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SHORT COMMUNICATION

CHARACTERIZATION OF WHEAT GERM LIPASE*

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Abstract—Some properties of wheat germ lipase were determined with a fluorometric assay of enzymatic cleavage converting the nonfluorescent 4-methyl umbelliferone butyrate (4-MUB) to the highly fluorescent 4-methyl umbelliferone (4-MU). Optimum reaction conditions were attained at buffer pH 7·5 and temperature 30°. Lineweaver–Burk plots were linear. Relative cation combination effectiveness as reaction activators was Ca + Mg + K > Ca + Mg + K + Na > Ca + Mg + Na > Ca + Mg > Mg > Ca, with no reaction effects of K, Na, and K + Na without Ca or Mg. Highly significant inhibitors of lipase reaction were CN^-, aflatoxin, Cu^{2+}, Fe^{3+}, S^{2-}, and EDTA.

INTRODUCTION

LIPASE activity has conventionally been determined with controlled incubation procedures utilizing triglyceride substrates with measurement of the fatty acid product or the cumulative glycerol formation. Although many other procedures have also been developed to estimate the hydrolytic activity of carboxylesterases such as measurement of released gas volumes and rate of pH change, fluorometric methods are generally several orders of magnitude more sensitive than chromogenic procedures.[1]

Jacks and Kircher[2] and Guilbault *et al.*[3] have described a simple, rapid and accurate procedure for the specific assay of lipase in the presence of the other esterases based on the hydrolysis of 4-methyl umbelliferone butyrate.

This procedure measures the change in substrate concentration directly and in addition, allows continuous monitoring of the reaction. Ca^{2+} and Mg^{2+} have been considered as activators[4] and EDTA and Cu^{2+} as inhibitors of lipase when determined by hydrolysis of oils.[5] The purpose of this study was to determine the optimum conditions for the wheat germ lipase-MUB reaction and to evaluate some specific activators and inhibitors of this reaction.

RESULTS AND DISCUSSION

The increase in fluorescence on addition of the enzyme was rapid and linear for 3 min. Guilbault *et al.* also reported the reaction to be linear for 3 min.

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[1] G. G. GUILBAULT and M. H. SADAR, *Analyt. Chem.* **41**, 366 (1969).
[2] T. J. JACKS and H. W. KIRCHER, *Anal. Biochem.* **21**, 279 (1967).
[3] G. G. GUILBAULT, M. H. SADAR and D. ARCENAUX, *Anal. Letters* **1**, 551 (1968).
[4] P. DESNUELLE, M. NAUDET and M. J. CONSTANTIN, *Biochem. Biophys. Acta.* **5**, 561 (1950).
[5] P. DESNUELLE, L. SARDA and G. AILHAUD, *Biochem. Biophys. Acta.* **37**, 570 (1960).

A plot of enzyme concentration versus activity for a fixed substrate concentration should be linear if the assay procedure is accurately measuring the results of enzyme action.[6] Fluorescence increased linearly with increase of lipase with $f(x) = 8{\cdot}018$ and a highly significant correlation coefficient. Wheat germ lipase at a concentration of $3{\cdot}30 \times 10^{-3}$ IU/ml was found to be optimal, and was used throughout this study.

Two additional criteria for determining if a procedure actually measures enzyme activity are linearity in a Lineweaver–Burk plot and consistent values for K_m with varying enzyme concentrations.[7] With five levels of wheat germ lipase and 4-methyl umbelliferone butyrate substrate, consistent values of K_m were obtained from Lineweaver–Burk plots, the K_m being $3{\cdot}1 \times 10^{-5}$ M. K_m values obtained by Guilbault *et al.* for fluorescein dibutyrate, *N*-methyl indoxyl butyrate, 4-methyl umbelliferone heptanoate and 4-methyl umbelliferone octanoate were respectively, 7×10^{-6}, $2{\cdot}9 \times 10^{-5}$, $7{\cdot}3 \times 10^{-6}$ and $8{\cdot}0 \times 10^{-6}$ M using porcine pancreatic lipase.

Enzymes are greatly influenced by pH and are only active within a limited pH range. In this study it was found that the optimal pH for the wheat germ lipase-4MUB reaction was 7·5. Troller *et al.* in their study of a *Staphylococcus* lipase found the optimal pH to be 7·5.[8] Fink and Koehler have suggested a pH range of 6–8.[9]

The effects of temperature on most enzyme systems are usually complex with extremes of temperature retarding the enzyme reaction; the optimum temperature for this assay was found to be 30°. This confirmed results obtained by Troller *et al.*[8] who found the optimum temperature for purified lipase to be 30–32°.

