

Chemically Reactive Liposomes as a New Type of Sensor for Proteins

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A new type of sensor for proteins has been developed by utilizing a liposome which contains molecules chemically reactive with protein on the membrane surface of the liposome. The covalently bound aggregation of proteins with liposomes has been observed by a quasi-elastic light scattering measurement at the concentration down to about 10^{-8} mol dm⁻³.

For a number of years, the bridging aggregation has been utilized in the field of immunoassay; the antigen-antibody reaction is detected by the observation of flock-formation of their complexes. Recently, we have succeeded in developing a new type of liposome which coagulates with proteins as described below. Liposomes are closed spherical bilayers of lipid molecules and utilized as model systems for biological membranes.¹⁾ As the liposomes are dispersed in water in colloidal particle size, various colloidal phenomena are observed, e.g., Tyndall phenomenon (light scattering) and aggregation due to the colloidal instability. According to the theory of colloidal stability, the mechanism for aggregation of colloidal particles is generally classified into two major categories, i.e., the neutralization of electric charge on particle's surface by oppositely charged ions and the bridging flocculation between colloidal particles by multi-functional molecules or polymers such as polycations.²⁾ In the case of liposome, it has been found that such mechanisms also play a significant role in the aggregation phenomenon.^{3,4)} Furthermore, as liposomes are one of the most easily functionizable colloidal particles, one can prepare various kinds of liposomes which might cause a new type of aggregation other than ordinary ones by modification of membrane surface, i.e., the embedding of functional molecules into the membrane layer or the binding of functional groups to the membrane surface. For example, liposomes of which surfaces are modified by biologically significant molecules (antigen, antibody, lectin, hapten etc.) have been prepared and various kinds of studies concerning the recognition of biological cells or molecules have been extensively carried out.⁵⁻⁷⁾

In this letter we demonstrate a new type of functional liposomes which can cause a covalently bound aggregation through a chemical reaction of liposomes

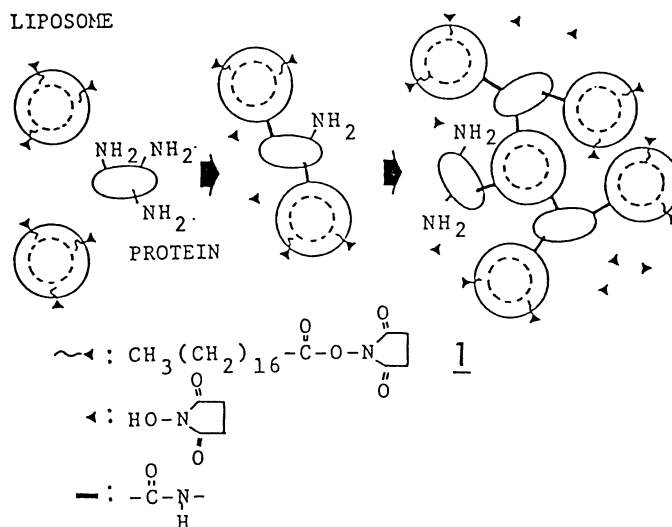


Fig. 1. Schematic representation of the new-type coagulation.

with protein. As a simple attractive system we chose a liposome which contained molecules chemically reactive with amino groups, i.e., stearoylhydroxysuccinimide (abbreviated as SHS (1)). Figure 1 shows a schematic representation of this type of aggregation. SHS molecules in liposome attack the free amino groups of protein and amide linkages are formed between SHS and protein. Such a chemical reaction makes a "bridge of protein" between two liposomes resulting in the network-like structure of liposomes which can strongly scatter the incident light beam.

SHS was synthesized from hydroxysuccinimide and stearic acid with the use of dicyclohexylcarbodiimide following the method as used for peptide synthesis reported in the literature.⁸⁻¹¹⁾ The product was purified by recrystallization twice from a mixture of chloroform/ethanol and obtained in the form of leaflet-like crystal. Its melting point is 91.5 °C and it is considered to be of good purity judging from DSC curve and elemental analyses. The chemically reactive liposomes were prepared by mixing SHS with egg phosphatidylcholine (abbreviated as PC; Sigma Chem. Co., USA) with the molar mixing ratio of SHS/PC of ca. 1/25 in a mixed solvent of chloroform/ethanol, followed by drying under N₂ gas flow, and by subjecting it to sonication in a bath-type sonicator. The dispersed amount of lipid was about 1 g per 100 ml. The dispersion medium for liposomes and the solvent of protein were both water deionized with the Barnstead NANO pure system. For the purpose of detecting the aggregation induced by proteins, the spectrum of a light scattered quasi-elastically from the dispersion after mixing the liposome dispersion with a protein (avidin; Sigma Chem. Co., USA) aqueous solution. The instrument of light-scattering measurement is made up of an Ar-ion laser (488 nm, 10 mW; NEC Co.), a goniometer system with a phototube, and a signal analyzer (Iwatsu Electric Co., SM-2100E). The homodyne spectrum of scattered

light at 90° from the incidence was measured and the "apparent" radius (r) of aggregated body was estimated from the half-height width (Δf) of spectrum after Stokes-Einstein equation;¹²⁾ in this experimental condition ($\eta = 10^{-2}$ poise in water), r is therefore given by $r = 3.7 \times 10^4 / \Delta f$ nm.

