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ACTIVATION OF CHICKEN ADIPOSE TISSUE DIGLYCERIDE LIPASE BY CYCLIC AMP-DEPENDENT PROTEIN KINASE AND ITS DEACTIVATION BY PURIFIED PROTEIN PHOSPHATASE

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SUMMARY: Diglyceride lipase of chicken adipose tissue was found to be activated by cyclic AMP-dependent protein kinase to the same extent as hormone-sensitive triglyceride lipase (3-to 10-fold) when lipase assays were carried out in buffers of low ionic strength. Sodium phosphate (50 mM) or sodium chloride (100 mM) preferentially enhanced the basal (nonactivated) form of diglyceride lipase, which minimized the apparent activation by protein kinase. The activated diglyceride lipase was readily deactivated by a pure protein phosphatase from bovine heart (MW 35,000) and the deactivated enzyme was then reactivated by protein kinase.

INTRODUCTION

It has been established that lipolytic hormones such as catecholamines, glucagon and ACTH increase rates of fatty acid mobilization from adipose tissue by increasing the activity of hormone-sensitive lipase. The term "hormone-sensitive lipase" has generally been used to refer to the neutral triglyceride lipase. This activity, but not that of diglyceride and monoglyceride lipase activities, was increased in homogenates prepared from adipose tissue previously exposed to lipolytic hormones (1). The neutral triglyceride lipase from the rat (2) and human (3) adipose tissue was clearly activated by cyclic AMP (cAMP)-dependent protein kinase, whereas that against partial glycerides appeared to be unaffected or only minimally increased (3,4). We have recently reported that by assaying at substrate concentrations well below saturation it was possible to obtain a clear-cut protein kinase activation of diglyceride lipase of chicken adipose tissue (5). However, the degree of activation (60 to 80%) was decidedly less than that of the triglyceride lipase (200 to 900%). In this communication, we demonstrate that under appropriate assay conditions diglyceride lipase can be

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activated to the same extent as triglyceride lipase and that it is reversibly deactivated by a pure protein phosphatase from beef heart.

MATERIALS AND METHODS

Adipose tissue from laying hens was homogenized (60 sec at 15°) in 2 volumes of a solution containing 0.25 M sucrose, 1 mM EDTA and 10 mM Tris-HCl, pH 7.4. The pH 5.2 precipitate fraction (designated 5.2 P) was prepared from the 100,000 x g supernatant fraction as described elsewhere (6) and was freed of lipoprotein lipase by treatment with heparin-Sepharose affinity gel (5).

Phosphorylase phosphatase was purified to homogeneity from bovine heart by the method of Brandt et al. (7). On sodium dodecyl sulfate polyacrylamide disc gel electrophoresis, a single protein with a molecular weight of 35,000 was observed. The preparation had a specific activity of 7,900 units/mg protein assayed (7) against phosphorylase a. It is referred to as protein phosphatase because of its broad substrate specificity (8-10). Protein kinase inhibitor was purified from rabbit skeletal muscle through the DEAE-cellulose chromatography step according to the method of Walsh et al. (11). cAMP-dependent protein kinase from bovine heart was purchased from Sigma Chemical Co.

The conditions for activation and deactivation of diglyceride lipase were essentially the same as those described for hormone-sensitive triglyceride lipase (6). The protein kinase activation reaction (in 0.1 ml) was terminated by addition of 0.7 ml of a diolein emulsion containing 0.1 mM [^{14}C] diolein (a mixture of 1,3 and 1,2 isomers), 2 mM EDTA, 5 mg/ml bovine serum albumin and, unless otherwise indicated, 5 mM of sodium phosphate at pH 7.0. After incubation at 30° for 30 min, free [^{14}C] oleic acid was extracted with a mixture of chloroform/methanol/benzene/water at pH 11.5 (5).

