STIMULATION OF TRIGLYCERIDE SYNTHESIS IN MAMMALIAN LIVER AND ADIPOSE TISSUE BY TWO CYTOSOLIC COMPOUNDS*

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SUMMARY

Two cytosolic compounds which stimulate neutral glyceride synthesis catalyzed by microsomal enzymes have been isolated from both rat liver and adipose tissue. In their absence, diacyl-sn-glycero-3-phosphate was the major product. They were separated from each other by Sephadex G-100 chromatography; in the case of each tissue, one had a molecular weight greater than 150,000, while the other was in the range 8,000-16,000. Both were inactivated by trypsin and the smaller one lost only 25% of its activity after incubation at pH 8.3, 90°, one hour. While Mg^{2+} also increased neutral glyceride synthesis, the cytosolic factors were active under optimal concentrations of these ions. These compounds may play a critical role in the regulation of neutral glyceride synthesis.

The regulation of glycerolipid biosynthesis is poorly understood. A stimulatory effect of the cytosol (100,000 X g supernatant fraction) on triglyceride synthesis catalyzed by membranous enzymes present in mammalian adipose tissue, liver and small intestine has been reported (1-3). The nature of the compound(s) responsible for the effect of the cytosolic fraction has been investigated and is the subject of this report.

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MATERIALS AND METHODS

Materials. Palmitic acid, rac-glycerophosphate, ATP (Na salt), CoA (Li salt), horse muscle myoglobin (Type I) (Sigma), lipid standards (Sigma and Serdary Research Laboratories), [U-^{14}C]-sn-glycero-3-phosphate (120 Ci/mol) (New England Nuclear), Blue Dextran 2,000 and Sephadex G-100 (fine) (Pharmacia) were purchased from the designated sources.

Methods. Livers and epididymal fat pads were obtained from fasted-refed (48-48 hours) male albino Wistar rats. At the time of sacrifice, they weighed 190-230 g. Homogenization was carried out at 0-4° in 0.005 M ammonium acetate adjusted to pH 8.3 with NH_4OH. In some experiments, the homogenizing solution consisted of 0.01 M potassium phosphate, pH 7.0, 0.15 M KCl, 0.25 M sucrose and 0.01 M 2-mercaptoethanol. Livers were homogenized with a Waring Blendor, while epididymal fat pads were homogenized with a motor-driven Potter-Elvehjem apparatus (Teflon pestle).

In the case of each tissue, after initial centrifugation at 15,000 X g for 20 min, the microsome-rich and the cytosolic (supernate) fractions were obtained at 100,000 X g for 60 min (all centrifugations at 0-4°).

Incubations were carried out using optimal concentrations of known substrates (sn-glycero-3-phosphate and potassium palmitate) and cosubstrates (ATP and CoA) as previously reported (1) except for the fact that the radioactive substrate was [U-^{14}C]-sn-glycero-3-phosphate (1 µCi per assay), the concentration of potassium phosphate was 0.1 M, 2-mercaptoethanol (0.01 M) was used and, unless otherwise specified, the concentration of $MgCl_2$ was 0.2 mM, in a total volume of 0.5 ml. Microsome-rich fractions of epididymal adipose tissue or liver were used in the presence or absence of adipose tissue or liver cytosol or partially purified fractions of the latter. Incubations were conducted at 25° for 15 min.

Lipid extraction was carried out by the method of Bligh and Dyer (4) using methanol which contained 0.01 M rac-glycerophosphate and 0.1 N HCl instead of water. The lipids were separated by thin-layer chromatography on Silica Gel H as reported (1). Fifty µg of authentic lipid were used as "carrier" for each glycerolipid formed. After visualization with I_2, the Silica containing the various lipid fractions was scraped and the radioactivity counted in a Nuclear-Chicago Mark II liquid scintillation spectrometer model 6847 using Bray's mixture.

Protein concentrations were determined by a microbiuret procedure (5). Magnesium concentrations were determined by atomic absorption spectrometry (6).

The liver cytosolic factors were resolved by chromatography on 2.5 X 60 cm Sephadex G-100 (fine) columns (Glenco Scientific, Inc.) equilibrated and eluted with 0.005 M ammonium acetate, pH 8.3, at 0-4°. Appropriate eluate fractions were concentrated by rotary evaporation prior to assay.

