CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determining Muscle Injury

Purpose

Muscle is a complex mixture of proteins, amino acids, lipids, glycogen, etc. The proteins consist of enzymes, which are largely involved with glycolysis, and non-enzyme proteins, which are involved in the contractile process.

The contractile proteins are made up mainly of two complex molecules, myosin and actin. Myosin is a high molecular weight polymer (500,000 daltons) consisting of two identical heavy chains and four light chains (i.e. polypeptide chains). Actin is a 42,000-dalton monomer. Both myosin and actin assemble (polymerize) into filaments under physiological conditions. The myosin and actin filaments combine to give the characteristic striations found in skeletal muscle, as seen under an electron microscope. The thick filament contains primarily myosin, while the thin filament contains actin.

Some time after the polypeptide chains of actin are synthesized, the histidine moiety is methylated. It does not appear that 3-methylhistidine is directly incorporated into the polypeptide. This 3-meHis in actin gives an internal tag for looking at the catabolism (breakdown) of muscle tissue.

After an accident involving muscle injury the chains of actin and myosin are broken down and hydrolyzed to their component amino acids. This releases 3-meHis into the plasma and urine. By quantitating the amount of 3-meHis released the extent of muscle damage can be ascertained.

Existing Methods

One existing method of determining 3-meHis employs ion-exchange chromatography and takes about 1 1/2 hours per run. This excludes sample handling time. Also, detection limits are rather poor, 200 pmoles injected. Another method uses pre-column derivatization with fluorescamine. Reverse-phase chromatography employing a concave gradient of sodium phosphate (pH 7.5) and acetonitrile was employed to separate the derivatives. This pH will of course drastically shorten the life of the column.

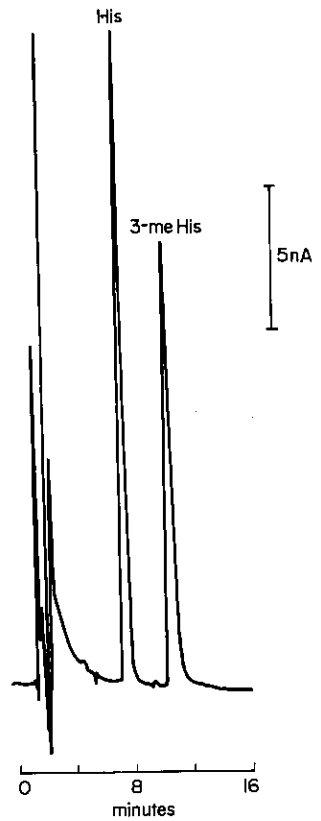

Figure 1. Chromatogram of OPA/t-BuSH derivatives of a standard solution of histidine (His) and 3-methylhistidine (3-meHis). The injection volume was 50 µL containing 366 pmoles His and 480 pmoles 3-meHis.

Notes

LCEC analysis of the OPA derivative of 3-meHis is a rapid and sensitive alternative method. Sample

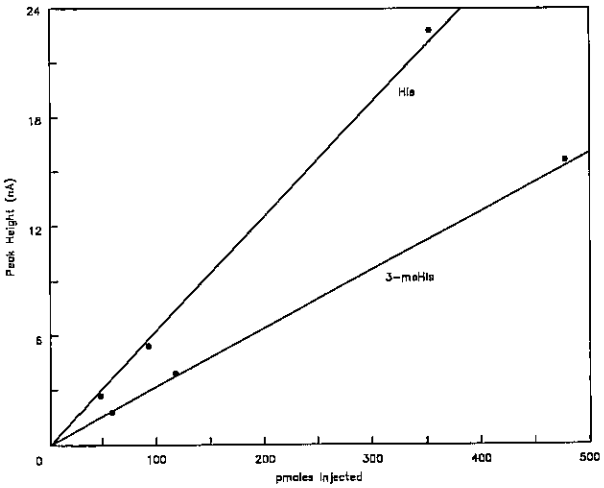

Figure 2. Linearity of LCEC analyzer for His and 3-meHis standards.

preparation should also be short and simple, employing a perchloric acid or methanol extraction (precipitation) similar to the method outlined by L.A. Allison, G.S. Mayer and R.E. Shoup in Anal. Chem. 56(1984) 1089-1096.

Conditions

System: LC-154T
Column: Biophase ODS, 250 x 4.6 mm
Mobile Phase: 0.1 M sodium acetate, pH 5.0, 15% with respect to acetonitrile. Flow was 1.3 mL/min.
Electrode: Glassy Carbon
Potential: +0.70 V vs. Ag/AgCl
Sample Volume: 50 µL

References

This work was carried out in the BAS demonstration lab and has not been verified by the BAS R&D Lab. Refer to the paper cited above for details of the OPA/t-BuSH derivatization reaction and detection limits.

Comments

This preliminary study was carried out using standards of His and 3-meHis to test the feasibility of derivatizing 3-meHis and of separating the derivative from the His derivative. Note that the chromatography will have to be adjusted to fit the sample matrix where 3-meHis is to be quantitated.

Related References

1. *W.A. Jacobs, Current Separations, 7 (1986) 39.*

2. *D. Pallister, Current Separations, 8 (1987) 53.*