Electronic supplementary materials *Mendeleev Commun.*, 2025, **35**, 592–594

SEM and SAXS characterization of recombinant lipase immobilized by adsorption on the surface of cellular carbon foam

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Materials and Methods

Recombinant *T. lanuginosus* lipase designed by Beklemishev[S1] using genetic engineering approach was immobilized on carbon cellular foam by two methods of adsorption: spontaneous and forced (impregnation-drying). *Spontaneous adsorption* was performed as follows. A sample of CCF support (2.1 mg) were poured into TLL solution in distilled water (2.1 mL) at a protein concentration of 0.2 mg·ml^{-1} in a closed vial; and kept under ambient conditions with periodic shaking for 24 h. The solution was then almost completely removed using an automatic dispenser; and samples of CCF with adsorbed lipase were placed on a filter paper to remove excess solution and dried under ambient conditions for one day, then stored in a desiccator. *Forced adsorption* was performed as follows. A sample of CCF support (ca.1 mg) was impregnated with an aqueous solution of TLL at a concentration of 0.2 mg/mL in an open vial using a drop (0.3 mL) of this solution. Then it was dried under ambient conditions for one days and stored in a desiccator. When drying, the processes of protein concentration with simultaneous fixation on a solid surface took place. The method is distinguished by its simplicity, fast execution and economical use of the enzymes (0.3 mL instead of 2.1 mL above).

An ultra-high-resolution field emission scanning electron microscope Regulus 8230 (Hitachi, Tokyo, Japan) was used in secondary electrons (SE) mode at low energy, E_0 = 5 keV, in order to destroy protein molecules of lipase as little as possible.

A small angle diffractometer S3 MICRO (HECUS), with point beam (CuKα, 50W) collimation was used for the measurements using a small-angle X-ray scattering (SAXS) method. The wave vector q, (defined as $q = 4\pi \cdot \sin(\theta)/\lambda$, where 2θ is the scattering angle and λ = 1.541 Å wavelength of the radiation used) was measured in the range $0.01 < q < 0.60$ Å^{-1}. The powder samples of CCF and adsorbed lipase (biocatalyst) were placed in a special cuvette with thin polymeric walls. High temperatures were NOT used in study in order to prevent sintering adsorbents and destructing protein molecule of adsorbed enzyme. Difference data were obtained to elucidate the structure of

aggregates of adsorbed lipase, namely, the SAXS curve for the original support (without lipase) was subtracted from the SAXS curve obtained for the biocatalyst, taking into account the absorption coefficient. For each sample, the measurement was carried out eight (8) times, then all data were averaged. The ATSAS software package[S2] was used for processing the SAXS data. The obtained SAXS curves were fitted by a set of polydisperse spheres with using the MIXTURE software[S2].

