mg of solid] was obtained from Sigma Chemical Co. 2,3-Dimercaptopropan-1-ol (DMP) tributyrate and *p*-nitrophenyl acetate (PNPA) were also purchased from Sigma. DMP tributyrate stock solutions were prepared fresh in distilled ethanol. Stock solutions of PNPA were prepared in acetonitrile.

Activity Measurements. Activity was measured at 25 °C, in 100 mM Tris-HCl buffer, pH 8.5, containing 5% v/v acetonitrile (when PNPA was used as substrate) or 5% ethanol and 0.3 mM DTNB [when DMP tributyrate was used as substrate (Kurooka et al., 1976)]. The hydrolytic release of *p*-nitrophenolate ion was monitored via the change in absorbance at 405 nm; the production of thionitrobenzoate dianion was monitored at 412 nm.

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The molar extinction coefficient for *p*-nitrophenolate ion was found to be 13 360 M^{-1} cm^{-1}, which is in a good agreement with the value obtained by Albro et al. (1976). For thionitrobenzoate dianion, ϵ was taken as 14 200 M^{-1} cm^{-1} (Riddles et al., 1979).

Fractionation of Lipase Preparations. The commercial enzyme was further purified by (a) gel permeation on Sephadex G-100 (0.9 × 26 cm column) equilibrated with 25 mM potassium phosphate buffer, pH 7.5, and (b) IEC on cellulose phosphate (1.4 × 20 cm column) equilibrated with 25 mM acetate buffer, pH 4.5, and eluted with a 100 mL linear gradient of 0−0.5 M NaCl. Fractions containing maximum specific activity were combined and used for kinetic studies after overnight dialysis against Tris-HCl buffer.

Protein Measurements. Protein concentration was measured at 280 nm.

Heat Inactivation. The commercial enzyme was incubated in 100 mM Tris-HCl buffer, pH 8.5, at 40 and 50 °C. After appropriate times of preincubation, the samples were rapidly cooled and the precipitates removed by centrifugation. Residual activity in the supernatant was assayed by using DMP tributyrate as substrate.

RESULTS AND DISCUSSION

Commercial wheat germ lipase was found to catalyze the hydrolysis of both PNPA and DMP tributyrate, with a much higher activity toward PNPA than toward DMP tributyrate.

A nonlinear Lineweaver−Burk plot was obtained with PNPA as substrate (Figure 1), indicating the presence of at least two esterases with low and high K_m. K_{m1} and K_{m2} were estimated as 17 μM and 2.3 mM and V_{max1} and V_{max2} as 17 and 37 μM/min, respectively. It appears that the enzyme with lower V_{max} has the lower K_m, and the reciprocal plot bends downward close to the vertical axis due to the contribution of the high-K_m enzyme to the total velocity at high substrate concentrations.

When the substrate was DMP tributyrate, the Lineweaver−Burk plot obtained was linear (Figure 2), suggesting a single enzymatic component. The kinetic parameters K_m and V_{max} were estimated as 6.7 μM and 3.5 μM/min, respectively. The Hill coefficient was unity at 25 °C (Figure 3). On the other hand, heat inactiva-

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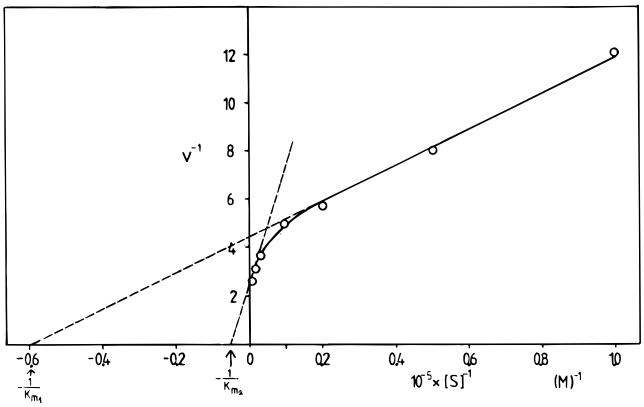

Figure 1. Lineweaver−Burk plot for PNPA. Enzyme, 0.5 mg/mL (v in units of ΔA_{405}/min).

