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A simple and efficient method to prepare thioesters in aqueous solutions

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Abstract—A simple method is described for the efficient synthesis of biologically-active thioesters in aqueous solutions. The method utilizes imidazole as a catalyst and easily synthesized acyl or aminoacyl adenylates to synthesize a variety of thioesters, from small molecules to macromolecules. Yields in excess of 90% can be achieved in less than 10 min at room temperature. Specifically, functional derivatization of RNA with biotin via thioester linkage is demonstrated.

1. Introduction

Biologically-active thioesters play central roles in living cells serving as essential metabolic intermediates due to their ability to act as excellent intermediates for acyl group transfer reactions. In addition, biosynthesis of polyketides and nonribosomal polypeptides is achieved via thioester intermediates of fatty acids and amino acids.[1] Over the past 50 years, extensive chemical and enzymatic methods have been developed to synthesize various thioesters for probing mechanisms of numerous thioester-utilizing enzymes.[2] However, most methods require the skill and setup of organic chemistry. In addition, they require protection and deprotection cycles if there exist multiple functional groups. For example, the synthesis of aminoacyl CoAs requires the protection and deprotection of amino groups.[3] Herein we report a simple method for the efficient synthesis of various thioesters using acyl or aminoacyl adenylates as the thioacylating reagents and imidazole as a catalyst in aqueous solutions. To demonstrate the simplicity, versatility, and utility of the method, four different thioesters with sizes from small to large are successfully synthesized and characterized.

In this report, a carboxylate–phosphate mixed anhydride (acyl or aminoacyl adenylate) was chosen as the thioacylating reagent because of their easy preparation through dicyclohexylcarbodiimide (DCC)-aided condensation of carboxylates/amino acids with adenosine 5′-monophosphate (AMP) without the need of protection of free amino groups.[4] Although an acylphosphate may also be used as a thioacylating reagent, we chose to use acyl adenylates because they are UV-active, can be easily analyzed by UV spectroscopy, and purified by HPLC.

2. Synthesis of acetyl-CoA

CoA is an essential coenzyme and its acetylation has been extensively studied.[2] Here, we provide a simple and efficient method to prepare acetyl-CoA (Ac-CoA) in aqueous solutions (Scheme 1). When a mixture (1:3 ratio) of CoA and acetyl adenylate (Ac-AMP, prepared from literature procedure[4,5]) was incubated in a solution containing 100 mM imidazole (pH 7) for 10 min at room temperature, more than 90% of CoA was converted into Ac-CoA, along with the side product AMP, as shown by HPLC (Fig. 1A, solid line). However, when imidazole was replaced with a HEPES (*N*-hydroxyethyl piperazine-*N*′-ethanesulfonic acid) buffer (100 mM, pH 7.0), no change in either CoA or Ac-AMP was observed (Fig. 1, dotted line), indicating that the reaction was catalyzed by imidazole. Furthermore, Ac-CoA was isolated and characterized by UV (Fig. 1B) and MS[6] analyses, whose results were consistent with the proposed structure. It was interesting to note that when the ratio of CoA:Ac-AMP was varied from 1:1, 1:2, to 1:3, thioester

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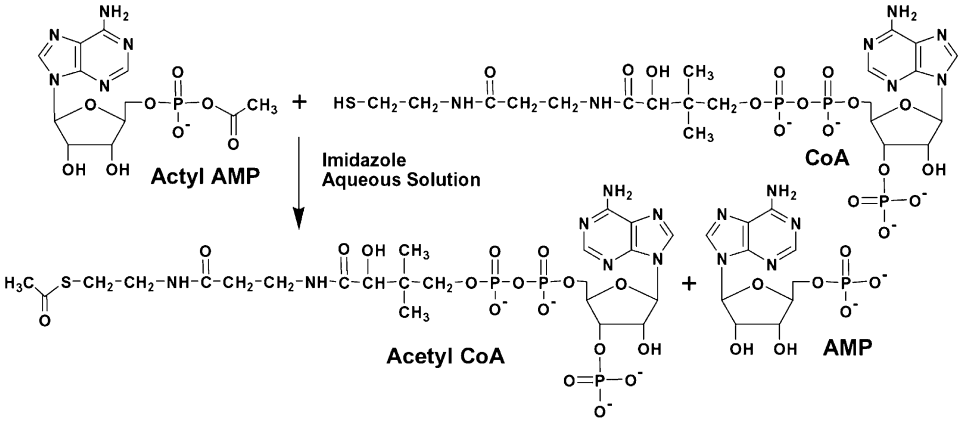


Scheme 1. Synthesis of acetyl CoA.

