Phosphate-Inhibition of Lipase Activity in Peanuts

ABSTRACT

Competitive inhibition of lipase activity in extracts of germinating peanuts occurred in media containing inorganic phosphate in concentrations above 10 mM. Lipase activity in germinating castor, cotton, and pumkin seeds and in quiescent castor seed was not similarly affected. Applications of this finding to peanut processing are suggested.

INTRODUCTION

In our earlier study of the localization of lipase activity in subcellular fractions from germinating peanuts (1), we observed that rates of lipolysis were lower in buffers that contained phosphate than corresponding rates in phosphate-free buffers of identical pH values. Results from examining this phenomenon are presented in this communication and are interpreted as the competitive inhibition of peanut lipase by inorganic phosphate.

EXPERIMENTAL PROCEDURES

Preparations of lipase from peanut seedlings, grown as described previously (1), were obrained as mitochondrial fractions in 0.25 M sucrose and as cell-free, liquid supernatants from centrifugations of homogenized tissues (tissue-water, 1:3 w/w) at 1600 g for 10 min (1). Hydrolytic activity was estimated by determining the production of fatty acids from emulsified triglycerides (1) and by measuring the fluorescence produced by hydrolysis of 4-methylumbelliferone (4-MU) from its heptanoyl derivative (2).

The effect of inorganic phosphate on the rate of hydrolysis of triglyceride catalyzed by peanut lipase was examined by including various amounts of phosphate in enzymic reaction mixtures. Results presented in Figure 1 show that the release of fatty acids from triglycerides decreased with increasing amounts of inorganic phosphate in each reaction medium. Inhibition was essentially complete at 50 mM phosphate.

To ascertain the type of inhibition of lipolysis produced by inorganic phosphate, rates of enzymic hydrolysis of heptanoyl 4-MU in the presence and absence of phosphate were measured as functions of substrate concentrations, when substrate concentrations were rate-limiting. Results shown in Figure 2 indicate that inorganic phosphate is a competitive inhibitor of peanut lipase. The apparent K_M was 4.3 x 10^{-6} M, and apparent K_i was 1.4 x 10^{-2} M. Results from rates of hydrolysis with twice the concentration of phosphate as that shown in Figure 2 essentially agreed with this latter value.

The effect of inorganic phosphate on lipolysis of storage tissues in other oilseeds also was examined. Lipase activities in preparations from 5 day old castor, cotton, and pumpkin seedlings and in quiescent castor seeds were not affected by up to 240 μmoles inorganic phosphate in reaction media during enzymic hydrolyses of heptanoyl 4-MU.

RESULTS AND DISCUSSION

Since somewhat large amounts of inorganic phosphate were required to inhibit lipase activity in peanut seedlings, the physiologic significance of this inhibition is uncertain. However, our results clearly show that uses of phosphate buffer in homogenizing and isolating media for tissue fractionation affect lipolytic activity of the fractions, depending upon the source of the tissue.

In addition, these findings suggest that processing peanuts into oil and protein with aqueous solvents (3-5) might be aided by use of phosphate in the solvents. In this manner, formation of free fatty acids by endogenous lipase

[1] ARS, USDA.

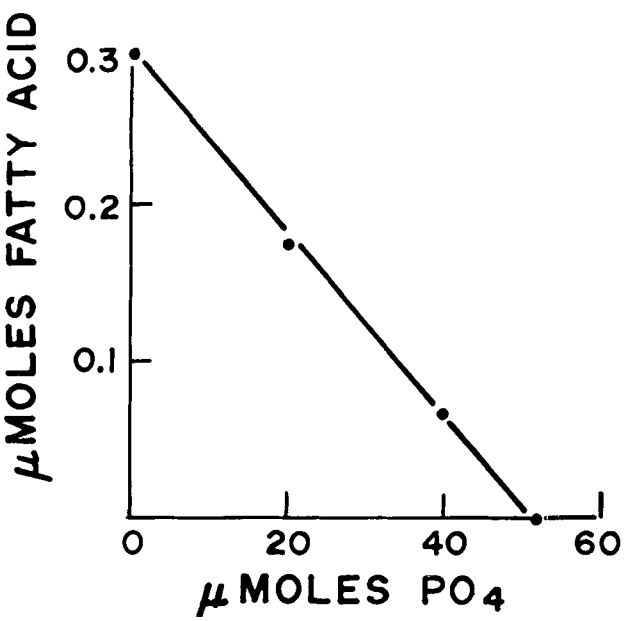

FIG. 1. Effect of inorganic phosphate on the enzymic hydrolysis of triglyceride. Each reaction mixture (1 ml) contained 40 μmoles acetate buffer (pH 5.2), 0.08% Poly-Tergent J-300 (nonionic detergent), 2% winterized cottonseed oil, 0-52 μmoles sodium orthophosphate, and mitochondrial fraction from 10 day old peanut seedlings. Oil was emulsified with detergent before addition to reaction mixtures. Rates of hydrolysis during 20 min of incubation at 25 C were determined by measuring the increases in contents of fatty acids in the enzymic reaction mixtures (1).

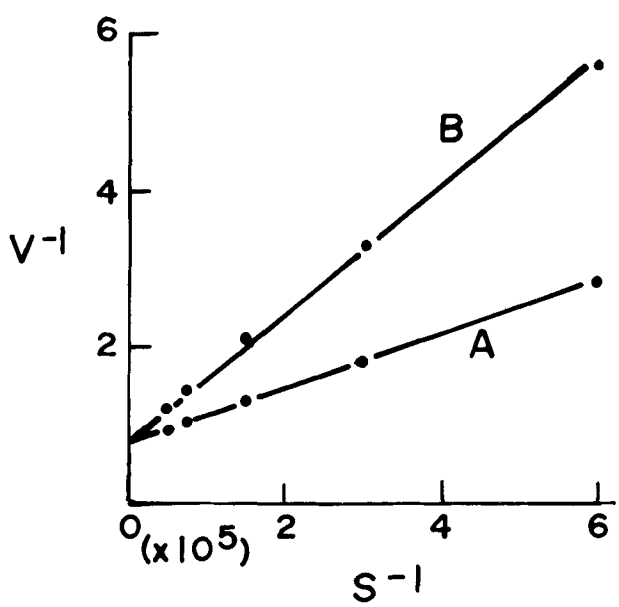

FIG. 2. Lineweaver-Burk plots of the rates of enzymic hydrolyses of heptanoyl 4-methylumbelliferone in the presence and absence of phosphate. Each reaction mixture (6 ml) contained 200 μmoles acetate buffer (pH 4.6), 0 (plot A) or 120 (plot B) μmoles of sodium orthophosphate (pH 4.6), cell-free homogenate of cotyledons from 7 day old peanut seedlings, and 0.01-0.12 μmoles substrate. Initial rates of hydrolysis at 26 C were determined spectrofluorometrically (2).

would be prevented. Prevention of the formation of free fatty acids in raw peanuts and comminuted meats during storage by the appropriate addition of inorganic phosphate may be of future interest.

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