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Selective Adsorption of Hemoglobin with Copper-Phthalocyanine Trisulfonate Derivative Immobilized on Gel Beads

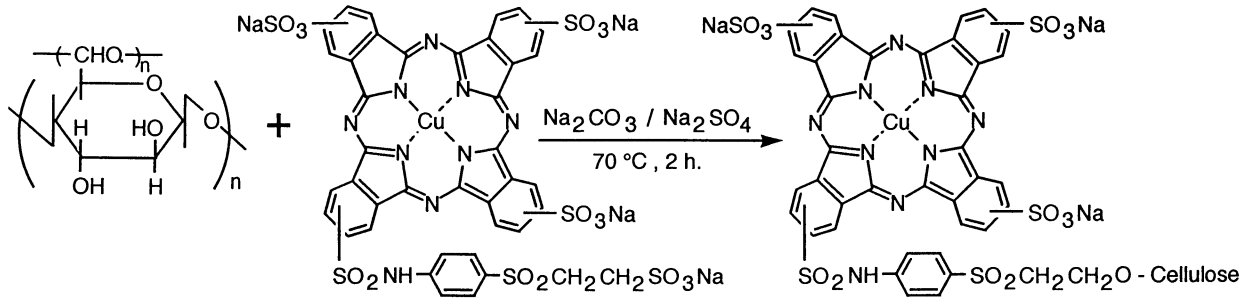
Yoshinari BABA* and Yoshinobu KAWANO
Department of Material Engineering, Faculty of Engineering,
Miyazaki University, Miyazaki 889-21

Copper phthalocyanine derivative was proposed as a new affinity ligand for the separation and purification of proteins. The adsorption equilibria of lysozyme, albumin and hemoglobin were studied with the affinity gel prepared by immobilizing its ligand to gel beads. The affinity gel was found to be highly selective for hemoglobin over albumin and lysozyme.

Affinity adsorption is a separation method of biological active substances such as proteins based on biological recognition.[1] The recognition comes from formation of a specific complex between a biological substance and a ligand.[2-5] Therefore, one of the most important problems in affinity adsorption is the development of a ligand that has a high selectivity to the special proteins to be separated.

In this study, an idea of using a metal-phthalocyanine trisufonate derivative as a new affinity ligand for the separation of proteins was proposed because three kinds of driving forces for the affinity adsorption may be expected from the structure of this ligand.[6] These forces are electrostatic, hydrophobic and metal-chelate formation interactions attributable to sulfonated anion, phthalocyanine framework and metal ion, respectively. The affinity gel was prepared by immobilizing this ligand to gel beads in order to examine its adsorption properties, especially, its selectivity for the protein to be separated. The ligand proposed in this study is the sulfonated derivative of a copper phthalocyanine, which is a commercial synthetic dye, C.I. Reactive Blue 21, possessing such a chemical structure as shown by 1 in Scheme. The gel beads used in this study as the matrices were Cellulofine 1120 for the gel filtration. The gel is spherical beads with an exclusion molecular weight of 10^7 in the order of magnitude. The gel was treated with C.I. Reactive Blue 21 in the alkaline solution according to the conventional method.[6] The concentra-

tion of the ligand immobilized on the gel was measured by the atomic absorption spectrophotometer after dissolving the affinity gel with sulfuric acid. The value was 1.1 x 10^{-6} mol g^{-1}. The affinity gel obtained like this is hereafter referred to as "blue gel".



Cellulofine 1120 C.I. Reactive 21 Blue gel 1

Scheme of synthesis of affinity gel("blue gel")

The adsorption equilibria of three proteins, bovine albumin, egg lysozyme and bovine hemoglobin, were measured by a batchwise operation. The pH and ionic strength of aqueous solution were adjusted using a phosphate buffer solution of 0.1 M(=mol dm^{-3}) and sodium chloride, respectively. The equilibrium concentrations of their proteins were determined by UV spectrophotometer at 280 nm for both lysozyme and albumin and 406 nm for hemoglobin. The adsorbed amounts of the proteins were calculated from the difference of the concentration in the aqueous solution between before and after the adsorption. The distribution ratio of the proteins was defined as the ratio of concentration of the protein on blue gel to that in the aqueous solution.