While the activity of some enzymes is not noticeably affected by the presence of salts, others are greatly influenced by the nature and concentration of the ions present. Among the cations used during this study, Ca^{2+} and Mg^{2+} significantly increased the activity of the lipase-MUB reaction (Table 1). Zn^{2+}, Pb^{2+}, Hg^{+}, Ag^{+}, Al^{3+}, Fe^{2+}, Co^{2+} and Mn^{2+} as acetate salts at 0·1 M did not have any significant effect on the rate of reaction. K^{+} and Na^{+} used singly were ineffective, but when combined with Ca^{2+} or Mg^{2+} increased the activity of lipase-MUB reaction. Combination of cations were also highly significant, particularly Ca + Mg + K, Ca + Mg + Na and Ca + Mg + K + Na. Ca^{2+} has been considered as a potential activator of lipase (Table 1). Troller *et al.*[8] also noted that the pure lipase activity increased by the addition of 10^{-3} and 10^{-4} M of Ca^{2+}. They further reported that Mg^{2+} produced similar results. Addition of NaCl to the reaction mixture produced a measurable decline in the hydrolysis of the tributyrin.

Six inhibitors, aflatoxin, EDTA, Cu^{2+}, Fe^{2+}, CN^{-} and S^{2-} gave a significant fluorescence inhibition (Table 1). Aflatoxin inhibited the reaction almost 87% whereas sodium sulfide inhibited only 25%. Troller *et al.*[8] have reported that the sulfhydryl group inhibitor, PCMB, does not produce inhibition of tributyrinase activity at concentrations at which it might be expected to be an inhibitor. Black *et al.*[10] observed that the development of gibberellic acid-induced lipase activity of germinating cotton seedlings could be completely inhibited with aflatoxin at 45 μg/ml.[10] These authors suggested that lipase activity in the germinating cotton seed is related to DNA-dependent RNA synthesis. Singer[11] reported

[6] M. DIXON and E. C. WEBB, in *Enzymes*, p. 10, Academic Press, New York (1959).
[7] R. S. WARREN and D. G. ROUTLEY, *Phytochem.* **9**, 311 (1970).
[8] J. A. TROLLER and M. A. BOZEMAN, *App. Microb.* **20**, 480 (1970).
[9] D. W. FINK and W. R. KOEHLER, *Analyt. Chem.* **42**, 990 (1970).
[10] H. S. BLACK and A. M. ALTSCHUL, *Biochem. Biophys. Res. Commun.* **19**, 661 (1965).
[11] T. P. SINGER, *J. Biol. Chem.* **174**, 11 (1948).

TABLE 1. RELATIVE ACTIVATOR AND INHIBITOR EFFECTIVENESS FOR ENZYME INDUCED FLUORESCENCE OF 4-MUB BY WHEAT GERM LIPASE

(Ca + Mg + K = 100)

Cation activators	Concentration	Ra	Inhibitors	Concentration	Ri
Ca		45	Cupric acetate	0·1 M	59
Mg		57	Ferric chloride	0·1 M	78
Ca + Mg		45	Sodium cyanide	0·1 M	55
Ca + Mg + Na		97	Sodium sulfide	0·1 M	25
Ca + Mg + K		100	EDTA	0·1 M	57
Ca + Mg + Na + K		93	Aflatoxin	120 μg	87

$$\%\ \text{Activation (Ra) or } \%\ \text{Inhibition (Ri)} = \frac{(\triangle F/\text{min})_{\text{blank}} - (\triangle F/\text{min})_{\text{i or a}}}{(\triangle F/\text{min})_{\text{blank}}} \times 100$$

inhibition by sulphydryl groups on lipase activity. Cu^{2+} and CN^- are biological poisons and may be expected to inhibit the enzyme reaction, while Fe^{3+} and EDTA may cause inactivation due to chelation.

EXPERIMENTAL

Wheat germ lipase having an activity of 0·1 IU/mg (Nutritional Biochemicals) was used. Enzyme solution ($3{\cdot}3 \times 10^{-4}$ IU in 0·1 ml) was mixed with 3·0 ml 0·1 M NaH_2PO_4 (adjusted to pH 7·5 with NaOH) in the fluorometer cell. After correcting the fluorometer scale to zero, 0·1 ml of 4-MUB in ethylene glycol monoethyl ether, was mixed and the rate of fluorescence increase was recorded for 3 min. Fluorescence was determined at 340 nm excitation and 450 nm emitted between cross polarizing lens.[12] Michaelis plot and Lineweaver–Burk determination of K_m were made using five different 4-MUB levels. In detecting the effects of activators and inhibitors the salt and lipase were thoroughly mixed for 5 min before adding 4-MUB.

[12] R. F. CHEN and R. L. BOWMAN, *Science* **174**, 729 (1965).

Key Word Index—Triticum sativum; Gramineae; wheat; lipase.