RESULTS

The effect of the cytosolic fraction on the composition of glycerolipids formed by microsomal enzymes is illustrated in Fig. 1. Various combinations of liver or adipose tissue microsome-rich and cytosolic fractions were used. Under the experi-

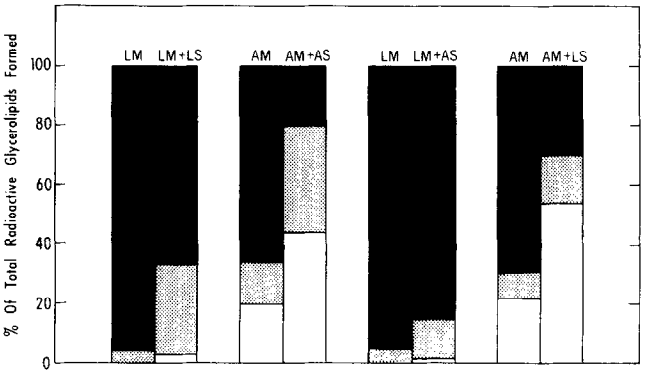

Fig. 1. Effects of liver or adipose tissue cytosol on glycerolipid synthesis catalyzed by liver or adipose tissue microsome-rich fractions. In the appropriate incubation mixtures, the following quantities of protein were used: liver cytosol (LS) 5.0 mg, adipose tissue cytosol (AS) 3.0 mg, liver microsome-rich fraction (LM) 0.8 mg and adipose tissue microsome-rich fraction (AM) 1.0 mg. ■ – 1,2-diacyl-sn-glycero-3-phosphate, ⊡ – diglyceride, □ – triglyceride.

mental conditions used in this study, the liver microsomal enzymes catalyzed predominantly the synthesis of 1,2-diacyl-sn-glycero-3-phosphate. Diglyceride constituted 4% of the total radioactive products, while triglyceride synthesis was negligible. The liver and adipose tissue cytosolic fraction resulted in an approximately 7- and 3-fold increase, respectively, in the diglyceride fraction.

In the case of the adipose tissue microsome-rich fraction, diacyl-sn-glycero-3-phosphate was also the major glycerolipid formed, but triglyceride constituted about 20% of the radioactive products (Fig. 1). Both adipose tissue and liver cytosol resulted in appreciable enhancement of both the diglyceride and triglyceride fraction and the latter became the major glycerolipid formed.

The resolution of two factors derived from liver cytosol and capable of enhancing neutral glyceride synthesis catalyzed by adipose tissue microsomal enzymes is illustrated in Fig. 2. The

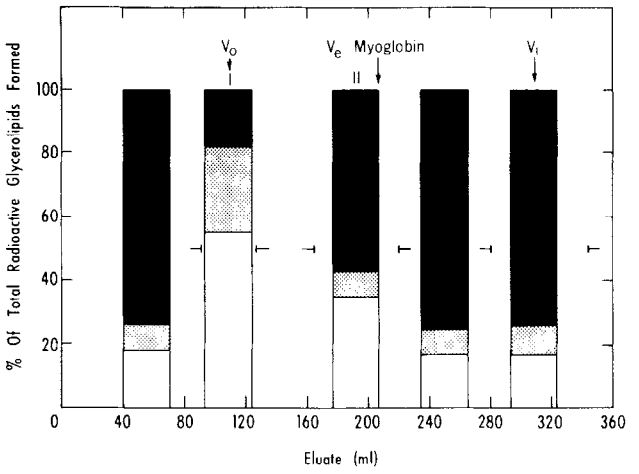

Fig. 2. Resolution of liver cytosolic factors which stimulate neutral glyceride synthesis by Sephadex G-100 chromatography. One ml of concentrated cytosol was applied onto a 2.5 X 60 cm column equilibrated and eluted with 0.005 M ammonium acetate, pH 8.3, at 4°. The fractions of the eluate between the horizontal T symbols were pooled and concentrated. In separate assays, the partially purified factors I and II (20 and 11 mg of total protein, respectively) were tested in the presence of 0.3 mg of adipose tissue microsomal protein. V_o-column exclusion volume, V_e myoglobin-elution volume of myoglobin added as a marker, V_i-internal column volume ("salt fraction"). ■ – 1,2-diacyl-sn-glycero-3-phosphate, ▩ – diglyceride, □ – triglyceride.

column eluting solution was used as control; in its presence, the quantity and glycerolipid composition were similar to those obtained with the "salt fraction" (V_i) portion of the eluate. Fig. 2 illustrates that two distinct regions of the eluate reproducibly enhanced neutral glyceride synthesis. The column exclusion volume (V_o) contained a factor(s) which will be referred to as factor I; it enhanced the proportion of both diglyceride and triglyceride formed by the microsome-rich fraction (Fig. 2). A smaller factor(s) (will be referred to as factor II) resulted in an appreciable increment in the triglyceride fraction, but had no effect on the proportion of diglyceride. The effect of each factor was reproduced in 30 experiments (Fig. 2).