The temporal variation of apparent radius (r) after the addition of avidin is shown in Fig. 2 where r_0 is the initial radius of liposomes. The light spectra in the absence of avidin were well approximated by a Lorentzian curve showing the quasi-monodispersity. The addition of avidin causes an increase in r and it is almost constant as shown in the figure; this means that the rate of aggregation is rather rapid. Such a feature is suitable for the sensing reagent. As a control system SHS-free liposomes were employed to detect avidin, but any aggregation was not observed in the same protein-concentration range as that in the above system. Therefore, it can be said that the mechanism of this aggregation is due to the chemical reaction of SHS-embedded liposomes with amino groups of protein. Figure 3 shows the change in the apparent radius of liposomes with the avidin concentration. Avidin affects the radius of liposome particles already at ca. $2 \times 10^{-8} \text{ mol dm}^{-3}$. The apparent radius is found to increase sigmoidally; this feature can arise from the polydispersity of the dispersion which depends on the concentration ratio of avidin/liposome, that is, the light spectrum is affected by the proportion of the scattered light from single liposomes to that from aggregated ones. To clarify the factors which determine the sensitivity of this type of sensor, some factors, i.e., the ratio of SHS/PC and the concentration of liposomes, were examined. It is found that the more the ratio of SHS/PC increases and the less the concentration of liposomes becomes, the more sensitive gets the sensor for avidin (further details will be published elsewhere).

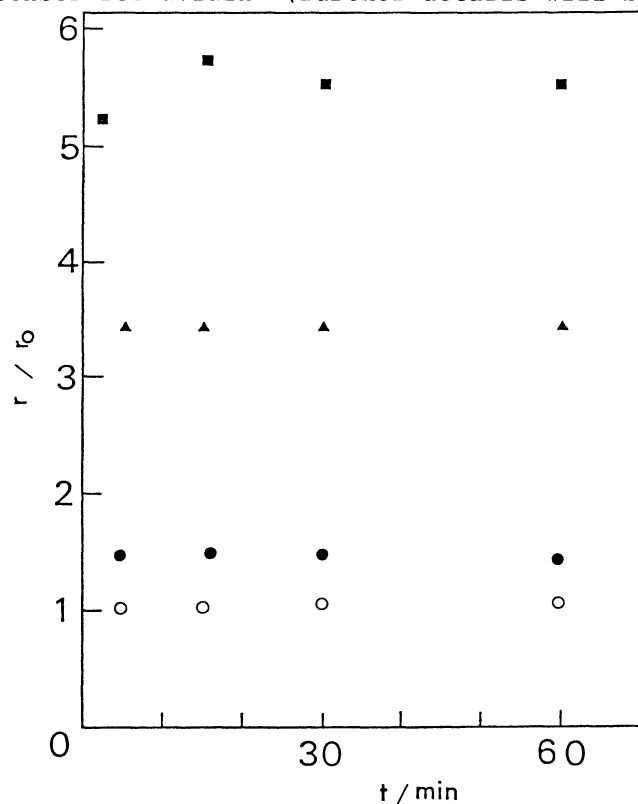


Fig. 2. Time dependence of apparent radius of aggregated body.

Avidin concentration:

(○) 1.0

(●) 6.1

(▲) 30.9

(■) 78.5

($\times 10^{-8} \text{ mol dm}^{-3}$)

All these results mean that a chemical reaction which is a microscopic molecular process can be visualized as a light-scattering phenomenon mediated by the "covalently bound aggregation" of liposomes. This new type of aggregation (in other words, a new type of polymerization) can be utilized as a sensing method for the chemical reaction system where the molecules to be detected have multiple reaction sites like proteins but any characteristic spectroscopic change does not occur by the reaction, i.e., no conventional colorimetric methods can be applied. The assembly of the above mentioned characteristics and the biocompatibility of reactive liposomes with an appropriate detection method will offer a novel class of bio-mimetic sensor systems.

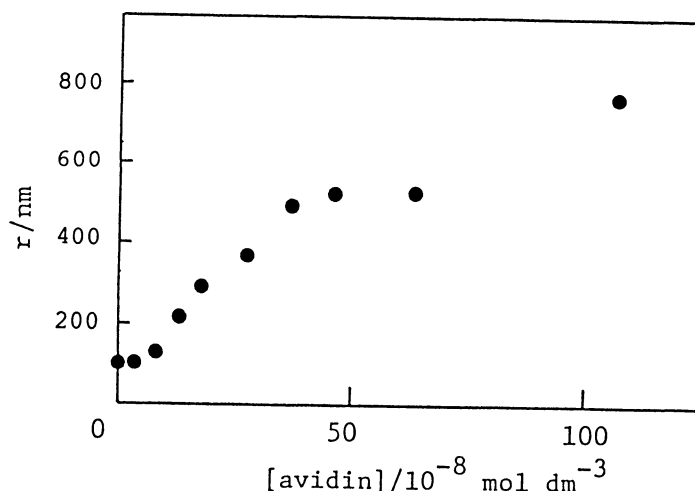


Fig. 3. Dependence of apparent radius on avidin concentration.

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