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Chemical Modification of Holey Carbon Supports for Cryogenic-Electron Microscopy

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Abstract
The structural determination of biological molecules has recently undergone a revolution with the Cryogenic-Electron Microscopy (cryo-EM) technique advances. However, a poor partitioning of macromolecules into the holes of holey carbon support grids frequently limits 3D structural determination by Single Particles Analysis through cryo-EM. This work present a method to deposit, on gold-coated carbon grids, a self-assembled monolayer whose surface properties can be controlled by chemical modification. There is a demonstration of this approach utility to maximize the grid wettability while drive partitioning lipossomes into the holes, thereby enabling better results using cryo-EM.

Key words: *chemical modification, wettability, cryo-EM.*

Introduction

A myriad of difficulties associated with the use of X-ray crystallography for structural studies of biological macromolecules (mainly protein complexes) could be solved with the advent of Cryogenic-Electron Microscopy (cryo-EM). In this technique, the sample remains hydrated and frozen in liquid ethane (-181 °C) inside the holes of an amorphous carbon support[1]. An efficient application of the cryo-EM technique to Single Particles Analysis requires a good dispersion and poly-orientation of the particles throughout the amorphous ice formed. For this, it is necessary that the support allow good dispersing of the sample aqueous suspension. The chemical modification of holey carbon supports for cryo-EM (essentially hydrophobic) has already been shown as an effective method to maximize the wettability of the grids while allowing the best dispersion of the particles throughout ice formed, making possible its application in 3D structural analysis by cryo-EM[2]. The goal of this work in developing novel methodologies of chemical modification in holey carbon cryo-EM supports using relatively low cost commercially available reactants is fundamental to obtain better results using cryo-EM.

Results and Discussion

C-flat™ (Electron Microscopy Sciences, USA) carbon grids were gold sputter coated for 30 seconds on only one side using a Sputter Coater Balzers SCD 050 (BAL-TEC, USA) to obtain ca. 13 nm of gold. Each gold-coated grid was immersed in 150 µL of self-assembly reaction mixture prepared using ethanol as a solvent with 14.3 mM 2-mercaptoethanol (Cat # M3148, Sigma-Aldrich/Merck, USA) for 24 h under periodic stirring. All reaction tubes were closed, sealed with Parafilm and covered with aluminium foil. Finally, the grids were dunked in water and ethanol to wash away unreacted thiol, and then air dried for 3 minutes to allow the ethanol to evaporate.

Upon applying a droplet of water, it was observed from the drop shape that Glow Discharge increases the wettability of carbon grid. However, gold-coated grids wich had undergone reaction with 2-mercaptoethanol reaction were the most wettable (Fig. 1). The water was seen to equilibrate to both sides of the SAM grid after Glow Discharge by passing through the grid holes. This observed equilibrium was attributed to the lower surface tension of the aqueous medium on the 2-mercaptoethanol surface, to the point that the water could spread through the holes in the chemical modified support film.

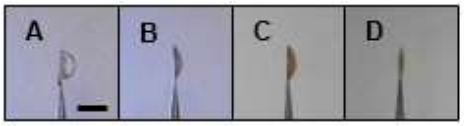

Figure 1. Wettability test. Wettability of (A) carbon grid is poor and (B) increase after Glow Discharge as (C) after SAM formation. The best surface wettability is with (D) SAM grid after Glow Discharge. Scale bar is 3 mm.

Vitrified specimens were prepared into a FEI Mark IV Vitrobot (FEI Company, USA) adding 3.0 µL of liposomes suspension to the grid and then immediately blotting the grid for 3 seconds before plunge-freezing in liquid ethane. The liposome species are observed in the holes of SAM grid prepared with 2-mercaptoethanol (Fig. 2). Actually, the use of a hydrophilic SAM is a viable way to mitigate liposome adhesion at the carbon supports. Images shown in Fig. 2 was acquired in a FEI Talos F200C (Thermo Fischer Scientific, USA) operated at 200 kV in low dose mode.

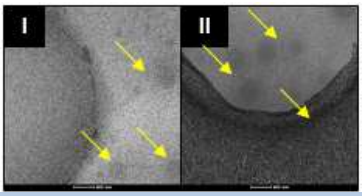

Figure 2. Cryo-EM. (I) Cryo-EM projection image of liposomes on holey carbon grids showing that the protein has a strong preference for carbon film. (II) Liposomes are observed in the holes of SAM grids prepared with 2-mercaptoethanol. The yellow arrows indicate some liposomes.

Conclusions

Gold-coated grids with 2-mercaptoethanol yields a hydrophilic surface to holey carbon cryo-EM supports. The method is simple and accessible requiring commercially available items at relatively low costs. The acquired cryo-micrographs for the liposome sample prepared on the SAM grids confirm the success on the drive partitioning of the species into the holes. This approach may enable structure determination of macromolecular targets that suffer from high affinity for holey carbon support.

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[1] Russo, C. J.; Passmore, L. A. Progress towards an Optimal Specimen Support for Electron Cryomicroscopy. *Curr. Opin. Struct. Biol.* **2016**, *37*, 81–89.
[2] Meyerson, J. R. et al. Self-Assembled Monolayers Improve Protein Distribution on Holey Carbon Cryo-EM Supports. *Sci. Rep.* **2014**, *4*, 1–5.