Synthesis of fatty acyl CoA and other thiol esters using *N*-hydroxysuccinimide esters of fatty acids

ADHID AL-ARIF and MELVIN BLECHER

Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington, D. C. 20007

SUMMARY *N*-Hydroxysuccinimide esters of long-chain fatty acids have been used to synthesize the CoA and thioglycolic acid thiol esters of palmitic and 3-ketopalmitic acids in high yield and with a minimum of untoward side reactions.

SUPPLEMENTARY KEY WORDS palmitoyl-*S*-CoA · 3-ketopalmitoyl-*S*-thioglycolate · ketal of 3-ketopalmitic acid

SEVERAL METHODS have been used to prepare fatty acyl esters of CoA and other thiols: reaction of acid anhydrides with the mercaptan in aqueous solution at pH 7.5 (1); thiol ester exchange reactions (2, 3); and the use of either mixed anhydrides of ethyl hydrogen carbonate (1) or acid chlorides (4). With the exception of the thiol ester interchange method, the acylating reagents described above have low specificity; they react also with amino, hydroxyl, and phosphoric acid ester groups, all of which are present in CoA and in acyl carrier protein. Additional disadvantages are the need constantly to adjust the pH to 7.5–8.0 by the addition of alkali, and the existence of competing hydrolysis reactions of the acylating agents under the conditions employed (1, 4).

Lapidot, Rappoport, and Wolman (5) recently described the synthesis of *N*-hydroxysuccinimide esters of long-chain fatty acids, and their use in the synthesis of *N*-acyl amino acids. The esters are easily prepared, crystalline, stable compounds, and react vigorously with amino groups in nonaqueous as well as aqueous solutions; they are particularly reactive in aqueous bicarbonate solutions. These esters do not react with phosphoric acid monoesters to form mixed anhydrides, nor do they form esters with hydroxyl groups, or amides with the free amino groups of adenine or cytosine (5). These properties suggested the use of these esters in the synthesis of thiol esters. In this communication, we describe the synthesis of palmitoyl-*S*-CoA and other thiol esters by this method.

Materials. All solvents were 99+ mole% pure, and were dehydrated before use. Palmitic acid (99% pure) was obtained from the Hormel Institute, Austin, Minn. *N*-Hydroxysuccinimide and dicyclohexyl carbodiimide were purchased from the Aldrich Chemical Company, Milwaukee, Wisc. Palmitoyl-*S*-CoA (95 ± 4% pure by spectral and chemical analyses) and Coenzyme A (lithium salt, 85–94% pure by spectral, chemical, and enzymic analyses) were obtained from P–L Biochemicals, Inc., Milwaukee, Wisc.

N-hydroxysuccinimide ester of palmitic acid. The ester was synthesized by the method of Lapidot et al. (5). The purified compound, mp 90°C, moved as a single spot (R_f 0.62) in thin-layer chromatography with chloroform on Silica Gel G.

Synthesis of palmitoyl-S-CoA. To a solution of 50 mg (57.4 μmoles) of CoA in 3 ml of water we added 46 mg (0.5 mmole) of thioglycolic acid, followed by 168 mg (2 mmoles) of $NaHCO_3$. To this solution we added a solution of 2 mmoles of the *N*-hydroxysuccinimide ester of palmitic acid[1] in 6 ml of tetrahydrofuran that had been freshly distilled from lithium aluminum hydride. The mixture, a single phase, was agitated by a stream of N_2 gas. Since it is imperative that the mixture remain a single phase throughout the reaction in order to obtain the product in high yield, it was sometimes necessary to add additional tetrahydrofuran to maintain homogeneity. Completion of the reaction, as indicated by disappearance of free sulfhydryl groups (6), was usually reached in 3–4 hr at room temperature.

After the reaction was complete, 12 ml of 5% $HClO_4$ was added to precipitate palmitoyl-*S*-CoA, palmitoyl-*S*-thioglycolic acid, and excess *N*-hydroxysuccinimide ester; any palmitoyl-*S*-glutathione or palmitic acid

[1] The use of thioglycolic acid and the consequent need for a large excess of acylating agent is related to the presence of variable amounts of oxidized CoA in commercial preparations of reduced CoA (4). When isotopically labeled fatty esters of *N*-hydroxysuccinimide are used, only a 20% excess of this acylating agent need be employed, and thioglycolic acid can be omitted; however, the yields of labeled acyl CoA will be lower, and will depend upon the amount of reduced CoA present.

present as contaminants also precipitates under these conditions. To achieve complete precipitation of these compounds, we concentrated the mixture under reduced pressure in a rotary evaporator. The precipitate was collected by filtration, and washed with 25 ml of 0.8% $HClO_4$.

The precipitate was transferred to a centrifuge tube and extracted with acetone (4 × 15 ml) and peroxide-free diethyl ether (3 × 10 ml); palmitic acid, palmitoyl-*S*-thioglycolic acid, and the *N*-hydroxysuccinimide ester of palmitic acid were completely extracted from the precipitate by these solvents. The white residue was extracted with three 4-ml portions of water (the solution was maintained at pH 5.0 by the addition of $NaHCO_3$) to dissolve palmitoyl-*S*-CoA, while any palmitoyl-*S*-glutathione present remained insoluble. Palmitoyl-*S*-CoA was precipitated from the pooled clear aqueous extracts by the addition of 6 ml of 5% $HClO_4$; the precipitate was washed with 10 ml of 0.8% $HClO_4$, and finally with acetone (3 × 5 ml). The yield of the dried white powder was 50 mg (87%, based on CoA).

