LYSOLECITHINASE ACTIVITY IN SUBCELLULAR FRACTIONS OF RAT ORGANS

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Summary: Lysolecithinase activity was measured in subcellular fractions of rat liver, kidney, lung and intestine and compared to similar findings in brain. To obtain optimal assay conditions, each fraction was subjected to a kinetic analysis in the absence and presence of albumin. Among the particulate preparations, lysolecithinase activity of the intestine exceeded by far similar fractions of other organs. Among the soluble fractions, the 100,000xg supernatant of lung had the highest activity. Under the assay conditions used, most of the lysolecithinase activity of all organs was particulate.

The activity of lysolecithinase (lysolecithin acyl hydrolase EC 3.1.1.5), an enzyme which is widespread in nature, was determined in a variety of organisms (summarized in (1)). The distribution of this enzyme in rat organs was also studied (2, 3, 4). Several investigators reported that the enzyme was inhibited by its substrate (2, 4-8, summarized in 9). This substrate inhibition by lysolecithin, which belongs to the class of soluble, amphiphilic lipids, resulted in non-hyperbolic, biphasic V/S curves (7). Using rat brain Leibovitz-Ben Gershon et al. (7, 10) showed that, because of the substrate inhibition, the specific activities of subcellular fractions of this tissue were not fixed values, but varied markedly, depending on the concentrations of the substrate, enzyme or an added protein such as albumin. Therefore, in order to obtain optimal activities, the various subcellular fractions had to be assayed under differing conditions. "Irregular" kinetics was also obtained using purified

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enzyme preparations (11). These observations on rat brain warranted a comparable investigation of lysolecithinase activity of other organs of the rat, as well as a reevaluation of the data reported in the literature. In this paper we investigated lysolecithinase activity of homogenates and subcellular fractions of several rat organs. The effects of substrate concentration and of serum albumin on the kinetics of lysolecithin hydrolysis of these fractions were studied. The results were used to assess optimal conditions of assay and specific activity values for each fraction.

EXPERIMENTAL PROCEDURE

Subcellular fractionation. Rats were killed by cervical dislocation, the organs were removed and chilled in 0.32 M sucrose. Each organ, except intestine, was homogenized in 9 volumes of 0.32 M sucrose and the subcellular fractions were prepared by differential centrifugation, as follows: Fraction 1, debris which sediments at 800 xg for 10 min; Fraction 2, particles which sediment by subsequent centrifugation at 8400 xg, for 10 min; Fraction 3, particles which sediment by subsequent centrifugation at 35,000 xg for 15 min; Fraction 4, microsomes which sediment by subsequent centrifugation at 100,000 xg for 60 min; Fraction 5, the supernatant of the above. Each of the sediments was suspended in 0.32 M sucrose and stored at -20°. The intestinal homogenate was prepared according to the procedure of Epstein and Shapiro (12) with the following two modifications: Microsomes were sedimented at 100,000 xg and the sediments of all fractions were suspended in 0.32 M sucrose. This procedure yields four fractions, debris (fraction 1), particles (fraction 3), microsomes (fraction 4) and supernatant (fraction 5).

Assay of the reaction: Incubation mixtures, in volumes of 0.2 ml each, contained 15 μmoles of Tris-HCl, pH 8.1, 1-[9, 10-$^3H_2$] palmitoyl-sn-glycero-3-phosphoryl choline, enzyme and sucrose. In those experiments in which albumin was added, its concentration equalled half the molar concentration of the lysolecithin. The mixtures were incubated at 37° for 15 min and the radioactivity of the released fatty acid was determined (13). A control tube without enzyme was added to each experiment. In those experiments which measured the effect of substrate concentration on the reaction rates, a control tube without enzyme was used for each concentration of lysolecithin.

MATERIALS AND METHODS. Tritium-labelled lysolecithin was prepared from livers of rats which had been injected with [9, 10 $^3H_2$] palmitic acid (13). The specific activity of the substrate was 3.8 x 10^6 dpm/μmole. Albumin was Cohn's fraction V, fatty acid poor (Pentex). Protein content was determined according to the procedure of Lowry et al. (14) and phosphorus according to the procedure of Bartlett (15). One unit is defined as that amount which released 1 nmole of fatty acid in 1 hr.

RESULTS. Subcellular fractions of several organs of adult rats were prepared as described in Methods. To determine the optimal conditions for measuring lysolecithinase activity of the individual organs and fractions, the rate of hydrolysis of lysolecithin was measured as a function of substrate concentration, at a fixed concentration of enzyme (v vs. S). This was then followed by similar experiments in which the concentration of the subcellular fraction was varied, at a fixed concentration of substrate (v vs. E).

Fig. 1A shows that, in accord with results which had been obtained with particulate preparations of rat brain (7) the v vs. S curves of similar preparations of liver, kidney, intestine and lung were biphasic. The concentration of substrate at which the maximal hydrolysis occurred depended on enzyme concentration when using microsomes of liver, kidney and lung but not intestine. It was shown previously that addition of albumin to the incubation mixtures increased the rates of hydrolysis of lysolecithin by particulate preparations of rat brain and converted the biphasic v vs. S curves to rectangular hyperbolas (7). Fig. 1B shows a similar effect of albumin on the microsomal enzyme of liver and kidney; the optimal effect of this protein was observed at an albumin to lysolecithin molar ratio of 0.5. This was however not true for particulate preparations of intestine and pancreas which were inhibited by albumin. Using lung microsomes, the effect was variable. The activity of the microsomal enzyme of lungs of some animals was increased while that of others was decreased in the presence of albumin.

