Protein Assembly on Solid Surfaces by Gel-Assisted Transfer (GAT) Technique

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Water soluble proteins – ferritin and hemoglobin - were spread on the surface of agarose gel, and transferred to solid substrates via contact with solid substrates. Formation of monolayer and multimeric layers of proteins was confirmed by electron microscopy and UV-Vis spectroscopy.

Immobilization and 2D organization of proteins on solid surfaces have been an important research target in biorelated chemistry and in the development of protein-based devices. Conventional approaches for this purpose include the use of air-water interface[1-4] and mercury surface[5] to organize water-soluble proteins and their successive transfer to solid substrates. 2D crystals of ferritin were formed at the air-water interface by injecting the protein into a subphase of aqueous glucose.[6] Perfluorocarbon oil provided a fluorocarbon-water interface that allowed formation of ordered domains, due to convective transport and lateral capillary forces.[7]

Alternatively, adsorption of proteins at solid-liquid interfaces[8,9] was recently extended to the layer-by-layer adsorption technique, and multiple protein layers have been prepared.[10] These adsorption techniques require strong electrostatic interaction or efficient ligand binding, and they cannot be generally applied to a wide spectrum of proteins.

We have recently developed a novel technique for two-dimensional organization of water-soluble silica nano-particles on hydrogel surfaces and their gel-assisted transfer (GAT) onto solid substrates.[11] In the GAT process, transfer of hydrophilic silica particles is assisted by surface network structures that act as *molecular glue*.[11] In this study, we extended the GAT principle to water-soluble proteins.

Agarose (1 wt%) was dissolved in hot water, and the solution was poured into a petri dish and cooled to room temperature to give gel. A rectangular frame made of fluorocarbon membrane film (1 x 1 cm, Sumitomo Electric, Fluoropore, type FP-010) was placed on the surface of agarose gel, and 100 μl of protein solutions (ferritin, 1 μg ml^{-1}, hemoglobin (Hb), 1 mg ml^{-1}) was spread over an area of 1 cm^2. The red color of the proteins remained on the surface without diffusion into the gel interior. After 12 h when water disappeared both by evaporation and by penetration into the gel, solid substrates (a carbon-coated copper grid for ferritin and a quartz plate for hemoglobin) were pressed against the gel and were allowed to stand for 0.5 to 2 h. The substrates were then detached from the gel surface (Figure 1). The carbon-coated grid was then observed by transmission electron microscopy without staining (Hitachi H-600). The Hb-transferred quartz plate was rinsed in water (10 ml, 1 min), and dried under a stream of nitrogen. The transfer procedure was repeated with fresh Hb-spread gel surfaces, and UV-Vis spectra were measured at every transfer cycle.

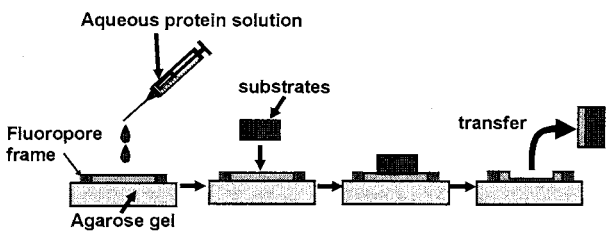

Figure 1. Gel-assisted transfer of proteins.

Figure 2 displays an electron micrograph of carbon-coated copper grid that has been detached from a ferritin-spread gel surface. Ferritin is a spherical protein (diameter, 12 – 13 nm) that stores an iron oxide core of ca. 8 nm in diameter.[12] It can be visualized under electron microscopy without staining.[1] In this micrograph, electron-dense cores with diameters of 5-7 nm are abundantly observed, and the average center-to-center separation of these cores is ca. 11 nm for 22 samples in a densely-packed area (the box in Figure 2). These figures are consistent with the presence of closely-packed ferritin molecules as monolayer.