The product had the following properties:

(*a*) Its UV spectrum was identical with that of palmitoyl-*S*-CoA obtained commercially or synthesized by the acid chloride or mixed anhydride methods (1, 4).

(*b*) Its UV spectrum measured against a reference solution of CoA showed a peak at 232 nm, which disappeared after treatment with methanolic NaOH, indicating hydrolysis of the thiol ester bond (1).

(*c*) It gave a positive nitroprusside test for free sulfhydryl groups only after treatment with methanolic NaOH.

(*d*) Chromatography on No. 1 Whatman filter paper in butanol–glacial acetic acid–water 5:2:3 revealed a single spot of R_f 0.53, identical with that of authentic palmitoyl-*S*-CoA. This spot gave a positive nitroprusside test only after addition of methanolic NaOH.

(*e*) With methyl palmitate as the standard, the product had an ester content (hydroxamic acid method) of 85%, identical with that of our commercial preparation of palmitoyl-*S*-CoA.

(*f*) Molar absorptivities of the product were 9.1×10^6 M^{-1} cm^{-1} at 232 nm and 16.2×10^6 M^{-1} cm^{-1} at 260 nm; the corresponding literature values are 9.2×10^6 and 16.4×10^6 M^{-1} cm^{-1} (1).

Other thiol esters synthesized. The procedure described above was also used to prepare palmitoyl-*S*-thioglycolic acid and 3-ketopalmitoyl-*S*-thioglycolic acid. In the synthesis of the latter compound, the *N*-hydroxysuccinimide ester of the ketal of 3-ketopalmitic acid was employed in order to protect the keto group. The ketal of 3-ketopalmitic acid was synthesized from methyl 3-ketopalmitate either by azeotropic distillation of the ester with ethylene glycol plus *p*-toluenesulfonic acid (7) or by reaction of the ester with ethylene glycol and boron trifluoride etherate (8), followed by base-catalyzed hydrolysis and acidification to obtain the ketal acid. After synthesis of the thiol ester, the protective ketal group was removed by heating the compound in 20 ml of acetone containing 100 mg of *p*-toluenesulfonic acid. In the synthesis of palmitoyl-*S*-thioglycolic acid, we used a 100% molar excess of thioglycolic acid; the yield of product was virtually quantitative based on acylating agent. The perchloric acid insoluble product was recrystallized from *n*-hexane to yield a compound with mp (62–63°C), UV and IR spectra, and chemical behavior identical with those observed with palmitoyl-*S*-thioglycolic acid prepared by the acid chloride method (4).

Discussion. This method of thiol ester synthesis offers a simple and specific method for the acylation of CoA with fatty acids in high yield without many of the side reactions and other disadvantages attendant upon previously employed methods. Preliminary experiments suggest that this procedure can also be used for preparing the thiol esters of unsaturated and hydroxy fatty acids; we have made the *N*-hydroxysuccinimide ester of 2-hexadecenoic acid, and the synthesis of 2-hydroxystearic acid has been reported recently (9). This method may also prove valuable in the synthesis of fatty acyl thiol esters of acyl carrier protein, since it may avoid the undesirable acylation of other portions of the protein—e.g., tyrosine hydroxyl groups—brought about by other procedures (10); however, it is likely, although not altogether certain, that free amino groups of acyl carrier protein (e.g., the ε-amino groups of lysine) will be acylated by the method described.

This work was supported by Research Grant AM-06208 from the National Institutes of Health. The data are taken from a doctoral dissertation to be submitted by Adhid Al-Arif to the Graduate School of Georgetown University.

Manuscript received 11 December 1968; accepted 20 February 1969.

References

1. Stadtman, E. R. 1957. *In* Methods in Enzymology. S. P. Colowick and N. O. Kaplan, editors. Academic Press, Inc., New York. **3:** 931.
2. Wieland, T., and H. Köppe. 1954. *Ann. Chem.* (*Justus Liebigs*). **588:** 15.
3. Wieland, T., and W. Schäfer. 1952. *Ann. Chem.* (*Justus Liebigs*). **576:** 104.
4. Seubert, W. 1960. *Biochem. Prep.* **7:** 80.
5. Lapidot, Y., S. Rappoport, and Y. Wolman. 1967. *J. Lipid Res.* **8:** 142.
6. Grunert, R. R., and P. H. Phillips. 1951. *Arch. Biochem.* **30:** 217.
7. Vagelos, P. R., and A. W. Alberts. 1960. *Anal. Biochem.* **1:** 8.
8. Adams, W. J., D. N. Kirk, D. K. Patel, V. Petrow, and I. A. Stuart-Webb. 1954. *J. Chem. Soc.* (*London*). 2209.
9. Kawanami, J. 1968. *Chem. Phys. Lipids.* **2:** 330.
10. Majerus, P. W. 1968. *Science.* **159:** 428.