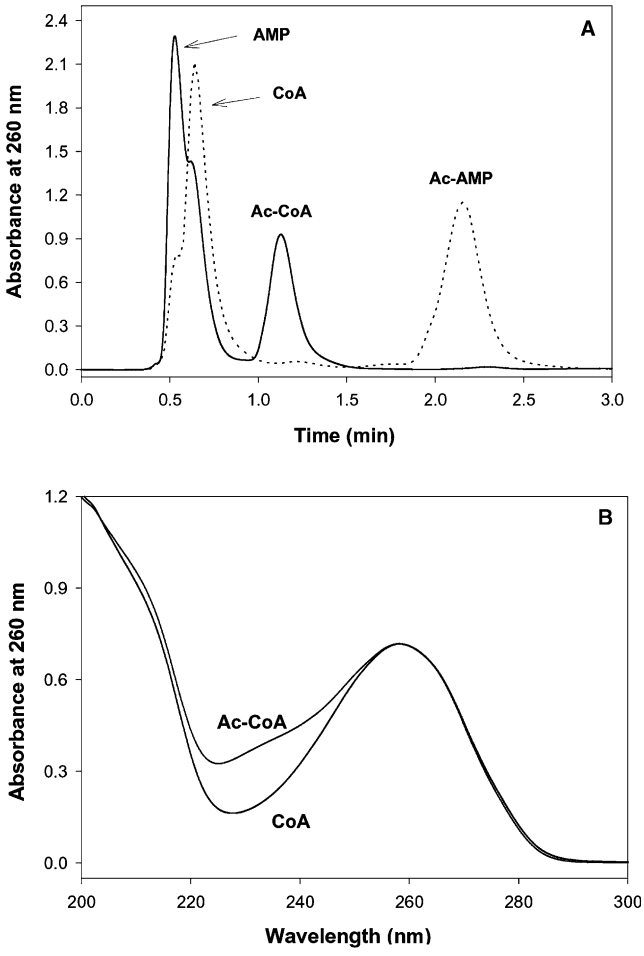


Figure 1. HPLC and UV spectroscopic analyses of Ac-AMP reaction with CoA in the absence (A, dotted line) and presence (A, solid line) of 100 mM imidazole. The reaction product Ac-CoA eluted at 1.13 min. HPLC conditions were: Alltech Expedite C18, 10 × 4.6 mm; 7% MeOH/93% 20 mM phosphate, pH 7.0; flow rate 0.5 mL/min. B, UV spectrum comparison of CoA and Ac-CoA. Ac-CoA has a characteristic UV shoulder between 230 nm and 240 nm[5] and the thioester product could be readily hydrolyzed to CoA by hydroxylamine.

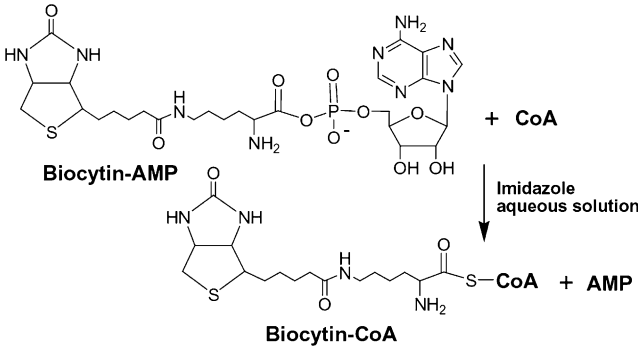
yields reached 50%, 80%, and 90 ± 10% (based on CoA), respectively. Therefore, 3 equiv of thioacetylating reagent are both necessary and sufficient for completing the reaction.

