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INHIBITION OF DNA POLYMERASES OF SEA URCHIN BY PALMITOYL COENZYME A.

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SUMMARY Palmitoyl CoA noncompetitively inhibited the activities of DNA polymerase α and γ, prepared from sea urchin germ cells, with Ki values of 28 μM and 116 μM, respectively. Myristoyl CoA also inhibited DNA polymerse α and γ, while coenzyme A, short chain fatty acyl CoA's, Na-myristate and Na-palmitate failed to inhibit the enzymes. It was concluded that both the long hydrocarbon chain and CoA moiety of long chain fatty acyl CoA's are necessary for inhibition of DNA polymerase activity. DNA polymerse β was not inhibited by long chain fatty acyl CoA's.

Long chain fatty acyl CoA's are known to inhibit the activities of several enzymes involved in fatty acid synthesis (1), citrate metabolism (2) and carbohydrate metabolism (3,4,5,6,7,8) as well as dihydrofolate reductase (9). Recently, inhibition by palmitoyl CoA of ATPase activities of heavy meromyosin of skeletal muscle was reported (10), suggesting the possible involvement of long chain fatty acyl CoA in the regulation of cell movement. The accumulation of long chain fatty acyl CoA in the livers of diabetic or starved rats was reported previously (11). The level of long chain fatty acyl CoA is also known to be twice higher in unfertilized eggs of sea urchin than in fertilized eggs (8).

In the present study, we found that DNA polymerase α and γ prepared from germ cells of sea urchin were inhibited by long chain fatty acyl CoA, such as palmitoyl CoA, while DNA polymerase β was little affected.

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MATERIALS AND METHODS

[Methyl-^{3}H]dTTP (47 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. Deoxyribonucleoside triphosphates and calf thymus DNA were purchased from Boehringer Mannheim GmbH, Germany. Coenzyme A and other acyl CoA's were obtained from Sigma Chem. Co., St. Louis, U.S.A. Activated DNA was prepared by the method of Aposhian and Kornberg (12).

DNA polymerase assay

Assay of DNA polymerase activity was carried out in a total volume of 50 µl at 26°C in the reaction mixture containing 50 mM Tris-maleate buffer at pH 8.0, 7 mM $MgCl_2$, 20 µM each of dATP, dCTP and dGTP, 10 µM (0.5 µCi) [^{3}H]dTTP, 40 mM NaCl, 0.4 mg/ml bovine serum albumin, 100 µg/ml activated DNA and 5 µl of DNA polymerase solution. Reaction was terminated by spotting 40 µl of the reaction mixture on a filter disc (Whatman 3MM, previously soaked in 10 mM Na-pyrophosphate and dried) which was then quickly thrown into ice-cold 5% trichloroacetic acid solution. The filter was washed 5 times with the acid, twice with ethanol and dried. The acid-insoluble radioactivity was determined by a liquid scintillation system.

Enzymes

DNA polymerase α and β were prepared from eggs of sea urchin, Hemicentrotus pulcherrimus, by the method described previously (13,14). DNA polymerase γ was prepared from sperm of sea urchin of the same species. Washed sperm (12.5 g) suspended in 100 ml of 30 mM K-phosphate buffer(pH 7.4) containing 2 mM 2-mercaptoethanol, 10 mM EDTA, 1 M NaCl and 20% glycerol was sonicated for 30 sec 6 times and the homogenate was centrifuged at 30,000 rpm for 1 hr. The supernatant was dialyzed against 50 mM K-phosphate buffer (pH 7.4) containing 2 mM 2-mercaptoethanol, 10 mM EDTA and 20% glycerol and centrifuged at 10,000 xg for 30 min. The resultant supernatant was applied onto a phosphocellulose column (1x8 cm) equilibrated with dialysis buffer, and DNA polymerase γ was eluted from the column at K-phosphate buffer concentration of 0.35 M. DNA polymerase γ thus prepared was characterized as described elsewhere (15). Since the present preparation of DNA polymerase γ was still contaminated with a small amount of DNA polymerase β , as checked by sensitivity to inhibition by ddTTP (16,17), the total incorporation of [^{3}H]dTTP obtained was corrected for DNA polymerase β .

