

Biological Chemistry I

A solution containing eight kinds of globular proteins was injected into a size-exclusion (or gel) chromatography (SEC) column and then eluted as shown in Figure 1. Proteins in each peak fraction were analyzed and assigned as listed in Table 1. Answer the following questions under the assumption of no interactions among different protein molecules.

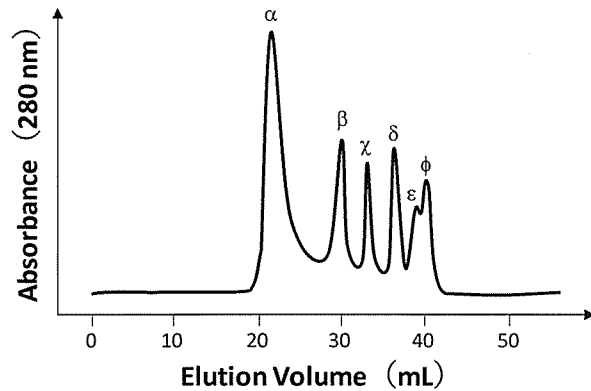


Figure 1

Table 1

| Peak | Elution Volume (mL) | Protein | Molecular Mass (kDa) |
|------|---------------------|----------------------|----------------------|
| α | 22.1 | Thyroglobulin | 669 |
| α | 22.1 | Ferritin | 440 |
| α | 22.1 | Catalase | 232 |
| β | 30.0 | Aldolase | 157 |
| γ | 33.4 | Bovine Serum Albumin | 66 |
| δ | 36.8 | Ovalbumin | 44 |
| ε | 38.9 | Myoglobin | 18 |
| φ | 40.3 | Ribonuclease A | 14 |

I – a

Explain the reason why the larger proteins are eluted off the column earlier than the smaller proteins, in relation to the underlying principle of SEC.

I – b

Explain the reason why the three proteins in peak α were not separated from each other.

I – c

What kind of experiments and analyses are necessary to measure a molecular mass of an unknown protein with SEC on the basis of the data shown in Table 1?

I – d

In a separate experiment, a solution containing a ‘protein X (with a molecular mass of 64 kDa)’ was eluted at the elution volume of 31.1 mL. Discuss and explain the possible structure of the protein X.

I – e

In order to improve the separation of peaks ϵ and ϕ , the performance of the SEC column has to be optimized in consideration of the number of theoretical plates (NTP).

- (1) Describe the relationship between NTP and resolution in chromatography.
- (2) Propose a method to evaluate NTP experimentally.

Biological Chemistry II

I Answer the following questions.

- (1) List all standard 20 amino acids in proteins by the one-letter symbol.
- (2) Classify sidechains of leucine, arginine, methionine, and asparagine as hydrophobic or hydrophilic.
- (3) Draw a structural formula for the phenylalanine-glycine-valine-glutamine-alanine peptide.
- (4) Explain the reason why glycine residue has larger allowable ranges in the backbone torsion angles than other amino acid residues.
- (5) Explain the reason why proline residue is a secondary-structure breaker.
- (6) Discuss the difference in structural features between water-soluble and membrane proteins.