CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

## *Cholinergic Enzymes and Carnitine*

**Detection of Acetyl Coenzyme A, Coenzyme A, Carnitine, Acetylcholinesterase, and Choline Acetyl Transferase**

**Purpose**

Discuss the feasibility of using LCEC for the detection of coenzyme A (CoASH), acetyl coenzyme A (CoASAc), carnitine, acetylcholinesterase (also called cholinesterase, AChE), and choline acetyl transferase (CAT). The latter two enzymes catalyze hydrolysis of acetylcholine or the synthesis of acetylcholine (choline + CoASAc ---> acetylcholine + CoASH).

**Existing Methods**

There are existing UV methods for detecting CoASH and CoASAc, but these are not very selective. There is no convenient assay for carnitine of which we are aware. The existing assay for AChE results in the formation of a colored product. Many common constituents of the sample will interfere with color formation or will react themselves to form the colored product. The assay for CAT depends upon the formation of a radioactive product, which has to be separated from the labeled precursor before it can be counted. All this requires extensive sample handling.

**LCEC Methods**

***For Coenzyme A and Acetyl Coenzyme A:***

CoASH contains a free thiol and should be detected selectively and with good detection limits on an Hg/Au electrode. The chromatography would have to be worked out.

CoASAc is another problem, but the following reaction would produce the free thiol for detection.

$$\text{Oxaloacetic Acid + CoASAc} \xrightarrow[\text{synthetase}]{\text{citrate}} \text{citric acid + CoASH}$$

The method for analysis would proceed something like this: extract the tissue with buffer (pH 5.0 or so), add $HClO_4$ to get a protein-free supernatant, and inject. This gives you the amount of CoASH. An aliquot of the buffer extract would be incubated with citrate synthetase (under the proper conditions). The reaction will proceed far to the right, and so is very favorable for the formation of free CoASH. A protein-free supernatant is obtained and injected. The increase in the CoASH peak would indicate the amount of CoASAc in the original extract (free CoASH + CoASH formed from CoASAc).

***For Carnitine:***

Carnitine would be extracted from the tissue and an aliquot incubated, under appropriate conditions, with carnitine acetyl transferase. This enzyme catalyzes the following reaction:

$$\text{CoASAc + Carnitine} \leftrightarrow \text{CoASH + Acetylcarnitine}$$

CoASH would be determined as above. Carnitine can not be detected via the enzymatic production of $H_2O_2$; at least using choline oxidase.

***For Acetylcholinesterase:***

This enzyme would be extracted from the tissue under conditions conducive to maintaining its enzymatic activity. An aliquot would be incubated with the substrate acetylthiocholine (again in the appropriate medium). The enzyme would catalyze the following reaction:

Acetylthiocholine + AChE ---> AChE + Thiocholine + Acetic Acid

The thiocholine contains a thiol group and is thus detectable at an Hg/Au electrode. The enzyme reaction would be cleared of proteins by $HClO_4$ precipitation and the supernatant injected.

The chromatography would be similar to that used for the thiols.

***For Choline Acetyl Transferase:***

This enzyme would be extracted from the tissue and an aliquot incubated in a medium containing, among other things, choline and CoASAc. CAT catalyzes the following reaction:

CoASAc+ Choline ---> Acetylcholine + CoASH

The amount of CoASH in a protein-free supernatant would be determined as outlined above.

**Notes**

None of these assays have been worked on at BAS. This preview was prepared because there has been a fair amount of interest in these compounds and enzymes from such areas as pharmaceutical companies and laboratories involved with nerve gases and brain functions (Armed Forces funded). These analyses should be do-able by combining enzymology with LCEC. The products of the enzymatic reactions are similar to analytes for which we have documented analysis procedures. Further, the enzymes and chemicals used in these proposed assays are available from commercial sources.

Laboratories investigating Alzheimer's disease are definitely interested in the assay for CAT and AChE, plus choline and acetylcholine turnover.

Carnitine is involved in fatty acid catabolism, muscle movement, biochemical energy transductions, etc. Also, it is used as a food additive.

CoASH is a molecule central in metabolism. It is involved in fatty acid metabolism, protein synthesis, group transfer reactions, energy transductions, etc.

**Related Reference**

1. LCEC Capsule 142 and 155 (assay for AChE and CAT, respectively).

2. LCEC Capsule 165 and 166 (assay for acetylcholine and choline).