Figure 1 shows the effect of the equilibrium pH(pH_{eq}) in the aqueous solution on the distribution ratio(D) of lysozyme. As seen from Fig.1, the addition of sodium chloride(I in figures denotes ionic strength) causes a decrease in the distribution ratio of lysozyme. The results suggest that the adsorption of lysozyme may be attributed to electrostatic interaction. However, judging from the fact that lysozyme was adsorbed even in the presence of sodium chloride, we have to take into account the hydrophobic interaction. Consequently, it appears that these two interactions

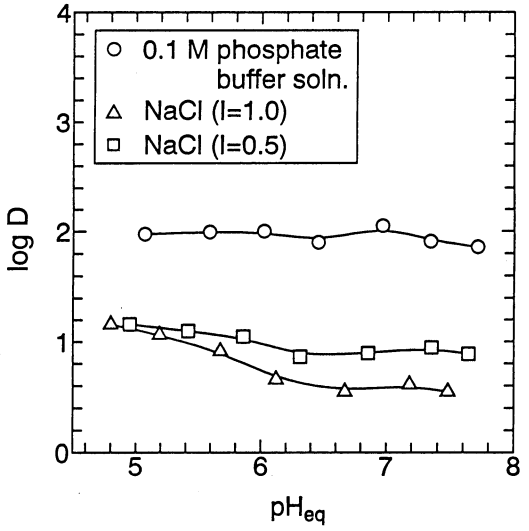


Fig.1. Effect of NaCl added to 0.1 M phosphate buffer solution of lysozyme.

mainly participate in the adsorption of lysozyme on blue gel.

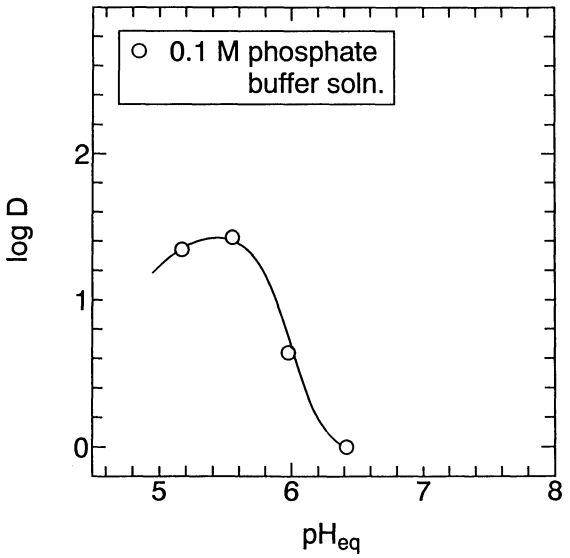


Fig.2. Effect of NaCl added to 0.1 M phosphate buffer solution of albumin.

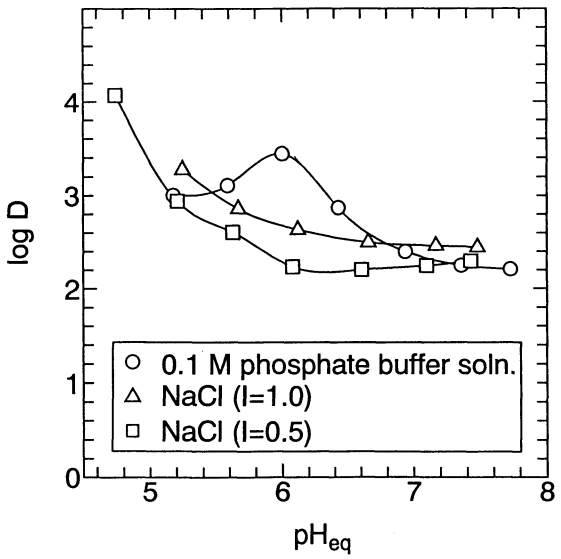


Fig.3. Effect of NaCl added to 0.1 M phosphate buffer solution of hemoglobin.

