Tetracycline Inhibition of a Lipase from Corynebacterium acnes

K. WEABER, R. FREEDMAN, AND W. W. EUDY

Eaton Laboratories, The Norwich Pharmacal Company, Norwich, New York 13815

Received for publication 14 December 1970

A lipase which hydrolyzes triglycerides (tricaprylin and trilaurin) and naphthyl laurate was obtained from the broth of Corynebacterium acnes cultures by ammonium sulfate fractionation. CaF²⁺ and sodium taurocholate stimulated activity of the enzyme. Ethylenediaminetetraacetic acid (EDTA) did not inhibit activity of the Ca²⁺-activated enzyme, but lipolytic activity was inhibited by EDTA in the absence of Ca²⁺. Tetracycline (10⁻⁴ M) produced a slight inhibition of the lipase activity with 5 × 10⁻⁴ M or less showing no effect on the lipase activity. However, complete inhibition by tetracycline at 10⁻⁴ M was observed for Ca²⁺-activated enzyme. Tetracycline inhibition of the C. acnes lipase could be demonstrated at concentrations as low as 10⁻⁶ M.

Although the pathogenesis of acne vulgaris is poorly understood, various factors associated with the disease process are known. Sebum has been implicated in the early inflammatory stages of acne (14). Of various fractions of sebum, the free fatty acids (FFA) have been found to be the most irritating (13). Newly formed sebum in the sebaceous gland contains no FFA (6). However, sebum found on the surface of the skin and in the pilosebaceous unit contains 20 to 35% FFA (12). These FFA are presumably produced within the pilosebaceous structure by one or more lipases of either epidermal or bacterial origin (9).

The inflammatory symptoms of acne are reduced with the systemic administration of tetracycline, which localizes in comedones (1). Systemic administration of tetracycline also results in a decrease of skin surface FFA (2). It has been postulated that tetracycline may not function solely as an antimicrobial; it may function also to block bacterial lipase (5) and thereby decrease the production of irritating FFA. Both chlortetracycline and tetracycline have been reported to inhibit pancreatic lipase (8, 11).

Perhaps, then, in the search for an ameliorative agent for acne, the effect of candidate substances on lipase should be considered. In this paper we report the inhibition of a lipase (EC 3.1.1.3) from Corynebacterium acnes by tetracycline.

MATERIALS AND METHODS

Organism and lipase production. C. acnes (ATCC 11828) was grown 18 to 20 hr at 37 C in 500 ml of eugon broth (BBL) containing 0.1% agar. The cells were collected by centrifugation at 9,300 × g for 10 min, resuspended in 50 ml of physiological saline, and frozen at −18 C. Ten milliliters of the thawed cell pool and 20 ml of a 20-hr C. acnes culture were inoculated into 1 liter of eugon broth containing 0.1% agar and incubated for 20 hr at 37 C. The culture was then centrifuged at 16,300 × g for 20 min at 4 C. The clear supernatant was used as the crude enzyme source.

Lipase preparation. Crystalline ammonium sulfate was added to the supernatant to 20% saturation. After storing for 18 hr at 4 C, the precipitate was removed by centrifugation at 27,300 × g for 20 min at 4 C. The pellet was discarded, and crystalline ammonium sulfate was added to the supernatant to 95% saturation. The solution was agitated and then stored for 18 hr at 4 C. The resulting precipitate was removed by centrifugation at 27,300 × g for 20 min at 4 C and dissolved in 8 ml of 0.2 M sodium phosphate buffer (pH 7.4). Ion concentration of the enzyme preparation was reduced by dialysis against 0.05 M sodium phosphate buffer (pH 7.4) at 4 C for 18 hr.