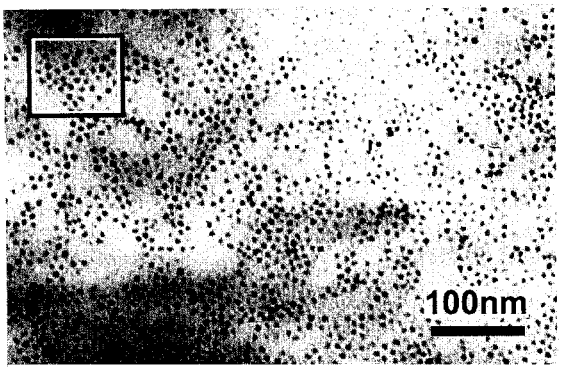

Figure 2. Electron micrograph of ferritin transferred on a carbon-coated TEM grid.

Interestingly, the close-packed domain is extended unevenly over a larger area. A similar structure has been observed for gold nanoclusters (diameter, 30 nm) which were transferred from agarose gel to highly oriented pyrolytic graphite (HOPG) surface.[13] Apparently, formation of such network-like particle distributions is attributed to the glue effect of agarose gel in the transfer process, as discussed before for the gel-assisted transfer of silica nanoparticles.[11]

Figure 3a shows absorption spectra of Hb on quartz slides measured after repeated transfers. Absorbance of the Soret band at 412 nm is enhanced with increasing numbers of transfer. This indicates that Hb is reproducibly transferred on the quartz plate. The average increase in absorbance, $\triangle$A at 412 nm, is 3.3 x 10^{-3} for a single transfer, and this value corresponds to 3-4

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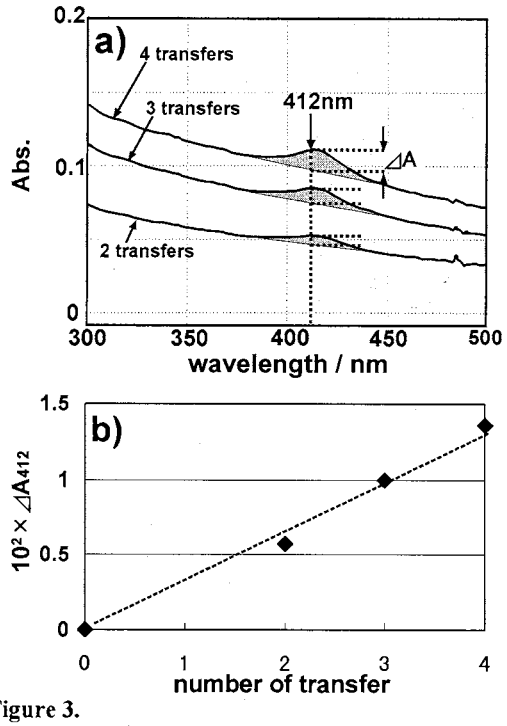

Figure 3.

a)UV-Vis adsorption spectra of Hb successively transferred on a quartz plate.

b)Dependence of ⊿A(at 412 nm) on number of transfers.

multimeric layers of closely packed Hb molecules.[14] The average thickness of the Hb layer would be affected by spreading conditions, as in the case of transferred silica nanoparticles.[11] In addition, enhancement of the spectral base line with the number of transfer observed in Fig. 3a is consistent with the presumption that Hb is transferred together with agarose networks.

It is desirable to point out the difference between the present GAT mechanism and that of the blotting process of biopolymers. In the latter case, biopolymers are electro-phoretically separated on agarose gel, and are blotted onto nitrocellulose paper by capillary attraction from gel to paper.[15] Thus, the agarose gel is merely used as a matrix for the separation. On the other hand, the agarose gel is utilized for 2D organization of hydrophilic materials in the GAT process, and its surface layer acts as *"molecular glue"* to hold water-soluble proteins together and to paste them onto solid substrates regardless of surface charge and hydrophobicity.

Surface-to-surface transfer of protein molecules has been reported earlier by Kuhn[3] and by Rothen.[16] They stripped off monolayers and protein multilayers from the surface of fatty acid LB films by the use of adhesive polymer films. The use of hydrogel surfaces as described here not only allows organization of water-soluble proteins under mild conditions, but also enables their facile transfer to varied solid substrates. These features make the current approach quite attractive.

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