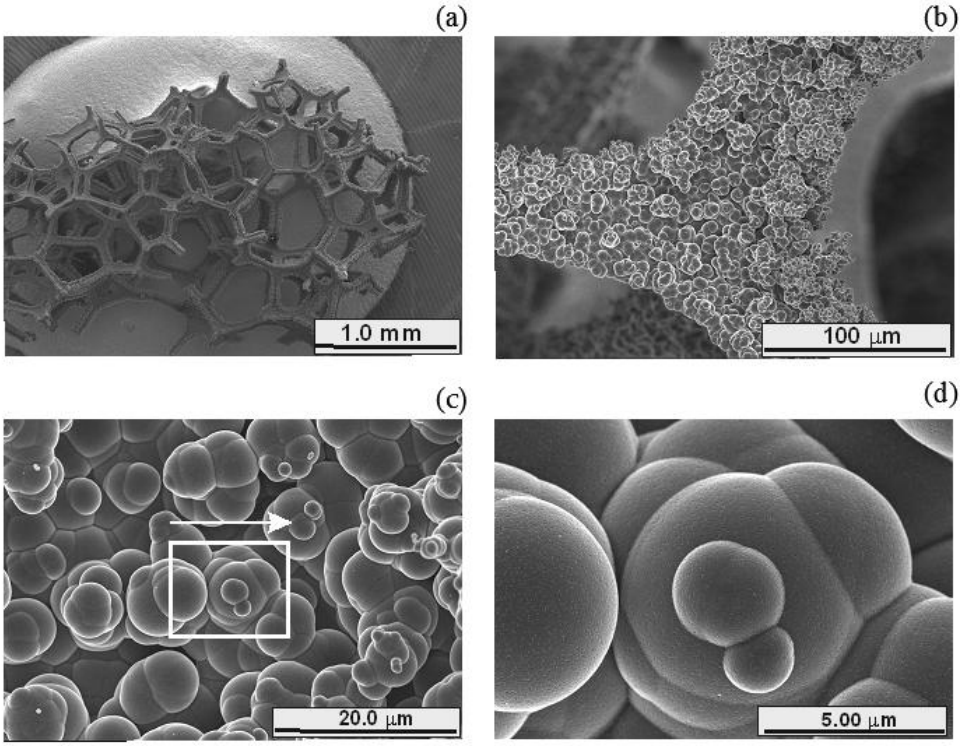

Figure S1. SEM images of cellular carbon foam at different magnifications

Additional evidence was obtained by SEM studying the supramolecular aggregation of immobilized lipase globules on a smooth aluminum metal surface (Figure S2). By analogy with the SEM study described[S3], the authors visualized the aggregation of TLL molecules on a flat, smooth aluminum surface. As can be seen in Figure S2, molecules of lipase associated into the large aggregates up to 1 μm in size, sometimes look like “butterfly” or “rice grain”, which covered the aluminum surface evenly. At high magnification, one can examine the fine structure of the aggregates, consisting of smaller nanoparticles (TLL monomer or dimer), seems to be of protein origin (Figure S2 b, c). Surely, as mentioned above, this study on aggregation and orientation of enzymes on supports of various chemical origin will allow us to formulate practical approaches to the preparation of highly active and stable biocatalysts.

