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Intestinal phosphatidate phosphatase*

Recent evidence has accumulated that the mechanism for fatty acid absorption and its conversion into triglycerides in the intestinal mucosa is quite similar to the system proposed for liver triglycerides as suggested by KENNEDY[1]. Labeled phosphatidic acids have been isolated from the intestinal mucosa following the incubation of segments of this tissue with labeled inorganic phosphate or ^{14}C-labeled fatty acids[2]. The enzymes involved in this process and in this tissue have not been fully investigated with the exceptions of the demonstrated presence of thiokinase by SENIOR AND ISSELBACHER[3] and the failure to find the enzyme glycerolkinase as reported by BRUELL AND RISER[4]. L-α-Phosphatidate phosphohydrolase (phosphatidate phosphatase, EC 3.1.3.4), an enzyme involved in these reactions, was first described by SMITH *et al.*[5] and its distribution in a variety of tissues, as well as the specificity, was examined. These investigators did not report on the occurrence of this enzyme in the intestinal mucosa. In addition, when the specificity of this enzyme was examined, utilizing such substrates as phosphatidyl choline, only the liberation of inorganic phosphate was determined. If the enzyme displays a similar action on this substrate as on phosphatidic acids, the expected product would be phosphorylcholine which would not be determined in the analysis for inorganic phosphate. It is the purpose of this paper to describe the presence of this enzyme and its intracellular distribution in intestinal tissues. In addition, the specificity of this enzyme has been examined not only from the aspect of inorganic phosphate liberation but also with regard to the production of certain phosphorylated bases.

Phosphatidic acids were isolated by the method of CHIBNALL AND CHANNON[6]. Phosphatidic acids were synthesized according to the procedure of WAGNER-JAUREGG AND ARNOLD[7]. The lysophosphatidic acid was prepared by the method of KABASHIMA[8]. Phosphatidyl choline was isolated by the method of HANNAHAN, TURNER AND JAKO[9]. Phosphatidyl ethanolamine, phosphorylethanolamine, phosphorylcholine, and α-glycerophosphate were obtained from commercial sources and their purity checked by chromatography.

* Previously referred to as phosphatidic acid phosphatase.

The enzyme was obtained from the intestinal mucosa of male golden hamsters in the following manner. The animals were killed by a blow on the head and a cannula inserted near the gastroduodenal junction. The intestine was then cut at the iliocecal junction and approx. 75 ml of cold isotonic saline allowed to perfuse through the small intestine. The intestine was then removed from the animal by stripping off the mesentery, cut longitudinally, and the mucosa scrapped with aid of Stadie blade and weighed. The mucosal scrappings were homogenized in 9 vol. of 0.25 *M* sucrose containing 1 m*M* EDTA. The intracellular fractions were obtained by differential centrifugation according to the procedure of SCHNEIDER AND HOGEBOOM[10]. The pH-5 fraction was separated by adjusting the remaining supernatant fluid to pH 5 with acetate, after the microsomal fraction had been removed, followed by centrifugation at 104000 × *g* for 1 h. The contents of the incubation flasks are given in Table I.

Inorganic phosphate was determined in a trichloroacetic acid filtrate by the method of KOSHLAND AND CLARK[11]. The presence of phosphorylated bases of choline and ethanolamine were detected by the chromatographic method of DAWSON[12].

As can be seen in Table I, the phosphatidate phosphatase activity was confined primarily to the mitochondrial and microsomal fractions.

When the mitochondrial fraction was incubated with a series of substrates under the conditions indicated in Table II, and the incubation mixture analyzed for P_i, only phosphatidic acid and lysophosphatidic acid were found to be suitable substrates.

TABLE I

DISTRIBUTION OF PHOSPHATIDATE PHOSPHATASE IN SUBCELLULAR FRACTIONS OF INTESTINAL MUCOSA

Each fraction contained the subcellular constituents obtained from 0.75 g of mucosal scrappings incubated with 3.4 μmoles of phosphatidic acids in 0.05 *M* maleate buffer (pH 6.2) containing 1 m*M* EDTA for 1 h at 37°.

Subcellular fraction	*μmoles of P_i formed*	*% conversion of phosphatidic acid*
Mitochondria	1.5	44
Microsomes	1.3	38
pH-5 fraction	0.7	21
Supernatant fluid	< 0.2	< 6

TABLE II

SPECIFICITY OF INTESTINAL PHOSPHATIDATE PHOSPHATASE

Mitochondrial fraction obtained from 200 mg mucosal scrappings incubated with 3.5 μmoles of the indicated substrate in 0.05 *M* maleate buffer (pH 6.2) containing 1 m*M* EDTA for 1 h at 37°

Substrate	*μmoles of P_i formed*	*% conversion to P_i*
α-Glycerophosphate	0.063	1.7
Phosphatidyl choline	0.021	0.6
Phosphorylcholine	0.085	2.4
Phosphatidyl ethanolamine	0.00	0.0
Phosphorylethanolamine	0.00	0.0
Phosphatidic acid (cabbage)	1.23	36.1
Phosphatidic acid (synthetic)	1.65	46.5
Lysophosphatidic acid	1.41	39.7

The values were corrected for blanks which were incubated with each substrate under identical conditions except that the enzyme was inactivated by heating for 10 min at 100° prior to the incubation. In addition, the flasks containing phosphatidyl choline and phosphatidyl ethanolamine were examined for the production of phosphorylcholine and phosphorylethanolamine. Under conditions of the incubation, no phosphorylcholine or phosphorylethanolamine was produced from these two phosphatides when examined by the method of DAWSON[12]. This finding would indicate a rather marked specificity for the enzyme(s).

From the reported result as well as those previously published by SMITH *et al.* it would appear that the enzyme(s) has at least two requirements for activity. First of all the presence of at least one acyl group on glycerophosphate is necessary since α-glycerophosphate is not a suitable substrate. Secondly, it would appear that a monosubstituted phosphate is required since phosphatidyl choline and phosphatidyl ethanolamine are both inactive.

The demonstration of the presence of rather specific phosphatidate phosphatase in the intestinal mucosa provides additional evidence for the occurrence of the suggested sequence of reactions involving phosphatidic acids in the intestinal absorption of fatty acids[13].

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Determination of glycerol in phosphatides

Our laboratory's need for a simple, yet sensitive and accurate method for determination of glycerol in phospholipids prompted the development of the method reported here. The method is based on a complete, single-stage acid hydrolysis of phosphatides under such conditions that no destruction of glycerol takes place. The free glycerol is subsequently determined by HIO_4 oxidation and spectrophotometric