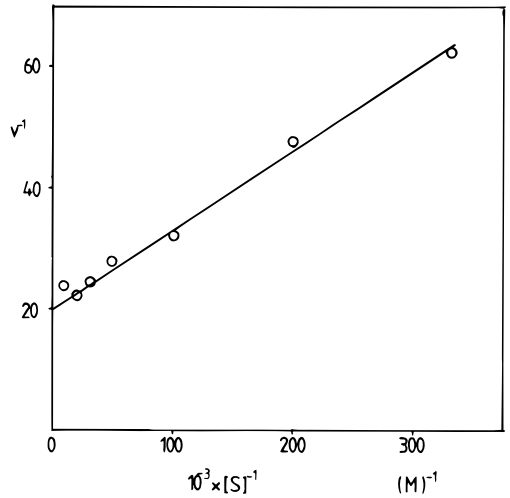

Figure 2. Lineweaver−Burk plot for DMP tributyrate hydrolysis. Enzyme, 2 mg/mL (v in units of ΔA_{412}/min).

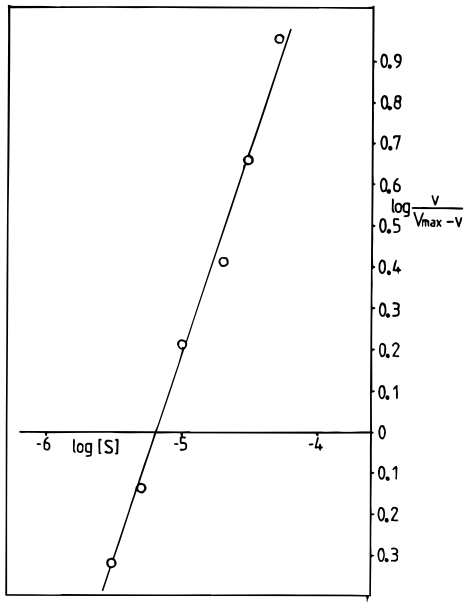

Figure 3. Hill plot for DMP tributyrate hydrolysis.

tion at 40 and 50 °C did not follow first-order kinetics (Figure 4). The observed curvature during heat inactivation gave evidence for the presence of more than one enzyme activity. A probable explanation for the linearity observed in Lineweaver−Burk plot is that the different enzymic activities have similar K_m values for DMP tributyrate.

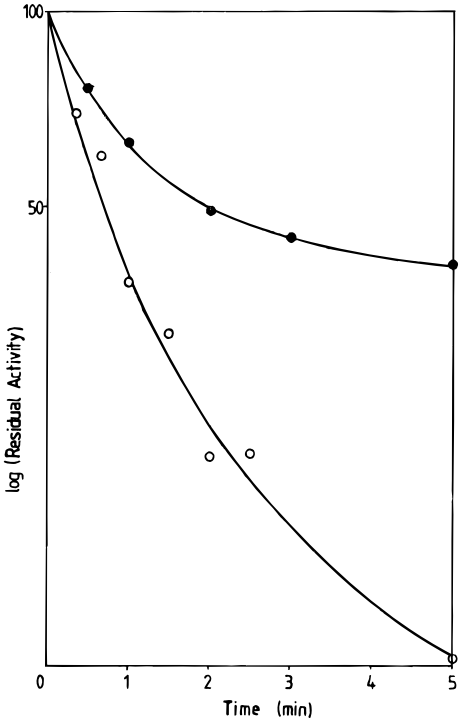

Figure 4. Heat inactivation of DMP tributyrate hydrolyzing activity. Preincubation was at (●) 40 °C and (○) 50 °C. Residual activity was assayed at 50 μM DMP tributyrate.

Table 1. Purification of Wheat Germ Lipase

	total activity[a]	
purification step	PNPA	DMP-tributyrate
commercial enzyme	202	3.2
GPC	137	2.3
IEC		
pool 22−26	70	0.38
pool 31−32		0.26
pool 40−44	45.5	

[a] Total activity expressed as micromoles of PNPA and DMP-tributyrate hydrolyzed per hour at pH 8.5 and 25 °C.

