Chemical modification of cytochrome C

Materials:

cytochrome C

acetic anhydride

acetic acid

acetate buffer

Sephadex G-50

Methods:

chemical modification of cytochrome

column gel chromatography – size exclusion

mass spectrometry

Theory:

1. Chemical modifications of proteins: reagents and their target groups.
2. Chemical modifications of proteins: analytical methods.
3. Chemical modifications of proteins: applications.
4. Chromatographical methods: size exclusion gel chromatography. Order of elution.
5. Mass spectrometry of proteins: ESI-MS method.
6. Protein databases and sequence analysis.

Introduction:

1. Locate and download the sequence of cytochrome C (from horse heart). Analyze the sequence and select the functional groups which can react with acetic anhydride.
2. Write the reaction between the cytochrome (represented by selected amino acid side chain) and acetic anhydride. How many amino acid residues could be modified by acetic anhydride in cytochrome?
3. Why is the column chromatography used in this experiment? What other methods could be used for this purpose?
4. Think about the detection method applicable for column chromatography of acetylated cytochrome.
5. Taking into account the molecular weight of cytochrome and the character of ESI ionization, calculate the m/z values for multiple charged cytochrome C (substrate) and the series of possible acetylated cytochromes (e.g. Excel spreadsheet).

Chemical modification of cytochrome C

Experimental:

Column preparation:

The suspension of Sephadex G-25 was prepared 24h before the experiment. Place the loose plug of cotton wool at the bottom of the column. Pour the suspension of Sephadex into the column, to form a gel bed of about 30 cm. Stabilize the gel by washing with 1% acetic acid in water (appr. 300 ml of acetic acid solution is required to prepare the column for experiment). The eluent layer over the Sephadex bed should be carefully maintained to prevent the drying of the column.

Cytochrome modification:

Place the specified amount of cytochrome C into vial, add 2 ml of 1M acetate buffer and agitate the vial till the cytochrome dissolves. Cool the vial in ice and add the specified amount of acetic anhydride solution. Stopper the vial and incubate the mixture at 4° for 1 hour.

When the incubation of cytochrome is complete, let the eluent level in the column drop to the gel level and close the column. Using Pasteur pipette, transfer the contents of the vial on top of the gel bed in the column (after removing the excess of eluent to avoid dilution of the sample), let it sink into the Sephadex bed, wash the vial with 1 ml of 1% acetic acid and add the residual solution on the column. Keep adding small volumes of eluent till the cytochrome layer is well within the column bed, build the eluent layer in the column and continue the chromatography till the cytochrome layer is eluted. Wash the column thoroughly with 1% acetic acid.

Measure the volumes of the fractions (dead volume and cytochrome fraction), transfer the cytochrome solution into Falcon vial and freeze the solution in preparation for MS experiment.

Report (part A):

1. Short description of the experiment.
2. Calculate the molar ratio of acetic anhydride to cytochrome.
3. Prepare the m/z prediction for acetylated cytochrome.