Hydrolysis of substrates. Tricaprylin, tributyrin (Nutritional Biochemical Corp.), and trilaurin (Mann Research Laboratories) were subjected to enzyme hydrolysis, and the hydrolysis products were separated by thin-layer chromatography. One milliliter of tricaprylin or tributyrin or 0.1 g of trilaurin was dissolved in 1.0 ml of acetone. Each of the triglycerides (0.5 ml) was added to each of two tubes containing 4.5 ml of 0.1 M Veronal buffer (pH 7.4) and 1.0 ml of water. The enzyme preparation, 0.2 ml in 0.2 M sodium phosphate buffer (pH 7.4), was added to one set of each of the triglyceride substrate tubes. This set of tubes along with the duplicate tubes, each containing triglyceride in Veronal buffer with no enzyme present, was incubated at 37 C for 4 hr. Triglyceride and hydrolysis products were ex-
electrolytes existed with 0.5 ml by evaporation with nitrogen and spotted on silica gel H plates of 0.25 mm thickness. The plates were developed in hexane-ether-acetic acid (70:30:1) for 20 min by the method of Kellum and Strangfeld (4). Spots were visualized by spraying with 0.2% dichlorofluorescein (Hellige Inc.) in 50% ethanol and viewed under an ultraviolet lamp.

Lipase assay. Lipase activity was determined by the colorimetric method of Seligman and Nachlas at pH 7.4 (10). For each experiment, a standard curve was constructed of the 560-nm absorbance per reaction tube after dirothaisidine (Naphthanil Diazol Blue B, Dajac Laboratories) coupling to 6 to 60 μg of β-naphthol. From this standard curve, activity of the enzyme was measured by determining the micrograms of naphthol released per hour at 37°C from β-naphthyl laurate (naphthol free, Mann Research Laboratories).

In this assay, lipase activity, expressed as micrograms of naphthol released per hour from naphthyl laurate at 37°C, was related linearly to enzyme concentration. Concomitantly, a linear relationship between time of enzyme substrate incubation and product formed was obtained for incubation periods up to 7 hr. Incubation was limited to 5 hr in subsequent studies.

Activation and inhibition studies. The effects of Ca^2+ and sodium taurocholate (Pfaltz and Bauer) on the enzyme were determined. The effects of ethylenediaminetetraacetic acid (EDTA) and tetracycline on the enzyme in the presence and absence of optimum Ca^2+ were also examined. EDTA or tetracycline was incubated with the enzyme for 1 hr at 37°C, and the remaining activity was subsequently determined. In the taurocholate study, the taurocholate was added at the time of substrate addition.

RESULTS

Hydrolysis of substrates. Chromatographic evidence for hydrolysis of triglycerides by this bacterial lipase has been demonstrated. Kellum, Strangfeld, and Ray (5) identified the products of trilaurin hydrolysis by various C. acnes isolates utilizing thin-layer chromatography. RF values for reference standards were: trilaurin, ~0.89; lauric acid, ~0.60; 1,3- and 1,2-diglyceride isomers, ~0.31 and 0.25, respectively. By utilizing their procedure, authentic samples of trilaurin and lauric acid gave RF values of 0.80 and 0.46 in our hands. Hydrolysis products of trilaurin had RF values of 0.82, 0.45, 0.25, and 0.20. On this basis, it was assumed that the lipase hydrolyzes trilaurin to lauric acid and the two diglyceride isomers. Chromatographic evidence of the tributyrin hydrolysis products was not found. Butyric acid migrated with tributyrin in the solvent system used. The end products found in the hydrolysis of tricaprylin could correspond to the diglyceride isomers and FFA.

Effect of Ca^2+ and taurocholate on lipase activity. The effects of various concentrations of Ca^2+ on the activity of the enzyme are illustrated in Fig. 1. Concentrations of Ca^2+ equal to or greater than 5 × 10^{-3} M produced inhibition of lipase activity. However, at 10^{-4} M and 5 × 10^{-4} M, Ca^2+ stimulated activity by 174% and 35%, respectively, over that of the control, whereas concentrations of 10^{-4} M or less apparently had no effect.

Sodium taurocholate activated the lipase at concentrations of 1.7 × 10^{-5} to 1.7 × 10^{-2} M, but, at 1.7 × 10^{-2} M, taurocholate showed little effect on the lipase activity (Fig. 2). Due to its activating effect, taurocholate at 1.7 × 10^{-4} M was incorporated into the assay system utilized in the tetracycline and EDTA studies.