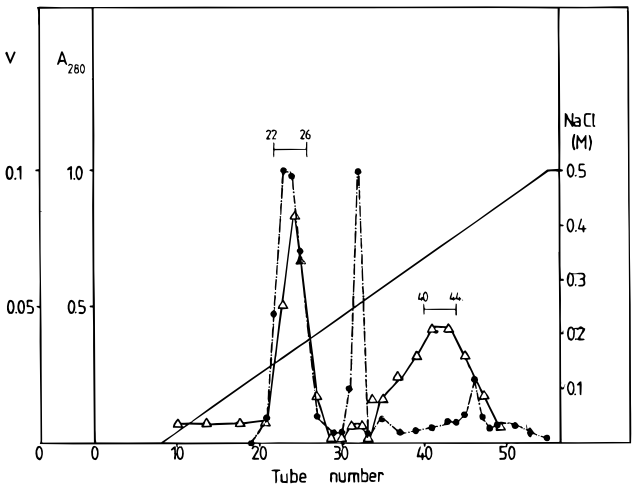

Figure 5. IEC of the pool from gel filtration on cellulose phosphate: (●) absorbance at 280 nm; (△) activity with 1 mM PNPA (v in units of ΔA_{405}/min).

Due to the observed heterogeneity in the commercial wheat germ lipase, further purification was required for the separation of active fractions. Two-fold purification was achieved by gel permeation chromatography (GPC) using Sephadex G-100 (Table 1). The commercial enzyme and the pooled enzyme preparation had identi-

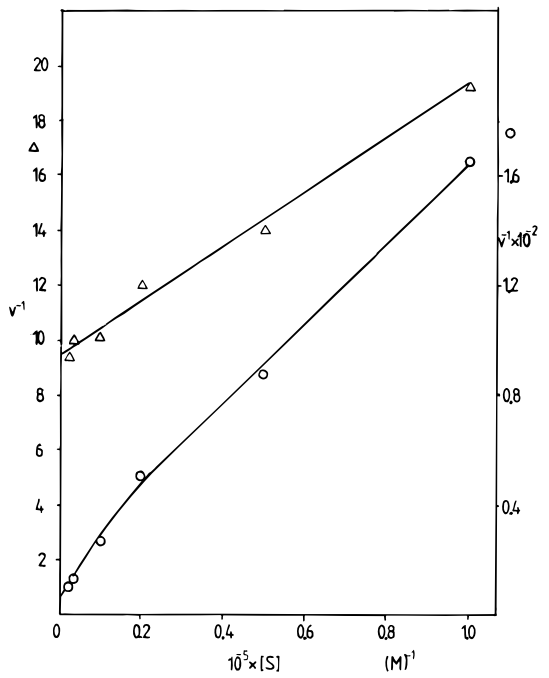

Figure 6. Lineweaver−Burk plots for PNPA hydrolysis by pooled fractions from IEC: (○) pooled fractions 22−26; (△) pooled fractions 40−44.

cal PNPA/DMP tributyrate activity ratios. About 33% of the protein (in A_{280} units) and 70% of the activity against PNPA and DMP tributyrate were recovered in the pooled preparation.

IEC of the pool from GPC yielded three active fractions (Figure 5). The first component (fractions 22−26) catalyzed the hydrolysis of both PNPA and DMP tributyrate; the second component (fractions 31−32) catalyzed only the hydrolysis of DMP tributyrate and the third component (fractions 40−44) catalyzed the hydrolysis of PNPA.

The hydrolysis of PNPA was studied in the fractionated enzymes. The kinetic behaviors of these enzymes were quite different (Figure 6). The first enzyme fraction (22−26) gave a nonlinear Lineweaver−Burk plot. The deviation from linearity is most likely to have arisen from the presence of more than one enzymic species. However, it is also possible that activity is due to a single, multisite enzyme, either showing negative cooperativity or having more than one class of site with different substrate binding affinities. These alternatives remain to be evaluated. The third enzyme fraction (40−44) gave a linear double-reciprocal plot with no evidence for multiple species. The K_m was estimated as 10 μM.

In conclusion, commercial wheat germ lipase preparation used in this study contains at least three fractions that may be resolved by IEC and that differ from one another with respect to substrate specificity. The first fraction, which hydrolyzes both PNPA and DMP tributyrate, may be heterogeneous and contain further components. Further study is needed to characterize these components regarding structure and catalytic specificity.

ABBREVIATIONS USED

DMP, 2,3-dimercaptopropan-1-ol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); GPC, gel permeation chromatography; IEC, ion-exchange chromatography; PNPA, *p*-nitrophenyl acetate.

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