Fig. 2 shows v vs. S curves of 100,000 xg supernatants of four rat organs. Most curves were not hyperbolic but showed a discontinuity above a certain concentration of the substrate; the same was true using the soluble fraction of rat brain (7). Addition of albumin decreased the reaction rate of soluble lysolecithinase of all rat organs tested; albumin was therefore not added to the assay sys-

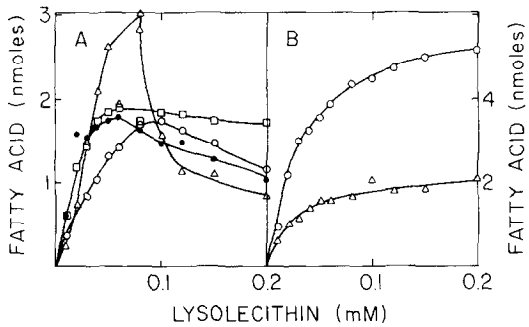

Fig. 1: Effect of substrate concentration on the hydrolysis of lysolecithin by microsomal preparations of rat organs. Standard assay conditions were used. Fig. 1A - no albumin was added. △ kidney (61μg), ● intestine (0.4μg), ○ liver (108μg), □ lung (31μg). Fig. 1B - albumin was added, at half the molar concentration of the substrate. △ kidney (6μg), ○ liver (108μg).

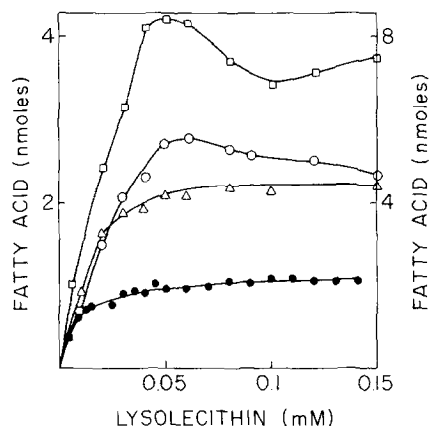

Fig. 2: Effect of substrate concentration on the hydrolysis of lysolecithin by soluble fractions of rat organs. Standard assay conditions were used, albumin was not added.
● intestine (72.5μg), □ lung (14μg), △ kidney (154μg), ○ liver (133μg, right ordinate).

tem in which the soluble preparations were used.

To determine lysolecithinase activity of the subcellular fractions of rat organs (Table I), particulate fractions of rat liver and kidney and lung were assayed in the presence of albumin, while similar fractions of intestine as well as all 100,000 xg supernatants were assayed in the absence of albumin. Each value in the Table

Table I: Lysolecithinase Activity in Subcellular Fractions of Rat Organs.

Subcellular fractions were prepared and the enzymatic activity was determined as described under Experimental Procedure. Lysolecithin concentration - 0.15 mM. 0.075 mM albumin was present in each reaction mixture, except in those specified in the footnote to the table.

	Total Activity (u/g)	Specific activity of subcellular fraction (u/mg protein)			
		Heavy particles	Light particles	microsomes	supernatant[1]
Liver	23780	120	460	195	70
Kidney	41976	300	800	750	40
Lung	37800	90	340	700	740
Intestine[2]	412086		15880[4]	20300	550
Brain[3]	17147		776	2117	66

1. All the 100,000 supernatants were assayed without albumin. 2. All intestinal fractions were assayed without albumin. 3. Values for brain are derived from a previous publication (7). 4. This fraction contained both heavy and light particles.

was derived from a curve which describes the rate of hydrolysis of lysolecithin, at 0.15 mM lysolecithin (and 0.075 mM albumin, when this protein was added to the reaction mixture) as a function of concentration of the respective fraction. The rate of hydrolysis of lysolecithin was directly proportional to the concentration of respective fractions.

Table I shows that the activity of the particulate fraction of intestine exceeded that of all other fractions many-fold. Among the other organs, liver showed the lowest while brain had the highest specific activities. The 100,000 xg supernatants of all organs had lower specific activities than the corresponding particulate preparations. Lung was an exception in that the specific activity of the supernatant equalled or exceeded that of the particulate preparations of these organs.

Stability of the enzyme preparations. The 100,000 xg supernatants retained almost full activity when stored for at least 4-7 months at -20°. In contrast, the micro-

somal preparations were not stable when stored in isotonic sucrose at -20°C. The preparation from liver retained about 25% of its activity after 4 months and that from lung retained 30% after one month and 20% after 2 months; the preparations from kidney and intestine lost practically all their activity within 2 months.

DISCUSSION: This paper shows considerable variability in the effects of enzyme, substrate or albumin on the hydrolysis of lysolecithin by subcellular fractions of several rat organs. The properties of the soluble lysolecithinase differed markedly from that of the particulate fractions. Furthermore, particulate fractions of different organs also showed considerable variability. The kinetic data of this and of former papers (7,10) could be used to analyze the above as well as variable and frequently conflicting reports found in the literature (2-6). De Jong et al. (11) purified two lysolecithinases from liver which resemble in some of their properties those of the particulate and soluble enzymes of rat brain (7,16). It is thus possible that more than one enzyme with lysolecithinase activity is present in each mammalian organ and that this contributes to the varying kinetic behaviour of the subcellular fractions. Furthermore, some of the enzyme may be solubilized thereby changing both the kinetic properties (4,7) and the relative activities of the soluble enzyme.

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