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A Capillary Electrophoretic Assay for Acetyl CoA Carboxylase

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Abstract

A simple off-column capillary electrophoretic (CE) assay for measuring acetyl coenzyme A carboxylase holoenzyme (holo-ACC) activity and inhibition was developed. The two reactions catalyzed by the holo-ACC components, biotin carboxylase (BC) and carboxyltransferase (CT), were simultaneously monitored in this assay. Acetyl coenzyme A (CoA), malonyl-CoA, adenosine triphosphate (ATP), and adenosine diphosphate (ADP) were separated by CE, and the depletion of ATP and acetyl-CoA as well as the production of ADP and malonyl-CoA were monitored. Inhibition of holo-ACC by the biotin carboxylase inhibitor, 2-amino-N,N-dibenzylloxazole-5-carboxamide, and the carboxyltransferase inhibitor, andrimid, was confirmed using this assay. A previously reported off-column CE assay for only the CT component of ACC was optimized, and an off-column CE assay for the BC component of ACC also was developed.

Keywords

capillary electrophoresis; enzyme assay; acetyl CoA carboxylase; enzyme inhibition

1. Introduction

Acetyl coenzyme A carboxylase (ACC) catalyzes the first committed and rate-limiting step in fatty acid biosynthesis in all bacteria, plants, and animals. The biotin-dependent carboxylation of acetyl coenzyme A (CoA) to form malonyl-CoA is carried out in two half-reactions (Fig. 1) [1]. In the first half-reaction, biotin carboxylase (BC) catalyzes the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), and the carboxylation of biotin, which *in vivo* is covalently attached to biotin carboxyl carrier protein (BCCP). In the second half-reaction, carboxyltransferase (CT) catalyzes the transfer of the carboxyl group on biotin to acetyl-CoA to form malonyl-CoA. In bacteria, BC, CT, and BCCP are separate proteins, and BC and CT retain their activity using free biotin in place of BCCP [1]. In eukaryotes, these three components of ACC are combined in one polypeptide [1]. Acetyl coenzyme A carboxylase is a target for the development of antibiotics [2,3], herbicides [4], and therapeutic agents for metabolic syndrome (including obesity and type 2 diabetes) [5–9] and cancer [10,11]. Thus, effective assays to measure the inhibition of ACC are desirable.

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Several assays have been developed to monitor the activity of isolated bacterial BC and CT, as well as holo-ACC from either bacterial or eukaryotic sources. Hereafter, holo-ACC will refer to the case when all three bacterial proteins, BC, CT, and BCCP are present in solution. Early studies of *E. coli* ACC utilized a radiolabeled bicarbonate fixation assay for determining the activity of holo-ACC and isolated BC [12]. Moreover, BC was also assayed with an enzyme-coupled assay, where ADP formation was monitored spectrophotometrically at 340 nm via NADH oxidation [12]. The enzymatic activity of isolated bacterial CT was commonly assayed in the reverse (nonphysiological) direction using either a radioactivity assay that measured the biotin-dependent decarboxylation of radiolabeled malonyl-CoA, or where acetyl-CoA production was coupled to the citrate synthase-malate dehydrogenase reaction and NAD⁺ reduction was monitored spectrophotometrically at 340 nm [12]. More recently, Kroeger *et al.* developed an NADPH based spectrophotometric assay for holo-ACC, where malonyl-CoA reductase was used to catalyze the NADPH-dependent reduction of the product, malonyl-CoA [13]. In addition, Alves *et al.* reported an assay for holo-ACC based on HPLC with tandem mass spectrometry (LC-MS/MS), where the enzyme and substrates were incubated for 3 hr at room temperature and malonyl-CoA formation was monitored [14].

Several of these assays for ACC have been adapted and optimized for high-throughput screening. Harwood *et al.* [7] developed a plate reader-based high-throughput screening assay for mammalian ACC based on the radiolabeled bicarbonate fixation assay of Guchhait *et al.* [12]. Subsequently, Santoro *et al.* developed a plate reader-based high-throughput screening assay for bacterial CT in the nonphysiological direction where citrate synthase was coupled to the production of acetyl-CoA and the product CoA was detected with DTNB (Ellman's Reagent) [15]. Soriano *et al.* developed a plate reader-based high-throughput assay for ACC that involved the use of ³²P-labeled ATP [16]. Seethala *et al.* developed a high-throughput assay for mammalian ACC where radiolabeled acetyl-CoA was used in an ACC/fatty acid synthase coupled assay and radiolabeled palmitic acid was detected [17]. Lui *et al.* reported two high-throughput assays for mammalian ACC where amplex red was used with two coupled enzyme-catalyzed reactions to detect phosphate by fluorescence or fluorescence polarization was used to monitor fluorescently labeled ADP binding to an anti-ADP antibody [18]. Chung *et al.* reported a high-throughput fluorescence assay where mammalian ACC activity was coupled to fatty acid synthase activity [19]. Recently Alves *et al.* developed a plate reader luminescence-based assay for assaying holo-ACC that monitored the depletion of ATP [14].