RESULTS

Effect of palmitoyl CoA on sea urchin DNA polymerases

The effect of the increasing concentration of palmitoyl CoA on DNA polymerase α , β and γ prepared from sea urchin germ cells are shown in Fig.1. Palmitoyl CoA inhibited the activities of DNA polymerase α and γ to various degrees depending on the concentration of palmitoyl CoA added, while it had little effect on DNA polymerase β . The concentrations of palmitoyl CoA necessary for 50% inhibition of DNA polymerase α and γ were 40 µM and 125 µM, respectively. Thus DNA polymerase α seemed more sensitive to palmitoyl CoA than DNA polymerase γ . The inhibitory effect of palmitoyl CoA on both enzymes appeared as soon as the start of incubation.

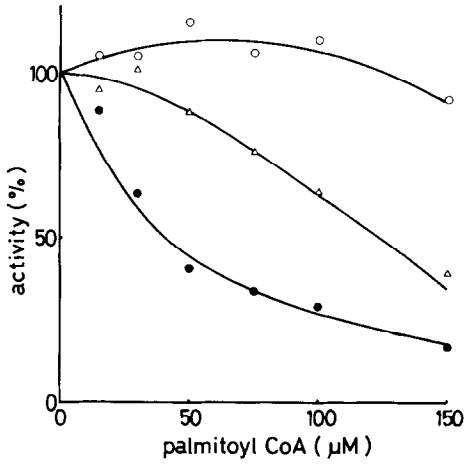

Fig.1. Effect of palmitoyl CoA on DNA polymerases of sea urchin. ●, DNA polymerase α ; o, DNA polymerase β; Δ, DNA polymerase γ . The amounts of dTMP incorporated into DNA during 30 min incubation in the absence of palmitoyl CoA by DNA polymerase α , β and γ were 5.9, 13.6 and 0.33 pmol, respectively.

Effect of coenzyme A, several acyl CoA's and related compounds on DNA polymerase α and γ.

As shown in Table 1, coenzyme A, acetyl CoA, butyryl CoA and propionyl CoA had no, or little if any, effect on the activities of DNA polymerase α and γ. Myristoyl CoA inhibited both DNA polymerases as well as palmitoyl CoA. Sodium salts of myristic acid and palmitic acid had no effect on both

Table 1. Effects of several acyl CoA thioesters and related compounds on the activities of DNA polymerase α and γ . The concentration of each of these compounds was 100 μM. Reaction was carried out at 26°C for 30 min.

Additions	DNA polymerase activity (pmol dTMP incorporated)	
	α	γ
None	6.55	0.44
CoA	4.85	0.49
Acetyl CoA	6.3	0.47
Butyryl CoA	6.43	0.50
Propionyl CoA	6.89	0.45
Myristoyl CoA	1.2	0.18
Palmitoyl CoA	1.48	0.25
Na-myristate	7.0	0.47
Na-palmitate	6.49	0.47

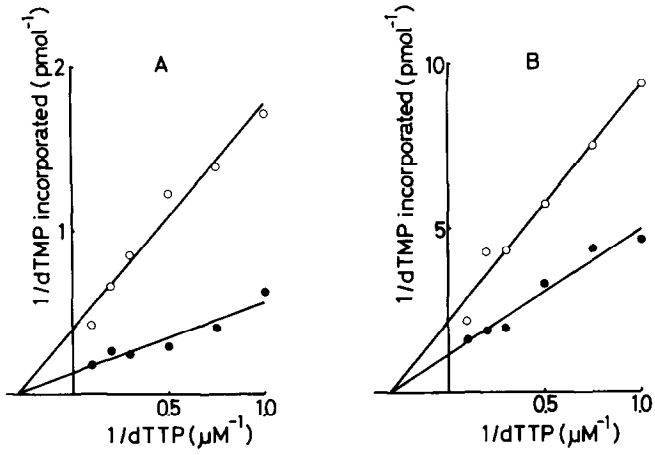

Fig.2. Inhibition of DNA polymerase α (A) and γ (B) by palmitoyl CoA as a function of dTTP concentration. (A) o, 60 μM palmitoyl CoA; ●, without palmitoyl CoA. (B) o, 100 μM palmitoyl CoA; ●, without palmitoyl CoA.