Effect of EDTA and tetracycline on lipase activity. The effects of EDTA and tetracycline on the enzyme in the presence and absence of 10^{-4} M Ca^2+ are shown in Table 1. EDTA, in the absence of Ca^2+, produced a slight inhibition of enzyme activity at 10^{-4} M and marked inhibition at 10^{-2} M. A slight increase of activity was observed at 10^{-5} and 10^{-6} M EDTA. EDTA (10^{-3} M) in the presence of Ca^2+ produced a slight increase of the activity of the enzyme at concentrations of 10^{-3} M and 10^{-2} M.

![Fig. 1. Effect of Ca^2+ on lipase activity. Each point represents the average of at least six determinations.](image1)

![Fig. 2. Effect of sodium taurocholate on lipase activity. Each point represents the average of at least six determinations.](image2)
ence of 10^{-3} M CaCl_2 had little effect on the lipase activity, but, as was seen in the absence of calcium, a slight stimulatory effect of EDTA at 10^{-4} to 10^{-2} M was indicated.

Tetracycline at 10^{-4} M, in the absence of Ca^{2+}, produced a slight inhibition of enzyme activity. Concentrations of tetracycline less than 10^{-4} M had virtually no effect on enzyme activity. Tetracycline at 10^{-4} M in the presence of 10^{-2} M Ca^{2+} produced 100% inhibition of enzyme activity. Inhibition of activity in the presence of 10^{-3} M Ca^{2+} decreased with diminishing concentrations of tetracycline, with enzyme activity slightly reduced by a tetracycline concentration of 10^{-4} M.

**DISCUSSION**

A lipase which hydrolyzes trilaurin and tri-caprylin has been partially purified from *C. acnes*, probably the most common inhabitant of the pilosebaceous unit. The effect of Ca^{2+} on the enzymatic activity of this lipase is similar to that for the staphylococcal lipase recently reported by Troller and Bozeman (15).

Taurocholate is often cited as a lipase activator (8). Taurocholate consistently activates this enzyme. A concentration of 10^{-2} M Ca^{2+} caused a 174% activation of the enzyme although a higher concentration of Ca^{2+} (5 \times 10^{-3} M) inhibited the enzyme, in agreement with the results of Oterholm et al. (7).

Because of the known chelating effects of the tetracyclines (3) and the Ca^{2+} activation of lipase, it was desirable to determine the effect of another chelator, EDTA, on the activity of this microbial lipase. In the absence of Ca^{2+}, 10^{-3} M EDTA markedly inhibited the enzyme (Table 1) in agreement with the results of Oterholm et al. (7), and EDTA at 10^{-4} M showed a slight inhibitory effect. Concentrations of EDTA less than 10^{-4} M produced slight stimulation of activity. In the presence of 10^{-3} M Ca^{2+}, 10^{-2} M EDTA showed little effect, but, at concentrations less than 10^{-1} M, EDTA appeared to stimulate the lipase activity. In the absence of Ca^{2+}, tetracycline (10^{-4} M) had nearly the same effect as EDTA (10^{-4} M); however, concentrations of tetracycline less than 10^{-4} M had no effect on activity. In these respects, EDTA and tetracycline, both chelators, appear to act similarly. EDTA in the presence of 10^{-3} M Ca^{2+} had little or no effect on enzyme activity at any concentration tested.

However, tetracycline in the presence of Ca^{2+} produced 100% inhibition of enzyme activity at 10^{-4} M, 41% at 5 \times 10^{-5} M, and 30% at 10^{-5} M. In this respect, the effects of tetracycline and EDTA on Ca^{2+}-activated enzyme are markedly different. The inhibition of lipase by tetracycline is mediated by Ca^{2+}. Two possible explanations of this observation are (i) tetracycline has an affinity for the calcium-activated lipase, and (ii) tetracycline-calcium complex is the specific inhibitor of lipase. These data support the hypothesis of Kellum, Strangfeld, and Ray (5) that tetracycline in substantial amounts limits the hydrolyzing activity of *C. acnes* by an enzymatic block, but data presented here indicate that tetracycline blocks only the Ca^{2+}-activated lipase. It has been reported that chlortetracycline and tetracycline also inhibit pancreatic lipase (8, 11), but the role of Ca^{2+} in this inhibition is unknown, and both antibiotics have more general effects on mammalian fat metabolism (16).

**LITERATURE CITED**