

## **Enzymatic hydrolysis of cytochrome and acetylated cytochrome**

### **Materials:**

cytochrome C (from horse heart)  
acetylated cytochrome, lyophilized from experiment 1  
trypsin (porcine, crystalline)

### **Methods:**

enzymatic digestion  
mass spectrometry

### **Theory:**

1. Proteolytic enzymes – classes and mechanisms of action.
2. Substrate specificity of proteases.
3. Proteases and their inhibitors.
4. Determination of primary structure of proteins.
5. Mass spectrometry of proteins and peptides: ESI-MS method.
6. Protein databases and sequence analysis.
7. Physicochemical aspects of lyophilization.

### **Introduction:**

1. Find the cleavage/digestion points for trypsin and other proteases in the sequence of cytochrome C.
2. Locate and download the sequence of porcine trypsin. Find the cleavage/digestion points for autodigestion of trypsin.
3. Find the webpage of MASCOT program and learn about its applications.
4. Find the applications of proteolytic enzymes in everyday life and medicine.

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### **Experimental:**

#### **Ammonium acetate buffer**

Dissolve 1.58 g of  $\text{NH}_4\text{HCO}_3$  in 50 ml HPLC grade water. The concentration of obtained buffer is 0.2 M and pH is approx. 8.5.

#### **Trypsin solution (0.1%)**

Dissolve 1mg of trypsin in 1 ml of HPLC grade water.

#### **Enzymatic hydrolysis**

Dissolve 2 mg of cytochrome C in 1 ml of 0.2 M ammonium acetate buffer (pH 8.5). Add 20  $\mu\text{l}$  of freshly prepared 0.1% trypsin solution. Transfer the 200  $\mu\text{l}$  samples of cytochrome-enzyme mixture after 30, 60, 90 and 120 min of incubation, and quench the reaction in the collected samples by adding 200  $\mu\text{l}$  of 1M acetic acid. Freeze and lyophilize obtained samples. Submit the dried samples for ESI-MS analysis.

Use the same procedure to digest the sample of acetylated cytochrome C from previous experiment.

### **Report (part B):**

1. Short description of the experiment.
2. Taking into account the specificity of trypsin, predict the cleavage points in cytochrome C sequence.
3. Prepare the m/z prediction for cytochrome C digest fragments.