BIOL 1104 Biological Techniques, Instrumentation and Data Processing

Experiment Laboratory Report

Name: mak nga ying
U.no: 2003273244
Group no: 5b
Date: 13-11-2003
Tittle: ion- exchange chromatography

Objective:

a. to know how the ion-exchange chromatography run
b. to determine which concentration of the ammonium hydroxide can elute the most tyrosine, thought the gradient elution technique.

Introductions:

the ion-exchange chromatography can separate the protein molecule, according to their molecular change. There are two phase present, which is a mobile phase and stationary phase. The mobile phase refer to concentration gradient of the ammonium hydroxide solution. the stationary phase just refer to the ion exchangers. The ion exchangers can exchange with the ion in the sample protein solution. there are two types of exchangers: cation exchangers and anion exchangers.

The group below demonstrate the process of the anionic exchange. At first, the chloride ion attaches on the A^+ ion on the anionic exchangers, later after the addition of the sample ion, chloride ion is replaced by X^- . the X^- are attached on the A^+.

<u>Graph that illustrate the process of anionic exchange</u>

later three methods can be used to elute ion from the exchangers: changing pH of the buffer, increasing the ionic strength and performing an affinity solution. When the pH or the ionic strength are changed, the electrostatic binding between the absorbed ion and the exchanger are weakened. The absorbed ion can be eluted. The affinity solution weaken the electrostatic interaction between the ion and the exchangers by lowering the net charge on the macromolecules, so that It can also elute the ion out.

Lastly, the amount of tyrosine eluted out is determined by the absorbance. The absorbance is directly proportional with the amount of tyrosine.

Result:

The volume of the tyrosine used : 0.5ml

Tube no.	Conductivity (1000 uS)	Absorbance of tyrosine at 280nm	Tube no.	Conductivity (1000 uS)	Absorbance of tyrosine at 280nm
1	33.5	0.007	11	38.9	0.236
2	36.0	0.017	12	39.2	0.137
3	36.3	0.006	13	39.4	0.021
4	36.7	0.022	14	39.7	0.003
5	37.1	0.009	15	39.9	0.005
6	37.4	0.020	16	40.2	0.001
7	37.8	0.051	17	40.3	0.055
8	38.1	0.007	18	40.4	0.020
9	38.5	0.008	19	40.6	/
10	38.7	0.043	20	40.7	0.034

Table that show the conductivity and the relative absorbance of the tubes

From the table, there is the highest absorbance at tube 11,
the respective conductivity is 38.9uS.
by the equation, $y= 5.758x + 31.778$:
where y is the conductivity and x is the concentration of the ammonium hydroxide that can elute most tyrosine.

$(38.9)=5.758\,x +31.778$

$x=1.24$ M.

Therefore 1.24 M ammonium hydroxide can elute most tyrosine.

Discussions:

The reason for why the tyrosine(Y) are changed to tyrosine ions(Y^+)

the amino acid has no net charge when it is on the isoelectric point. the pH of the cation-exchange column is adjusted to pH 4 by addition of the hydrochloric acid. In this slightly acidic environment, hydrogen ions are released. then the tyrosine become the tyrosine positive ions (Y^+).

About the process of the cation –exchange chromatography.

In this experiment, the tyrosine is changed to the tyrosine ion, which is positively charged .A cation exchangers are used for interacting with these tyrosine ions. at first, the cation-exchangers bind with the Na^+ ions and the Na^+ ions act as a counterion. Later, after the addition of the tyrosine, the tyrosine are changed to tyrosine ions. these ions are exchanged with the sodium ions and they bind with the C^- ions instead. The process is illustrated by the graph below. The tyrosine ions (Y^+) are said to absorb the cation-exchanges.

Graph show the tyrosine (Y) change to tyrosine ion (Y^+)

About the gradient elution setup

After the binding of the tyrosine ion to the cation exchangers, the ion should be eluted out by changing the ionic strength. It can be changed though increasing the concentration of the salt(i.e ammonium hydroxide) in the elution solution. in this experiment, the concentration of the elution solution is continuously changed by using the follow set up.

the set up for making the gradient elution solution

there are two buffer, A has 30ml distilled water and B has the 30 ml ammonium hydroxide solution(2 M). the ammonium hydroxide solution mix with the distilled water and the mixture enter the cation-exchanger column. There is a magnetic stirrer in A that can mix the distilled water and the salt well. Then the concentration of the salt in the column is rise from 0-2 M.

the ammonium hydroxide salt is alkaline, so the pH in the column increase when the concentration of the salt increase. The change in pH alter the net charge of the tyrosine ion and weaken the electrostatic bonding between the tyrosine ion (Y^+) and the cation exchangers. At a certain value, the tyrosine will be eluted out from the column.

However, different salt concentration can make different amount of tyrosine elute out. The concentration of the salt is monitored by the conductivity meter since the NH_4^+ and OH^- provide the conductivity.

the absorbance act as a indicator for the amount of the tyrosine. Since the tyrosine has the aromatic ring, it can absorb light at 280nm. The absorbance is directly proportional to the concentration of the tyrosine. In this experiment, 1.24M of the ammonium salt can elute the largest amount of the tyrosine.

<u>conclusion</u>

1.24 M ammonium hydroxide can elute the largest amount of the tyrosine in the cantion-exchange chromatography.