RESULTS AND DISCUSSION

The effect of ionic strength on the activated and non-activated forms of diglyceride lipase. In the course of searching for conditions that would optimize the degree of activation of chicken adipose tissue diglyceride lipase by cAMP-dependent protein kinase, we found that the apparent degree of activation obtained was very much dependent on the ionic environment in which the diglyceride lipase activity was assayed. Thus, the apparent activation of diglyceride lipase varied from as little as 10% when assayed in 100 mM sodium phosphate to as much as 1000% when assayed in 0-5 mM sodium phosphate (Fig. 1A). The source of these differences was found to lie in a differential effect of ionic strength on the activity of basal (non-activated) and protein kinase-activated diglyceride lipase. Thus, both sodium phosphate and sodium chloride preferentially activated the basal (non-activated) form of diglyceride lipase, with relatively little effect on the protein kinase-activated form of diglyceride lipase. Similar results were obtained with potassium phosphate or potassium

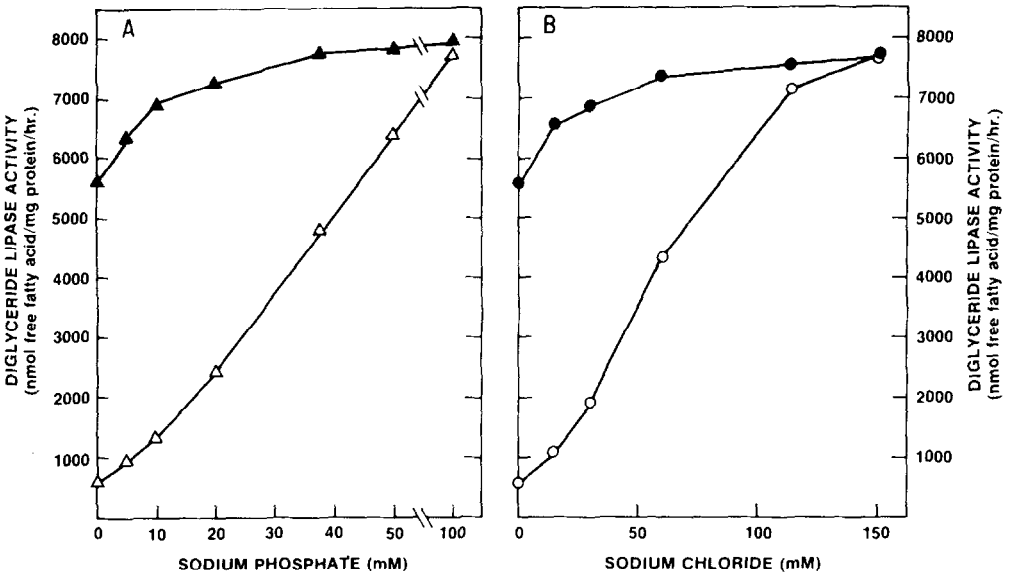

Fig.1 Differential effects of sodium phosphate (A) and sodium chloride (B) on the activated and nonactivated forms of diglyceride lipase. Adipose tissue 5.2 P (150 µg/ml) was incubated with and without additions of 10 µM cAMP, 0.5 mM ATP and 5 mM magnesium acetate for 5 min at 30°. The activated (▲,●) and nonactivated (△,○) forms of diglyceride lipase were measured in a lipid emulsion containing 0.1 mM [^{14}C] diolein, 5 mg/ml bovine serum albumin, 10 mM PIPES (Piperazine-N,N-bis (2-ethane sulfonic acid)) buffer, pH 7.0, and increasing concentrations of sodium phosphate (pH 7.0) as indicated in panel A and sodium chloride in panel B.

chloride, suggesting that the total ionic strength rather than a specific ion effect was the relevant parameter.

The apparent degrees of activation of chicken adipose triglyceride lipase and cholesterol esterase activities were also significantly increased when assays were carried out in buffers of low ionic strength, but the effects were less striking than those observed with diglyceride lipase. The activation of monoglyceride lipase (40-80%) by protein kinase was not influenced by ionic strength. It should be noted that ionic strength over the range used did not affect the protein kinase-catalyzed activation, but only the subsequent lipase activity.