3. Synthesis of biocytin-CoA

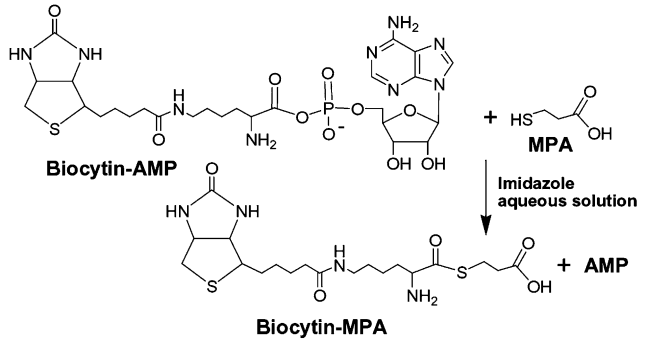
Once the reaction conditions and procedures were established, we used the same method to introduce a biotinyl group to CoA through the formation of the thioester, as shown in Scheme 2. Similarly, the incubation of 1:3 molar ratio of CoA:biocytin-AMP (synthesized from biocytin and AMP according to Berg[4]) under the same conditions gave the desired product, biocytin-CoA, which was confirmed by HPLC and MS analyses.[7] Furthermore, biocytin-CoA could be easily hydrolyzed to CoA by treatment with hydroxylamine, as monitored by HPLC analysis.

4. Synthesis of biocytin-MPA

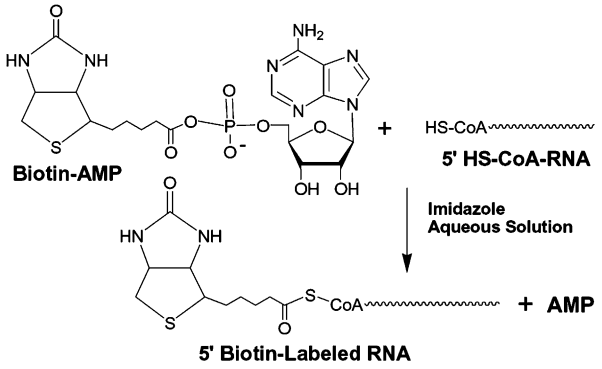
To extend the method to include other thiol-containing molecules, 3-mercaptopropionic acid (MPA) was used as a small molecule substrate. As described in Scheme 3, incubation of MPA with 3 equiv of biocytin-AMP in the presence of 0.1 M imidazole produced biocytin-MPA via a thioester bond, along with side product AMP. The product possessed the characteristic UV spectrum,[8] as shown in Figure 2, with λ_{max} and λ_{min} at 236 and 218 nm, respectively. After isolation by HPLC,



Scheme 2. Synthesis of biocytin-CoA.



Scheme 3. Synthesis of biocytin-MPA.



Scheme 4. Synthesis of 5′biotin-labeled RNA.

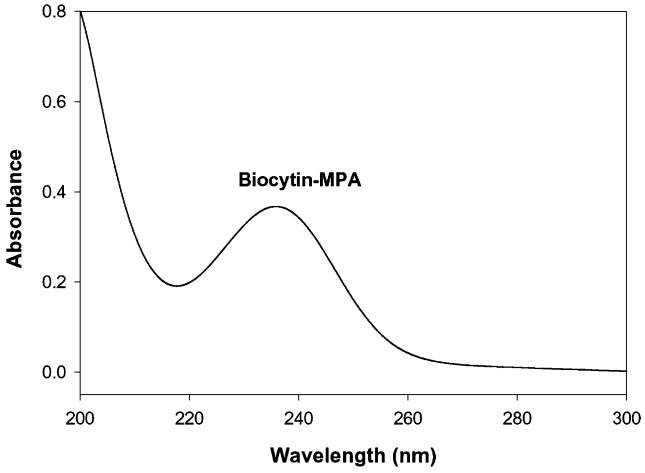


Figure 2. UV spectrum of biocytin-MPA with λ_{max} at 236 nm and λ_{min} at 218 nm. The 220–250 nm peak is indicative of a thioester functional group.

