A New Method for the Preparation of Acyl-CoA Thioesters

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A method is described for the preparation of CoA thioesters of fatty acids. Acyl-CoA thioester was synthesized by way of 1-acylimidazole and purified by high performance liquid chromatography. This method is applicable to the preparation of a variety of acyl-CoA thioesters of various chain lengths (from C₂ to C₁₈) and with varying degrees of unsaturation. It is particularly suitable for preparing acyl-CoA thioesters of labeled fatty acids or fatty acids available only in small amounts.

A variety of methods, including chemical and enzymic syntheses, are currently available for the preparation of CoA thioesters of fatty acids. Chemical syntheses most frequently employed involve the acid anhydride (1), the acid chloride (2), the mixed anhydride of ethyl hydrogen carbonate (3) and the N-hydroxysuccinimide ester of the fatty acid (4) as the acylating reagent. Product isolation in these procedures has been inadequate for small quantities of fatty acids. Several methods which utilize acyl-CoA synthetase as a catalyst have also been reported (5-7). However, these procedures for enzymic syntheses suffer from the following drawbacks: (a) crude enzyme preparations contain significant amounts of endogenous fatty acids (5, 6); (b) preparation of the enzyme in a form free of endogenous fatty acids is rather laborious and time-consuming (7, 8); (c) general applicability to acyl-CoA thioesters of different chain lengths is often difficult because of the specificity of the enzyme (8).

The present paper describes a method for the synthesis of acyl-CoA thioesters. It utilizes 1-acylimidazole as the acylating reagent and product purification is carried out by high performance liquid chromatography. This procedure has been in use in our laboratory for the past few years with success for preparations of acyl-CoA thioesters of labeled fatty acids available only in small amounts (9).

EXPERIMENTAL PROCEDURE

Materials—CoASH was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo; palmitoyl-CoA was from P-L Biochemicals Inc., Wisconsin; N,N'-Carbonyldiimidazole was from Fluka AG, Buchs SG. All other reagents and solvents were commercial products of analytical grade. To remove peroxides, tetrahydrofuran and ethyl ether used in these experiments were filtered through a column of Woelm alumina (alkaline, activity grade I) immediately before use.

Synthesis of Acyl-CoA Thioesters—A solution of fatty acids (5 µmol) in tetrahydrofuran (0.1 ml) was added to a solution of carboxyldiimidazole (6 µmol) in tetrahydrofuran (0.1 ml). After 30 min at room temperature, the solvent was evapo-
rated off and the residue was dissolved in tetrahydrofuran–H₂O (2 : 1, 0.2 ml). This solution was allowed to react with 5 μmol of CoASH which had been dissolved in 0.5 ml of tetrahydrofuran–H₂O (2 : 1). The pH of the reaction mixture was adjusted to 7.0–7.5 with 1 N NaOH. The reaction was carried out at room temperature under an argon atmosphere for 4 h. Tetrahydrofuran was evaporated off and the residual aqueous solution was acidified to pH 3 to 4 by adding small amounts of Dowex 50 (H⁺). Dowex 50 (H⁺) was removed by filtration, then the filtrate was extracted with ethyl ether (0.5 ml×3) to remove the unreacted fatty acid and the water layer was lyophilized.

High Performance Liquid Chromatography of Acyl-CoA Thioesters—The above preparation contained imidazole and unreacted CoASH as contaminants. Separation of acyl-CoA from contaminating imidazole and CoASH was performed with a Shimadzu LC-3AG liquid chromatograph that was fitted with a 25-cm Shimadzu LC PNH₂ column and equipped with a UV detector operating at 254 nm. Elution was performed at room temperature with a linear gradient from 0.04 M to 0.3 M KH₂PO₄ (adjusted to pH 3.3 with H₃PO₄).

Determination of the Content of Acyl-CoA Thioesters Synthesized—The thioester content of the preparations was determined by measuring the ratio of the 232 and 260 nm absorptions (2). In some cases, the thioester content was checked by measuring the decrease in absorption at 232 nm after alkaline hydrolysis of the sample at 95°C for 1 min in 0.1 N NaOH. The hydroxamate reaction was also used to determine the thioester content of the preparations (5).