The previously developed assays each have significant limitations for screening of ACC inhibitors. The assays based on radiolabelling can be expensive and complex in practice due to health, waste and regulatory issues. The method based on LC-MS/MS provides comparable selectivity without the health and regulatory issues associated with radiolabelling, but it relies on complex methodology and expensive instrumentation. Assays based on enzyme-coupled reactions are inherently indirect and more susceptible to false positives for inhibition studies. Additionally, spectrophotometric assays based on UV absorbance or fluorescence with UV excitation are prone to spectral interference by inhibitors and biological sample matrices.

Recently, we developed a simple capillary electrophoresis-based assay for the CT component of ACC using UV absorbance detection [20]. This method was developed to overcome spectral interference for particular inhibitors and botanical extracts that could not be assayed using the enzyme-coupled spectrophotometric method employed for the rest of the study [21]. Capillary electrophoresis (CE) has been used for a wide array of applications, and CE has several advantages including high separation efficiency, simplicity, small sample volume (nL) and rapid analysis times [22]. It has been demonstrated that CE can be

used effectively for performing quantitative enzyme assays and studying enzyme inhibition [23,24]. Because CE is an electrophoretic separation technique, spectral interference is reduced by separation of substrates, products, inhibitors and sample matrix components.

Herein, we report an off-column capillary electrophoretic assay to measure holo-ACC activity and inhibition that allows for simultaneous monitoring of the BC and CT catalyzed reactions. To our knowledge, this is the first report of simultaneous, direct monitoring of ATP and acetyl-CoA depletion, and ADP and malonyl-CoA production for determination of holo-ACC activity. Also, the CE assay for CT [20] has been optimized, and a CE assay for the BC component of ACC has been developed.

2. Materials and Methods

2.1 Reagents and assay solutions

Acetyl coenzyme A, malonyl-CoA, ATP, ADP, biocytin, dimethyl sulfoxide, potassium phosphate monobasic, potassium bicarbonate, benzoic acid, magnesium chloride and sodium dodecyl sulfate were purchased from Sigma-Aldrich (St. Louis, MO). (2E,4E,6E)-N-((S)-3-(((S)-3-methyl-1-((3R,4S)-4-methyl-2,5-dioxopyrrolidin-3-yl)-1-oxobutan-2-yl)amino)-3-oxo-1-phenylpropyl)octa-2,4,6-trienamide (andrimid) was a gift from Pfizer. 2-amino-N,N-dibenzoyloxazole-5-carboxamide was synthesized according to the protocol published by Mochalkin *et al.* [3]. Tris(hydroxymethyl)aminomethane (tris) was purchased from Acros Organics (Morris Plains, NJ). N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) was purchased from EMD Millipore (Billerica, MA), and sodium phosphate and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Biotin carboxylase and carboxyltransferase from *Escherichia coli* were purified as described previously [21]. Biotin carboxyl carrier protein was a gift from Pfizer. All solutions were prepared using ultrapure water (> 18 MΩcm, ModuLab water system, United States Filter Corp., Palm Desert, CA). All buffers were filtered through a 0.2 μm nylon membrane (Whatman; Hillsboro, OR) prior to use for CE.

2.2 Capillary electrophoretic enzyme assays

All assays were performed using a P/ACE MDQ system with UV absorbance detection (photodiode array detector) from Beckman Coulter, Inc. (Brea, CA). The data were collected and analyzed using 32 Karat 5.0 software from Beckman Coulter, Inc. Fused-silica capillary (50-μm i.d./360-μm o.d.) was purchased from Polymicro Technologies (Phoenix, AZ) and cut to a total length of 59.8 cm. The polyimide coating was removed using a MicroSolv CE window maker (Eatontown, NJ) to make a 3-mm detection window at 49.8 cm (effective length). The capillary was rinsed with 1.0 M NaOH, ultrapure water and 20.0 mM sodium phosphate buffer at pH 7.55 for 10.0 min at 20 psi for conditioning at the beginning of each day. All reaction vials were held at 25 °C and the capillary was held at 25 °C. All injections were performed hydrodynamically at 0.5 psi for 10.0 s. All separations were performed using 30.0 kV (508 V/cm). All electropherograms were plotted with absorbance at 256 nm.