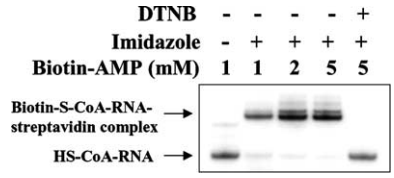


Figure 3. Streptavidin gel mobility shift assay for extent of imidazole-catalyzed biotinylation. RNA incubated with only biotin-AMP shows no reaction product (lane 1), while in the presence of imidazole, the reaction proceeds to nearly 100% (lanes 2–4). Blocking the free thiol with DTNB prior to reaction with imidazole prevents the thioester formation (lane 5).

the biocytin-MPA was further characterized by MS,[9] which was consistent with the identity.

5. Synthesis of biotin-CoA–RNA

Finally, this method was extended to a biopolymer for a validation. A CoA–RNA conjugate (92 mer, purified by thiopropyl Sepharose 6B affinity column)[5] was incubated with biotin-AMP[5] under different conditions (Scheme 4). The RNA and biotin-labeled RNA were then incubated with streptavidin and analyzed by polyacrylamide gel electrophoresis[5] (Fig. 3). From the gel, it is clear that imidazole is required for RNA biotinylation (lanes 2–4), and treatment of RNA with the Ellman's reagent 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) prevents the reaction (lane 5). The results show that an imidazole-catalyzed thioesterification reaction occurs specifically at the free thiol of RNA-linked CoA. At 1 mM biotin-AMP, RNA labeling achieves 90–95% yields (lane 2). Labeling yields can reach ~100% at higher biotin-AMP concentrations (lanes 3 and 4).

These example reactions have demonstrated that this method is simple to use for both chemists and nonchemists. It may be applied to a broad spectrum of thiols and carboxylic acids, both small and macromolecular, and can afford excellent thioester yields of greater than 90% within 10 min at room temperature in a simple aqueous imidazole solution. A distinguishing advantage of this method is the tolerance of other functional groups, such as free amino groups, which are normally quite nucleophilic and reactive but do not affect thioesterification reaction under the reaction conditions. Therefore, protection of amino groups is not necessary. In contrast to established methods for introducing functional groups to CoA, RNA, proteins, or small molecules through thioester linkages, the current method utilizes a very mild condition suitable for most biomolecules.

In summary, we have developed a simple and efficient method to introduce functional groups to small molecules and macromolecules such as RNA by imidazole-catalyzed thioesterification in aqueous solutions. Theoretically, any free thiol on a small molecule or macromolecule (protein, DNA, and RNA) of interest can be functionalized in this manner. The method is therefore robust in preparing a variety of biologically relevant or artificial thioesters for numerous applications. For example, the use of biotinyl adenylate or biocytinyl adenylate allows the tagging of thiol-containing molecules for easy separation by avidin affinity chromatography, which has proven to be useful for protein and nucleic acid affinity purification techniques.[10–15] Biotinylated macromolecules can also be used in conjunction with immunoprobes to identify or localize target

molecules.[16–18] In addition, tagging with fluorophore-modified acyl or aminoacyl adenylates may readily lend itself to target identification or localization studies. Further, CoA thioesters covalently attached to RNA by this method could be useful for attaching a variety of functional groups to RNA, which may be helpful for identifying novel RNA catalysts by SELEX techniques.[19–21]

References and notes

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6. MOLDI-TOF MS gave the thioester peak with $w/z = 810.0$ for $(MH)^+$, consistent with the MW of Ac-CoA ($C_{23}H_{38}N_7O_{17}P_3S$, 809.5).
7. Biocytin-CoA was analyzed with the following HPLC conditions: Alltech Expedite C18, 10×4.6 mm; 20% MeOH/80% phosphate, pH 7.0; flow rate 0.7 mL/min. Expected MW of 1122.0 ($C_{37}H_{62}N_{11}O_{19}P_3S_2$) was confirmed by MOLDI-TOF MS: 1122.5 for $(MH)^+$.
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9. Biocytin-MPA was eluted at 2.48 min under the following HPLC conditions: Alltech Expedite C18, 10×4.6 mm; 25% MeOH/75% phosphate, pH 7.0; flow rate 0.7 mL/min. MOLDI-TOF MS found $(MH)^+$ of 461.5, expected mass of ($C_{19}H_{33}N_4O_5S_2$) 460.2.
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