RESULTS AND DISCUSSION

Synthesis of Acyl-CoA Thioesters—The procedure described in this paper consists of two reactions:

1) \[ R-C=O + N-C=N + \text{carbonyldiimidazole} \rightarrow R-C-N-C-N + CO₂ + HN \]

2) \[ R-C-N + CoASH \rightarrow R-C-SCoA \]

The reaction of fatty acid with carbonyldiimidazole proceeded very rapidly to give a 1-acylimidazole with release of carbon dioxide. The acyl residue of 1-acylimidazole was transferred to the thiol group of CoA. The excess carbonyldiimidazole was hydrolyzed to imidazole in the presence of water.

Table I shows the yield of various acyl-CoA thioesters. These results indicate that this method is useful for the preparation of saturated acyl-CoA thioesters of chain length from C₂ to C₁₈. This method was also applicable to various unsaturated fatty acids, except for 2-enoic fatty acids. It is possible that the bonding between the 2-enoylacyl residue and imidazole is too stable to form the thioester. The data in Table I suggest that this procedure is suitable for the preparations of acyl-CoA thioesters from 0.5 μmol of fatty acids. We had tried to synthesize nonanoyl-CoA by way of

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Numbers of experiments</th>
<th>Yield (％)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic (5)</td>
<td>3</td>
<td>82±12</td>
</tr>
<tr>
<td>Nonanoic (5)</td>
<td>5</td>
<td>68±10</td>
</tr>
<tr>
<td>Nonanoic (0.5)</td>
<td>3</td>
<td>55±15</td>
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<tr>
<td>2-Decenoic (5)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Palmitic (5)</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Palmitic (0.5)</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Stearic (5)</td>
<td>3</td>
<td>65±10</td>
</tr>
<tr>
<td>Stearic (0.5)</td>
<td>2</td>
<td>38, 45</td>
</tr>
<tr>
<td>Oleic (5)</td>
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<td>70</td>
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<tr>
<td>Linoleic (5)</td>
<td>5</td>
<td>61±17b</td>
</tr>
<tr>
<td>Linolenic (5)</td>
<td>2</td>
<td>60, 72b</td>
</tr>
</tbody>
</table>

a Values in parentheses indicate the amount (μmol) of fatty acid used for the experiments. b These results were obtained by Dr. T. Okayasu and Prof. Y. Imai, Department of Biochemistry, School of Medicine, Hokkaido University.
either the mixed anhydride (3) or nonanoyl chloride (2) using 0.5 µmol of nonanoic acid. However, these methods gave very poor yields (less than 5%). The present method utilizing acylimidazole as the acylating reagent seems to be especially suitable for the preparation of acyl-CoA thioesters of fatty acids available only in small amounts.

**Purification of Acyl-CoA Thioesters Synthesized**—The above preparations of acyl-CoA thioesters contained imidazole, unreacted CoASH, and some compounds which have not been identified yet. Usually the preparations have been used in our laboratory without further purification, but it is sometimes necessary to separate acyl-CoA thioesters from these contaminants. The purification of acyl-CoA thioesters can be effectively carried out by high performance liquid chromatography. Figure 1 shows a typical elution profile of palmitoyl-CoA synthesized by the procedure described. The peak corresponding to palmitoyl-CoA was collected and the palmitoyl-CoA obtained was over 90% pure on the basis of the usual criteria used to determine the purity of preparations. Purification of acyl-CoA thioesters has been performed by acid precipitation (2), DEAE-cellulose column chromatography (10), paper chromatography (11), and thin-layer chromatography (12). However, these methods are rather laborious and time-consuming, and some of them are not suitable for the purification of acyl-CoA thioesters available only in small amounts. Although more detailed studies on the general applicability to acyl-CoA thioesters have not been made yet, high performance liquid chromatography described in this paper seems to be a convenient and rapid method to purify acyl-CoA thioesters synthesized by various methods.

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**REFERENCES**