Figure 2 illustrates the relation between the distribution ratio of albumin and the equilibrium pH. The distribution ratio shows the maximum value at a pH of about 5. This pH value is close to the isoelectric point of albumin. Above pH 5.5, the distribution ratio of albumin decreased with increasing pH. In this region, the adsorbent is negatively charged and albumin also is negatively charged. As a result, the electrostatic repulsion exerted by these charges may prevent the adsorption of albumin. Furthermore, the addition of sodium chloride caused no adsorption of albumin. These results suggest that the adsorption of albumin is attributable to electrostatic interaction.

Figure 3 shows a similar plot for the adsorption of hemoglobin. In a low pH region, the distribution ratio increased with decreasing pH under coexistence of sodium chloride. Above pH 6, the adsorption of hemoglobin is almost independent of pH in the aqueous solution. There is no decrease in the distribution ratio by the addition of salts. These results suggest that the hydrophobic interaction, which is attributable to the phthalocyanine framework, mainly acts between the ligand and hemoglobin.

Figure 4 shows the adsorption isotherms of lysozyme, albumin and hemoglobin at 30 ^{o}C. The ordinate stands for the amount of adsorption of proteins, q, the abscissa stands for the protein concentrations at equilibrium, C. These adsorption isotherms indicate the Langmuir type adsorption as expressed by Eq.(1). The adsorption equilibrium constant, K,

$$q = \frac{q_s KC}{1 + KC} \qquad (1)$$

and the saturation capacity, q_s, were calculated by the least squares method based on Eq.(1). The results are shown in Table 1. The solid lines are the theoretical lines calculated using their values according to Eq.(1). These lines are in good agreement with the experimental results. As seen from these figures, the amounts of lysozyme and albumin adsorbed are negligibly small compared with that for hemoglobin. The amount adsorbed of hemoglobin is about ten times those of albumin and lysozyme.

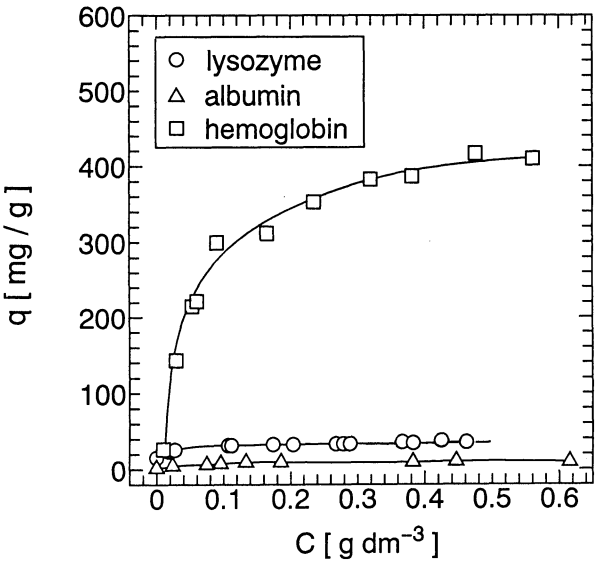


Fig.4. Adsorption isotherms of proteins from 0.1 M phosphate buffer solution at pH=5.5.

Table 1. Determination of adsorption parameters in the Langmuir equation

Proteins	K/dm^3 g^{-1}	q_s/mg g^{-1}
Hemoglobin	22.4	410
Lysozyme	68.2	36
Albumin	37.8	10

In conclusion, it was found that copper-phthalocyanine trisulfonate derivative examined in this study is a high selective ligand for hemoglobin over lysozyme and albumin. At present, the separation and purification of hemoglobin are commercially performed by the difference of isoelectric point and solubility of each protein. The selectivity based on these separation principles is not so high compared with that based on the affinity properties of copper phthalocyanine for hemoglobin. Therefore, "blue gel" developed in this study is expected as a affinity gel with high selectivity for hemoglobin.

References

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