

Label-Free Screening of Stearoyl-Coenzyme A Desaturase from an Enriched Rat Liver Fraction

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Abstract

Stearoyl-coenzyme A desaturase (SCD) is an important drug target with relevance to a variety of metabolic disorders. Unfortunately, difficulties in generating and isolating active enzymatic preparations, as well as problems developing practical screening techniques, have impeded progress in the development of viable drug candidates. To date, identification of new lead compounds showing inhibitory activity have been limited to radiometric (3H) assays, involving the generation of tritiated water. These protocols are time-consuming, expensive and generate significant amounts of radioactive waste precluding large scale screening efforts. Using an SCD enriched fraction from rat liver, we have been able to develop an effective high-throughput screening methodology utilizing a proprietary high-throughput mass spectrometry system as a detection tool. Mass spectrometry allows for the direct, quantitative detection of native, label-free enzymatic substrates and products (i.e. stearoyl-CoA/oleoyl-CoA) facilitating functional assay screening of previously intractable targets.

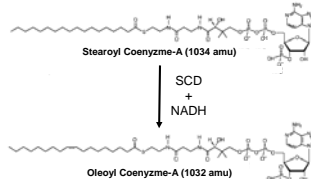
Native Stearoyl Coenzyme-A Desaturase Isolated from Fast-Fed Rat Liver

(Blue Sky Biotech, Inc. Cat #: 1091-003)

The enzyme is isolated using a multi-step extraction/ enrichment procedure and livers of Fast-Fed Rats. This product contains background proteins that co-purify with Stearoyl Coenzyme-A desaturase but do not negatively affect the activity.

Stearoyl Coenzyme-A Desaturase

The SCD enzyme converts stearoyl coenzyme-A into oleoyl coenzyme-A through a cytochrome B₅ mediated pathway



Materials

Stearoyl Coenzyme-A Desaturase	BlueSky Biotech*
Stearoyl Coenzyme-A	Sigma-Aldrich
Oleoyl Coenzyme-A	Sigma-Aldrich

*SCD enzyme was purified from fast-fed rat livers by BlueSky Biotech, Inc.

Assay Buffer

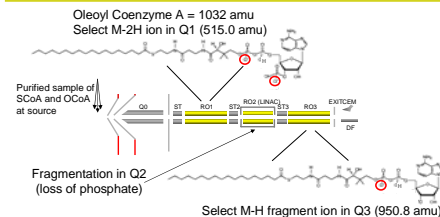
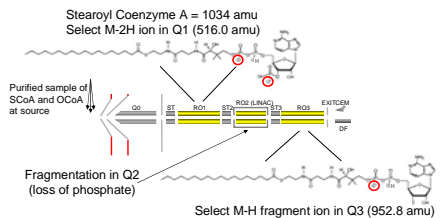
150 mM KCl	5 mM MgCl ₂
40 mM NaF	100 mM NaPO ₄ , pH 7.4
1.5 mM GSH	20 μM SCoA
200 μg/mL SCD enzyme	0.7 mg/mL NADH

Assay Conditions

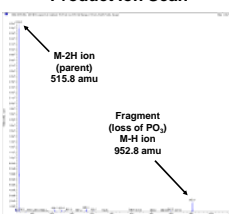
- Analytical methods for the detection of SCoA and OCoA were developed using BioTrove's proprietary RapidFire™ high-throughput mass spectrometry system
- Throughput = 1 sample per 7 seconds
- Reactions were run at room temperature
- Reactions were quenched with the addition of 9x volumes of 100 mM NaPO₄, pH 7.4; 0.5 N HCl; 0.05% Triton X-100 prior to analysis with the RapidFire system
- Addition of detergent helps solubilize the SCoA and OCoA from the microsomal membranes and increases the observed MS signal

Detection of SCoA and OCoA by MS

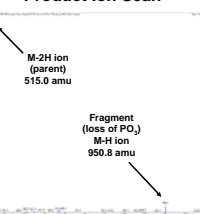
- The small difference in mass (2 amu) between SCoA and OCoA creates a challenge for simultaneous detection and analysis was done using electrospray ionization in negative ion mode
- Quantification of SCoA and OCoA was performed using multiple reaction monitoring (MRM) mode



Stearoyl Coenzyme-A Product Ion Scan

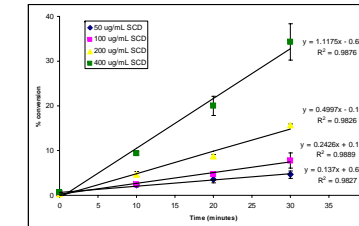


Oleoyl Coenzyme-A Product Ion Scan



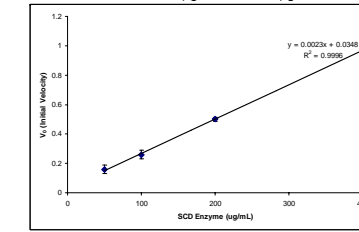
Time dependence

- 20 μM SCoA substrate, room temp incubation
- Reaction rates are linear up to 30 minutes



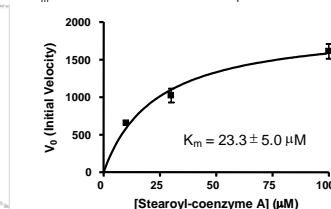
Enzyme dependence

- 20 μM SCoA substrate, room temp incubation
- Conversion is linear with respect to enzyme concentration between 50 μg/mL and 400 μg/mL SCD



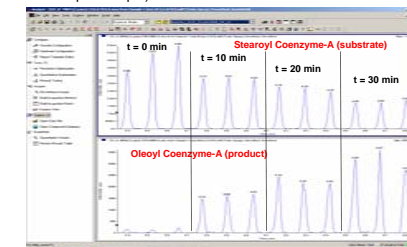
Substrate dependence

- 200 μg/mL SCD enzyme, room temp incubation
- K_m for SCoA determined as 23.3 μM



Example RapidFire HTMS Data

- Disappearance of SCoA and appearance of OCoA in a time dependent manner is shown (n=3)
- 12 samples have been analyzed in 1.4 minute (throughput of 7 seconds per sample)



Conclusions

- We have developed an assay using RapidFire high-throughput mass spectrometry that can be used for the discovery of inhibitors against SCD
- The assay has a sustained throughput of 7 seconds per sample
- We have determined that the conversion of SCoA to OCoA by the SCD enzyme is time, enzyme concentration, and substrate concentration dependent
- The assay can be easily adapted for HTS

BlueSky Biotech, Inc.

The mission of Blue Sky Biotech is to relieve scientists from the onerous and labor-intensive tasks that take them away from key experimentation, novel theory exploration, and other optimal uses of their time. Blue Sky is synonymous with *productivity* in the Early Discovery Biology processes known as genetic cloning, recombinant protein expression, and laboratory-scale fermentation (scale-up bioprocessing). We are motivated by our ability to impact the overall efficiency of the drug discovery process, thus contributing to a longer, healthier lifespan for human beings. 1(800)383-7795

BioTrove's RapidFire™ Lead Discovery Service

BioTrove scientists collaborate with researchers to design mass spectrometry-based high-throughput screening assays. These assays enable the identification of lead compounds against drug targets that may have previously been thought to be too expensive or technically impossible to screen. Over the past few years in multiple campaigns, BioTrove scientists have developed dozens of assays and leveraged mass spectrometry to screen millions of test compounds. 1(781)721-3605