All enzyme assay reaction samples were prepared in 5.00 mM potassium phosphate buffer at pH 7.55 (reaction buffer). The capillary was rinsed with the separation buffer for 30.0 s at 20 psi prior to each injection. All enzyme reactions were performed off-column in a 1.5 mL glass Beckman CE vial in a 1.0 mL total volume.

2.3 Carboxyltransferase CE assay

The assay mixture contained 250.0 μM malonyl-CoA and 4.00 mM biocytin in the reaction buffer. To initiate the reaction, CT was added to the reaction mixture to a final concentration of 10.0 μg/mL. The assay mixture was injected for CE analysis after 1.0 min and again after

9.5 min. The CT assay was carried out using 5.0 mM potassium phosphate buffer at pH 7.55, which was also used for the separation buffer.

2.4 Biotin carboxylase CE assay

The BC assay mixture contained 50.0 μ M ATP, 2.5 mM $MgCl_2$, 50.0 mM biotin, 5.0 mM potassium bicarbonate and 200 μ M benzoic acid (used as an internal standard) in the reaction buffer. To initiate the reaction, BC was added to the reaction mixture to a final concentration of 20.0 μ g/mL, and the assay mixture was injected for CE analysis after 1.0 min and again after 12.0 min. The separation buffer contained 10.0 mM sodium phosphate and 20.0 mM sodium dodecyl sulfate at pH 7.55.

2.5 Holo-ACC CE assay

The holo-ACC assay mixture contained 25.0 μ M ATP, 50.0 μ M acetyl-CoA, 2.50 mM $MgCl_2$, 2.0 μ M BCCP and 5.0 mM potassium bicarbonate in the reaction buffer. The reaction was initiated by adding BC and CT to a final concentration of 10.0 μ g/mL in the assay mixture. The assay mixture was injected for CE analysis after 1.0 min and again after 9.5 min. 2-amino-N,N-dibenzylloxazole-5-carboxamide (212 μ M) or andrimid (30.0 μ M) was used for studying inhibition of holo-ACC. Both inhibitors were dissolved in DMSO, and the assay mixtures contained 0.3% DMSO for those experiments. During preliminary studies, it was concluded that DMSO did not interfere with the enzymatic reaction or the separation of the substrates, products, and inhibitors. After adding BC (10.0 μ g/mL) and CT (10.0 μ g/mL) to the assay mixture containing inhibitor, the mixture was injected for CE analysis at 1.0 min and at 11.5 min. The separation buffer for all assays contained 10.0 mM sodium phosphate and 20.0 mM sodium dodecyl sulfate at pH 7.55.

3. Results and Discussion

3.1 Optimized CE assay for carboxyltransferase

We previously developed an off-column capillary electrophoretic assay for the CT component of ACC using UV absorbance detection [20]. The assay used in that study was functional but had not been optimized. The buffers for the sample (enzyme reaction mixture) and CE separation were substantially different in terms of composition and conductivity, which can lead to relatively poor CE peak shapes and separations [25–27]. The assay for CT was optimized primarily by matching the separation and enzyme reaction buffers. Both the enzyme reaction and separation buffers contained 5.0 mM potassium phosphate at pH 7.55. The reaction was monitored in the nonphysiological direction (malonyl-CoA reacts to form acetyl-CoA), and the reaction mixture contained the substrates, malonyl-CoA (250 μ M) and biocytin (4.00 mM). Because the K_m of malonyl-CoA for CT is 100 M [21], 250 μ M malonyl-CoA was used. In bacteria, CT retains its activity using free biotin in place of the BCCP [1]. Biocytin, a more reactive analog of biotin where lysine is attached to the valeric acid carboxyl group (Fig. S1, Supplemental Material), is commonly used when running the CT assay in the nonphysiological direction [21]. Figure 2A shows an electropherogram for 250 μ M malonyl-CoA and 4.00 mM biocytin just prior to the addition of CT to initiate the reaction. A large malonyl-CoA peak is evident at 6.6 min. Figure 2B shows an electropherogram 9.5 min after the addition of CT (final concentration, 10 μ g/mL). A large acetyl-CoA peak with a migration time of 5.3 min is observed, and a very small malonyl-CoA peak is observed, indicating that the reaction was near completion. The peak shapes and separation of malonyl-CoA and acetyl-CoA were significantly improved relative to previous work as a result of optimizing the assay by better matching the sample buffer and separation buffer [20].