enzymes. Since any precipitation of these long chain fatty acids was not found in the reaction mixture during incubation, the present result suggests that the failure of inhibition of DNA polymerases by sodium salts of these fatty acids may not be due to the low solubilities of these compounds. Thus, both long chain hydrocarbon residue and CoA moiety of fatty acyl CoA seem to be necessary for inhibition of DNA polymerases.

Mode of inhibition of DNA polymerases by palmitoyl CoA.

Lineweaver-Burke plots of the activities of DNA polymerase α and γ as a function of dTTP concentration in the presence of palmitoyl CoA (60 μM for α and 100 μM for γ) are shown in Fig.2A and Fig.2B, respectively. Both enzymes were inhibited noncompetitively by palmitoyl CoA with Ki values of 28 μM (α) and 116 μM (γ). From the comparison of the Ki values, it is suggested that DNA polymerase α is about 4 times more sensitive to inhibitory action of palmitoyl CoA than DNA polymerase γ.

Reversal of palmitoyl CoA inhibition by polyamines or by bovine serum albumin which was reported on several other enzymes (8,9,10) was not observed on DNA polymerases of sea urchin.

DISCUSSION

In the present study, DNA polymerase α and γ of sea urchin were found to be inhibited by long chain fatty acyl CoA's, such as palmitoyl CoA and

myristoyl CoA. Coenzyme A, acetyl CoA, butyryl CoA, propionyl CoA and sodium salts of long chain fatty acids had no effect on DNA polymerases, suggesting the necessity of long nydrocarbon chain and CoA moiety of palmitoyl CoA for the inhibition of DNA polymerase activity. DNA polymerase α seems to be 4 times more sensitive to palmitoyl CoA than DNA polymerase γ, judging from Ki values. It is interesting that DNA polymerase β was little inhibited by palmitoyl CoA.

The concentrations of palmitoyl CoA necessary for 50% inhibition of DNA polymerases (40 μM for α and 125 μM for γ) were much higher than those needed for the other enzymes reported previously (less than 5 μM) (3,6,7,8, 9), but were close to the value obtained on ATPases of heavy meromyosin from rabbit skeletal muscle (10). DNA polymerase I of Micrococcus luteus was also inhibited by palmitoyl CoA with 50% inhibition at 25 μM (unpublished data). These results suggest that, though DNA polymerases or meromyosin ATPases are inhibited by long chain fatty acyl CoA's, they have lower sensitivity to these thioesters than the enzymes involved in the other metabolic pathways.

The concentration of long chain fatty acyl CoA in unfertilized sea urchin eggs was reported to be about 3 nmol/10^6eggs, which declined to 1.5 nmol/10^5eggs after fertilization (8). A rough calculation of intracellular concentration of long chain fatty acyl CoA from these data gives the value of 6 μM for an unfertilized egg and 3 μM for a fertilized egg, assuming the diameter of an unfertilized egg and a fertilized eggs before cleavage to be 100 μm. Thus, the estimated intracellular concentration of long chain fatty acyl CoA is lower than the concentration needed to produce 50% inhibition of DNA polymerase activities. Since our preparation of DNA polymerases is still impure, it is conceivable that some parts of palmitoyl CoA may be broken down during incubation by the substance contaminating in the enzyme preparation, and, as a result, high concentration of the thioester is needed to inhibit DNA polymerase activities. The possible compartmentation of long chain fatty acyl CoA within a cell will also help to produce the localization of the

thioester in a highly concentrated state. However, more data are needed to assert a possible coupling of DNA synthesis and metabolism of fatty acids.

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