

## LAB 1

### Separation and Identification of Amino Acids

#### INTRODUCTION

##### 1.1 What are amino acids?

Amino acids are the fundamental building blocks from which all proteins are made. They all share certain structural characteristics, having an  $\alpha$ -amino group, an  $\alpha$ -carboxyl group, and a side chain or R group, which is unique for each amino acid.

Research done by an international team of scientists from Colombia, Germany, USA and Canada shows that many ecological studies like finding the eating habits of migratory birds can be done by doing the amino acid analysis of the feathers, claws and blood of the migratory birds (1). A team of researchers from Japan has studied the importance of D-amino acids as biomarkers in various mammalian tissues (2). A research out of University of Chicago has shown that amino acid analysis can also be used to characterize the chemical content of the extracellular fluids and the data can be used to probe the neurochemical signaling networks in brain (3).

The enormous number of different proteins existing in living cells are all linear polymers of just 20 amino acids, covalently joined by peptide bonds. Once polymerized in this way, the  $\alpha$ -amino and  $\alpha$ -carboxyl groups of the amino acids are no longer ionizable, being tied up in peptide bonds, and the chemical and physical properties of the amino acid R groups become of paramount importance. The R groups of the 20 amino acids cover a huge variety of molecular structure and physico-chemical properties. Some are positively charged at physiological pH, some carry no net charge, and some are negatively charged. Within these subgroups, some are relatively polar or hydrophilic, while others are quite nonpolar or hydrophobic.

It is these R groups, and the interactions between them, that determine the folding pattern of a protein, and thus its unique 3-dimensional conformation. This arrangement in space in turn controls the unique biological function of each protein, whether it be enzymatic activity, binding of small molecules or ions, electron transfer, etc.

##### 1.2 Separation and Identification of amino acids

A common first step in the investigation of any protein is the analysis of the **amino acid composition and amino acid sequence**, which are unique to that protein. Thus separating and identifying amino acids is a very important tool in the protein biochemist's arsenal of analytical techniques. The ability to separate different amino acids depends on a complete knowledge of their physico-chemical properties, such as acid-base behaviour, polarity etc.

One very commonly used technique for separating amino acids is **ion-exchange chromatography, which separates the amino acids on the basis of their charge**. An ion-exchange resin consists of beads of a synthetic (or natural) polymer, to which are covalently attached certain charged functional groups, such as  $-\text{SO}_3^-$ , or  $-\text{NR}_3^+$ . Counterions, such as  $\text{Na}^+$  or  $\text{Cl}^-$ , are present to balance the charge on the beads. These counterions can be exchanged for other ions of the same charge, leading to the term **ion-exchange**.

When a mixture of molecules carrying different charges is added to a column of these charged beads, those molecules with a charge opposite to that of the beads can displace the counterions, and bind to the beads. Molecules with the same charge, or with no net charge, fail to bind, and will pass through the

# Amino Acid Lab Report

**Douglas C. Somerville**



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