Characterization of the protein kinase activating system. As in the activation of hormone-sensitive triglyceride lipase (6), activation of the diglyceride lipase absolutely required cAMP, Mg^{2+}-ATP and an endogenous cAMP-dependent protein kinase.

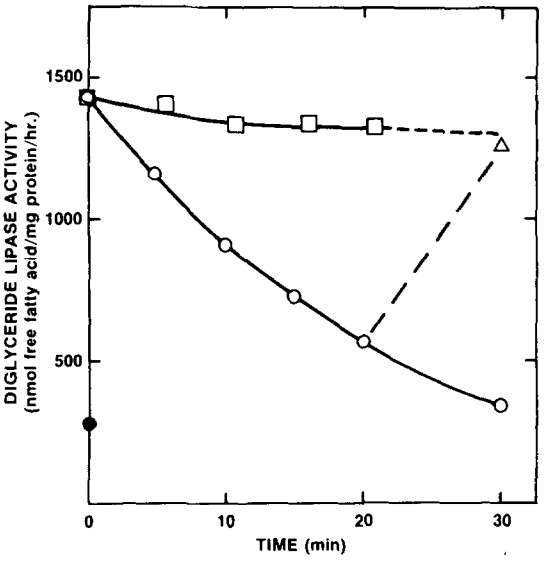

Fig.2 Reversible deactivation of activated diglyceride lipase. Diglyceride lipase was fully activated with cAMP-dependent protein kinase as described in Materials and Methods. Nucleotides were removed by Sephadex G-50 chromatography. Enzyme fractions collected at the void volume were supplemented with 5 mM Mg^{2+} without further addition (□) or with the addition of 3.5 units/ml of phosphorylase phosphatase (○). At time intervals indicated, aliquots of 0.1 ml were removed for assay of diglyceride lipase activity. At 20 min, a sample of 0.1 ml was removed and reactivated with 10 μM cAMP, 0.5 mM ATP and 10 units/ml of cAMP-dependent protein kinase (△). The protein phosphatase reaction and cAMP-dependent protein kinase reaction were terminated both by dilution and by the 5 mM sodium phosphate and 2 mM EDTA present in the 0.7 ml of diolein emulsion mixture. Lipase was assayed at 30° for 30 min. The basal diglyceride lipase activity prior to protein kinase activation is indicated by (●).

Activation was completely blocked by protein kinase inhibitor. Half-maximal activation was obtained at 4.5×10^{-7}M cAMP and 2.5×10^{-5} M ATP. Similar Km values were obtained previously for activation of chicken adipose tissue triglyceride lipase (6).

Reversible deactivation. Since the diglyceride lipase has not been obtained in purified form, it was not possible to directly correlate the rate of activation and the rate of phosphorylation. However, it could be shown that the activated form of diglyceride lipase, like other phosphorylated enzymes, could be readily deactivated by protein phosphatase and that the deactivation was a reversible process (Fig. 2). Addition of a highly purified phosphatase from bovine heart

to the fully activated diglyceride lipase preparation produced deactivation in a time-dependent manner. A 50% deactivation was observed at 15 min and the enzyme had returned to its basal activity by 30 min. To demonstrate reversible deactivation, an aliquot was removed at 20 min and again incubated for another 10 min with cAMP-dependent protein kinase. Lipase activity was restored to nearly that of the fully activated preparation. The deactivation process required Mg^{2+} and was inhibited by 10 mM phosphate, consistent with the general properties of protein phosphatases (7-10,12).

The marked effect of salts on the activity of basal diglyceride lipase with little effect on it after protein kinase activation suggests that the configurational change induced by phosphorylation is mimicked by high ionic strengt It may represent subunit dissociation or at least some unfolding of tertiary structure stabilized by ionic bonds. Changes in the diolein emulsion induced by salt may also play a role. The discovery of conditions that sharply distinguish the basal from the protein kinase-activated forms of the diglyceride lipase will be of practical significance in further studies of its properties. The present demonstration of reversible activation-deactivation of the diglyceride lipase, taken together with previously published evidence (5,13-15), supports the concept that both triglyceride and diglyceride lipase activities actually reside in a single enzyme protein.

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