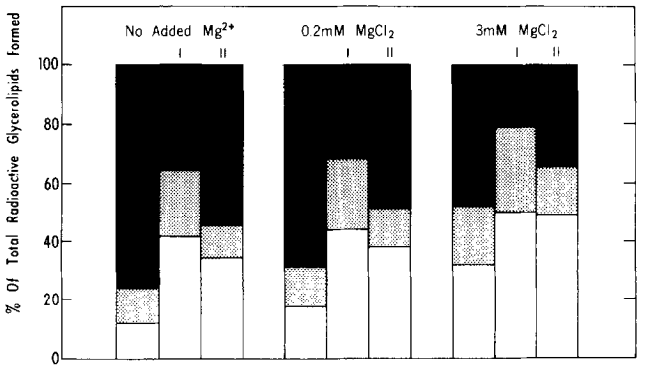

Fig. 3. Effects of liver cytosolic factors on glycerolipid synthesis catalyzed by adipose tissue microsome-rich fractions in the presence of different concentrations of added Mg^{2+}. After resolution by Sephadex G-100 chromatography, the partially purified factors I and II (34 and 15 mg of total protein, respectively) were assayed in the presence of 0.3 mg of adipose tissue microsomal protein. ■ – 1,2-diacyl-sn-glycero-3-phosphate, ▩ – diglyceride, □ – triglyceride.

The molecular weight of the larger compound was greater than 150,000, while that of the smaller one was in the range 8,000-16,000.

The effects of liver cytosolic factors I and II on the composition of glycerolipids formed by the adipose tissue microsome-rich fraction in the presence of different concentrations of added $MgCl_2$ is illustrated in Fig. 3. Three mM $MgCl_2$ resulted by itself in an increase in the fraction of newly formed diglyceride and triglyceride. The effect of each cytosolic factor persisted in the presence of $MgCl_2$ concentrations which were required for maximal neutral glyceride synthesis; when 10 mM $MgCl_2$ was used, the results were the same as those shown for 3 mM. The total endogenous magnesium concentration associated with the Sephadex G-100 eluate containing either factor I or II was in the range 0.3-0.4 meq/liter. When an equimolar concentration of $MnCl_2$ was substituted for 0.2 mM $MgCl_2$, each cytosolic compound resulted in a similar relative increment in the neutral glyce-

ride fraction. Each factor was inactivated by trypsin; addition of soybean trypsin inhibitor prior to incubation with membranous enzymes, substrates and cosubstrates prevented any further effect of the protease.

Liver cytosolic factor I was inactivated by preincubation in 0.005 M ammonium acetate, pH 8.3, at 60° for 1 hour; factor II was stable under these conditions and lost only 25% of its activity when preincubated at 90° for 1 hour.

DISCUSSION

The current study has demonstrated the presence of two rat liver cytosolic compounds which stimulate neutral glyceride synthesis catalyzed by membranous liver and adipose tissue enzymes. These compounds, which are at least partly protein in nature, are similar or identical to the two cytosolic adipocyte factors previously presented by this laboratory (7).

Earlier reports have ascribed the effect of the cytosolic fraction to a "soluble phosphatidate phosphohydrolase" (EC 3.1.3.4) (2, 3, 8). The properties of the smaller compound, such as its heat stability, indicate that it is not an enzyme, but an activator of catalytic activity. The precise nature of the larger factor remains to be elucidated. The relationship of these compounds to the liver cytosolic factor reported to be required for triglyceride synthesis from diglyceride, is not clear (9).

It has been reported that in rat adipose tissue Mg^{2+} account for the effect of the cytosolic fraction by activating microsomal phosphatidate phosphohydrolase (10). This study has revealed that, in addition to Mg^{2+}, two larger cytosolic compounds stimulate neutral glyceride synthesis; they are active at saturating concentrations of Mg^{2+}.

Hence, two cytosolic compounds which enhance neutral glyceride synthesis have been isolated from both liver and adipose tissue. They may play a critical role in regulating this biosynthetic process.

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