3.2 Development of a CE assay for biotin carboxylase

An off-column CE assay for BC also was developed. Although ACC normally functions with both enzymes as holo-ACC, it is important to have assays for BC alone and CT alone. For example, these assays can be used to determine which part of ACC is being inhibited by a molecule that inhibits holo-ACC. The assay reaction mixture contained ATP (50 μ M) and biotin (50 mM), as well as 2.5 mM MgCl_2 , 5.0 mM potassium bicarbonate and 200 μ M benzoic acid (internal standard). The K_m for ATP in isolated BC is 80 μ M [28], and an ATP concentration (50 M) was selected close to the K_m that resulted in a substrate peak with an adequate signal-to-noise ratio for the assay. Biotin is needed in the reaction mixture because BC catalyzes the carboxylation of biotin. Bicarbonate is the source of CO_2 for the carboxylation reaction, while MgCl_2 is needed because BC activity requires two equivalents of the metal ion. One equivalent is chelated to ATP such that the metal nucleotide chelate is the substrate for BC, while the other metal ion binds in the active site and participates in catalysis [29]. Figure 3A shows an electropherogram for 50 μ M ATP just prior to the addition of enzyme to initiate the reaction. Figure 3B shows an electropherogram at 12.0 min after the addition of BC (final BC concentration of 20 μ g/mL). An ADP peak with a migration time of 7.5 min is observed, indicating that the reaction is proceeding. The development of the separation conditions used for this assay is described in Section 3.3.

3.3 Development of a CE assay for holo-ACC

The first step in the development of a CE assay for holo-ACC assay was to separate all of the substrates (ATP, acetyl-CoA) and products (ADP, malonyl-CoA) that have significant absorbance at 256 nm. A number of separation conditions were tested with a goal of identifying a buffer system that would allow resolution of the four molecules and also would be appropriate for the enzyme-catalyzed reactions. Because ACC functions well from pH 7.0 and 8.0, buffers were tested that could be used in that pH range. When using HEPES buffer alone (10.0 and 20.0 mM) at pH 7.55 or HEPES buffer with 2.0 mM KCl at pH 7.55, the 4 peaks were not fully resolved and the ATP peak width would range from 15 to 30 s. The separation was also attempted using 20.0 mM tris at pH 8.00, but similar results were obtained. Sodium dodecyl sulfate (SDS) was added to the separation buffer to improve the separation based on previous reports of separations of ATP and ADP for off-column enzyme assays and other applications [30–34]; however, the 4 peaks were not resolved using 20.0 mM HEPES with 15.0 mM SDS at pH 7.55. Attempts were made to add SDS to 20.0 mM HEPES buffer (pH 7.55) containing 2.0 mM KCl since the reaction buffer contained KCl, but the SDS precipitated out of solution upon KCl addition. Next, 10.0 mM sodium phosphate at pH 7.55 was used for the separation, but only 2 of the 4 peaks were resolved (Fig. 4A). Finally, resolution of all 4 peaks was achieved using 10.0 mM sodium phosphate with 20.0 mM SDS at pH 7.55, as shown in Figure 4B.

The substrate concentrations used for the holo-ACC assay were different than the concentrations used for assaying the individual components, BC and CT. The K_m for ATP in holo-ACC is 4 μ M (T.C. Broussard and G.L. Waldrop, unpublished observations); however, a substantially higher ATP concentration of 25 μ M was required to obtain an adequate signal-to-noise ratio. While the K_m for acetyl-CoA in holo-ACC is 0.5 mM, the concentration of acetyl-CoA used in the CE assay was only 50 M because the ATP concentration was only 25 μ M. The second half-reaction is limited by the amount of product produced in the first half-reaction (Figure 1), and higher concentrations of acetyl-CoA would not result in more malonyl-CoA production because ATP is the limiting reagent in this two-step reaction. Additionally, the reaction solution contained 2.5 mM MgCl_2 , 2.0 μ M BCCP, and 5.0 mM potassium bicarbonate, but it did not contain SDS, which would denature the proteins. Figure 5A shows an electropherogram for 25 μ M ATP and 50 μ M acetyl-CoA just prior to the addition of enzyme to initiate the reaction. Figure 5B shows an