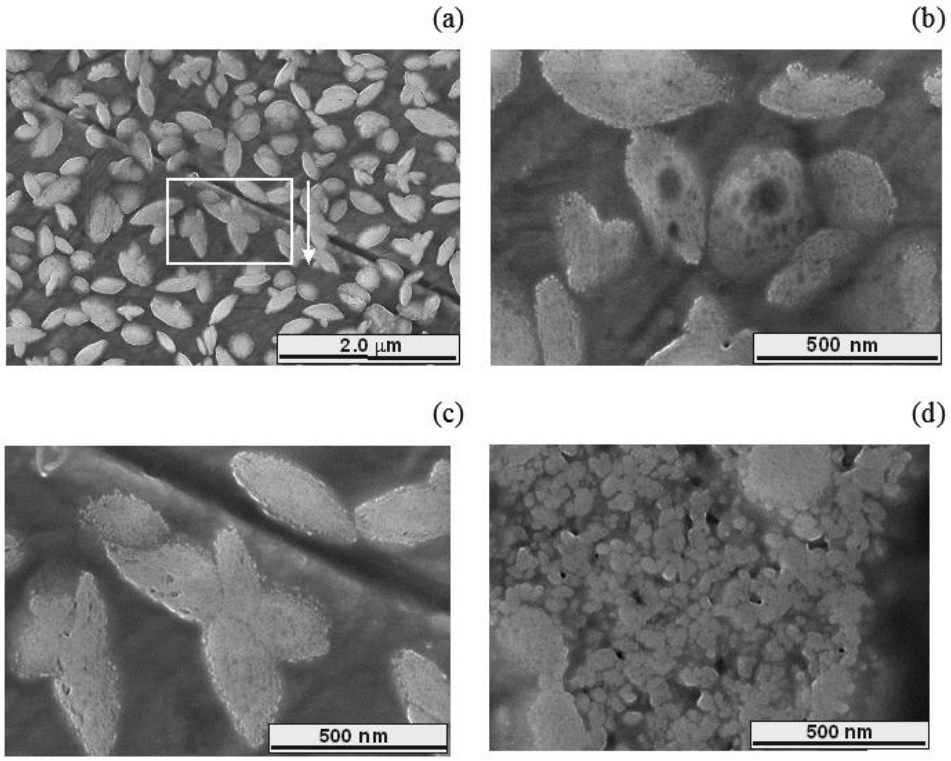

Figure S2. SEM images of TLL aggregates, formed on flat aluminum surface (a–c) and their internal fine structure (d). Rectangle and arrow indicate area with a higher magnification

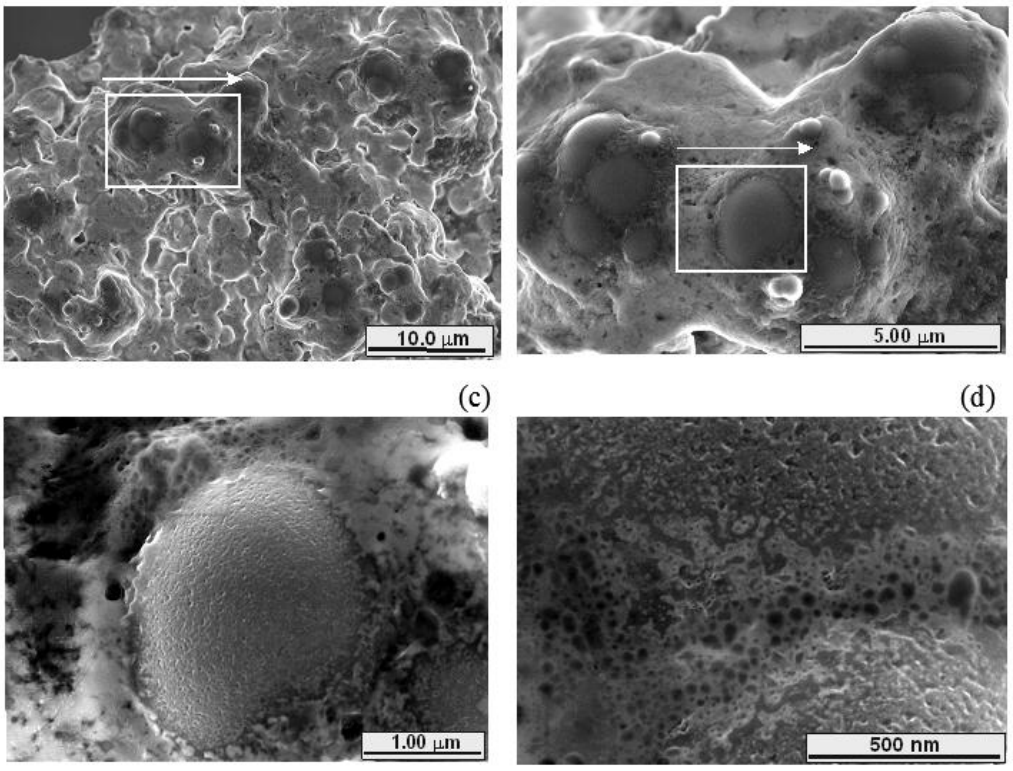

Figure S3. SEM images of protein films formed by TLL immobilized by forced (impregnation-drying) adsorption on pyro-carbon surface of cellular foam (CCF) (a–c), a high-magnification image of the external surface of the protein film (d). Rectangles and arrows indicate area with a higher magnification.

Probably to obtain purer monodisperse lipase molecules or its oligomeric fractions supported in the porous structure of different by chemical nature materials it makes sense to prepare biocatalyst samples using stabilizers, such as described[S4].

References

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