electropherogram 9.5 min after the addition of BC and CT (final concentration of 10 $\mu\text{g/mL}$ each). An ADP peak is observed with a migration time of 6.2 min, and a malonyl-CoA peak is observed with a migration time of 7.3 min, indicating that the reaction is proceeding. Furthermore, the necessary differences between the reaction solution and the separation buffer did not significantly degrade the separation as indicated in Figure 5B.

Two well documented inhibitors of BC and CT were used to determine the effectiveness of the new CE assay for detecting inhibition of holo-ACC. The BC inhibitor used as a positive control was 2-amino-N,N-dibenzylloxazole-5-carboxamide (Fig. S1), which was developed by Pfizer as an antibiotic [3]. This inhibitor concentration was tested first at 212 M, which is well above the IC_{50} value of 0.125 M reported by Mochalkin *et al.* [3]. The inhibitor has a strong absorbance at 256 nm, so 3 peaks are observed instead of the usual 2 for ATP and acetyl-CoA prior to the addition of the two enzymes (Fig. 6A). It is important to note the strong absorbance of 2-amino-N,N-dibenzylloxazole-5-carboxamide. If the inhibitor, substrate, and product absorb at the same wavelength, the inhibitor would significantly interfere with traditional cuvette-based or plate reader-based assays due to spectral interference. Because CE is a separation technique, the inhibitor can be physically separated from the substrates and products, eliminating spectral interference. After adding CT and BC to the reaction mixture and allowing the reaction to proceed for 11.5 min, no product formation was observed, indicating strong inhibition (Fig. 6B). This inhibitor was also tested near the IC_{50} value at 0.20 M using the same reaction time (11.5 min) and conditions. Product formation was observed at this concentration, but it was significantly reduced compared to experiments without inhibitor. The ratio of peak areas for ADP/ATP decreased from 1.2 (no inhibitor) to 0.57 (0.20 M 2-amino-N,N-dibenzylloxazole-5-carboxamide). Corresponding ratios for malonyl-CoA/acetyl-CoA were 0.79 (no inhibitor) and 0.55 (0.20 M inhibitor). The IC_{50} was not determined here, but other studies have shown that this is possible using off-column CE assays [23].

Inhibition of holo-ACC using a known CT inhibitor, andrimid, also was studied [2]. Andrimid (Fig. S1) is a natural product isolated from a marine isolate of *Pseudomonas fluorescens* that exhibits broad spectrum antibacterial activity [35]. A concentration of 30 μM was used, which is well above the IC_{50} value of 13 nM, reported by Freiberg *et al.* [2]. Electropherograms before addition of CT and BC and at 11.5 min after initiation of the reaction are shown in Fig. 7. No product formation was observed. Unlike the BC inhibitor, andrimid does not absorb strongly at 256 nm. These results with inhibitors of both BC and CT are important because they show the assay can detect inhibition of bacterial holo-ACC, regardless of which half reaction the inhibitor is targeting.

4. Conclusions

The first off-column capillary electrophoretic assay for holo-ACC that allows for simultaneous monitoring of the holo-ACC components, BC and CT, is described. For this assay, acetyl-CoA, malonyl-CoA, ATP, and ADP were separated by CE. The assay allows for simultaneous screening of inhibitors against BC and CT by directly monitoring the depletion of ATP and acetyl-CoA, and the concomitant production of ADP and malonyl-CoA. A CE assay for the BC component of ACC and an optimized CE assay for the CT component of ACC have been demonstrated. The CE assays for holo-ACC and the individual components BC and CT eliminate disadvantages that have been associated with previously developed ACC assays such as, spectral interference, the use of radioactive materials, consumption of large sample amounts, and indirect monitoring, which could lead to false positives. One drawback of this CE-based method is that the ATP and acetyl-CoA concentrations were limited to 50 M and above because UV absorbance detection was used with a 50 m internal diameter capillary. While several of the previously published assays for

holo-ACC used ATP and acetyl-CoA concentrations above 50 M, it is desirable to have the capability to use substrate concentrations below 50 M. Because ACC is a target for the development of therapeutic agents for metabolic syndrome (including obesity and type 2 diabetes) and cancer as well as antibiotics and herbicides, this assay will be beneficial for efficient inhibitor screening and could lead to new discoveries of antibiotics, herbicides, and treatments for metabolic syndrome and cancer. Finally, the development of methods that can directly monitor reactants and products for multiple enzyme-catalyzed reactions will have impact beyond studying ACC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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^aPi: Inorganic phosphate

Fig. 1.
Acetyl-CoA carboxylase reaction scheme.

Fig. 2.

Electropherograms for the CT assay. (A) The assay reaction mixture contained 250 μ M malonyl-CoA and 4.00 mM biocytin prior to addition of the enzyme. (B) At 9.5 min after addition of CT, a large acetyl-CoA peak was observed (product formation), and the malonyl-CoA peak had decreased. The sample buffer and separation buffer were both 5.0 mM potassium phosphate at pH 7.55. All injections were made at 0.5 psi for 10.0 s. The field strength for CE was 508 V/cm with a current of 11.1 μ A.

Fig. 3.

Electropherograms for the biotin carboxylase assay. (A) The reaction mixture contained 50 μ M ATP, 2.5 mM MgCl_2 , 50 mM biotin, 5.0 mM potassium bicarbonate and 200 μ M benzoic acid prior to addition of the enzyme. (B) At 12.0 min after addition of BC, an ADP peak is observed (product formation). The sample buffer was 5.0 mM potassium phosphate at pH 7.55, and the separation buffer was 10.0 mM sodium phosphate at pH 7.55 with 20.0 mM SDS. All injections were made at 0.5 psi for 10.0 s. The field strength for CE was 508 V/cm with a current of 26.8 μ A.

Fig. 4.

Electropherograms for separation of holo-ACC assay components. (A) Separation of a mixture of acetyl-CoA, malonyl-CoA, ATP and ADP (25 μ M each). The sample and separation buffer was 10.0 mM sodium phosphate at pH 7.55. (B) The same sample separated using 10.0 mM sodium phosphate, pH 7.55 with 20.0 mM SDS as the separation buffer. All injections were made at 0.5 psi for 10.0 s. The field strength was 508 V/cm with a current of 19.0 μ A for (A) and 25.2 μ A for (B).

Fig. 5. Electropherograms for the holo-ACC assay. (A) The reaction solution contained 25 μ M ATP, 50 μ M acetyl-CoA, 2.5 mM MgCl_2 , 2.0 μ M BCCP, and 5.0 mM potassium bicarbonate prior to the addition of 10.0 μ g/mL of CT and 10.0 μ g/mL of BC to initiate the reaction. (B) At 9.5 min after initiation of the reaction (addition of CT and BC), ADP and malonyl-CoA peaks were observed (product formation) and ATP and acetyl-CoA had decreased. The sample buffer was 5.0 mM potassium phosphate at pH 7.55 and the separation buffer was 10.0 mM sodium phosphate at pH 7.55 with 20.0 mM SDS. All injections were made at 0.5 psi for 10.0 s. The field strength for CE was 508 V/cm with a current of 24.6 μ A.

Fig. 6.

Electropherograms for the holo-ACC assay with 2-amino-N,N-dibenzylloxazole-5-carboxamide (A-O). (A) The reaction mixture contained 212 μM 2-amino-N,N-dibenzylloxazole-5-carboxamide, 25 μM ATP, 50 μM acetyl-CoA, 2.5 mM MgCl_2 , 2.0 μM BCCP, and 5.0 mM potassium bicarbonate prior to the addition of addition of 10.0 $\mu\text{g/mL}$ of CT and 10.0 $\mu\text{g/mL}$ of BC to initiate the reaction. (B) At 11.5 min after initiation of the reaction there was no product formation observed. All other conditions are the same as in Fig. 5.

Fig. 7.

Electropherograms for the holo-ACC assay with andrimid. (A) The reaction mixture contained 30 μ M andrimid, 25 μ M ATP, 50 μ M acetyl-CoA, 2.5 mM MgCl_2 , 2.0 μ M BCCP, and 5.0 mM potassium bicarbonate prior to the addition of 10.0 μ g/mL of CT and 10.0 μ g/mL of BC to initiate the reaction. (B) At 11.5 min after initiation of the reaction, no production formation was observed. All other conditions are the same as in Fig. 5.