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Bacterial surface layer proteins as a novel capillary coating material for capillary electrophoretic separations

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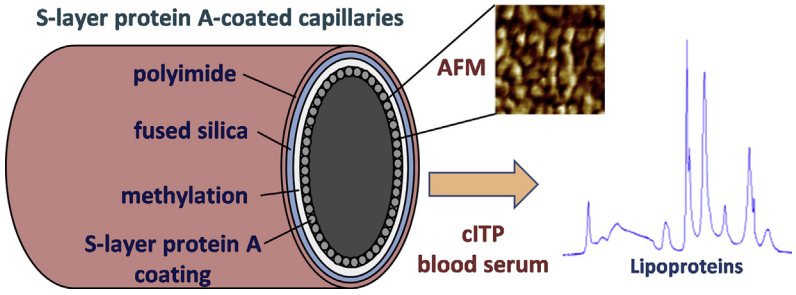
HIGHLIGHTS

- New coating using recrystallized surface-layer proteins on silanized fused silica.
- Very stable coated capillaries are produced, able to withstand high pH conditions.
- Highly repeatable isotachophoresis separations of serum lipoproteins are allowed.
- Very good inter-capillary reproducibility is also demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel concept for stable coating in capillary electrophoresis, based on recrystallization of surface layer proteins on hydrophobized fused silica capillaries, was demonstrated. Surface layer protein A (SlpA) from *Lactobacillus acidophilus* bacteria was extracted, purified and used for coating pre-silanized glass substrates presenting different surface wettabilities (either hydrophobic or hydrophilic). Contact angle determination on SlpA-coated hydrophobic silica slides showed that the surfaces turned to hydrophilic after coating (53 ± 5°), due to a protein monolayer formation by protein-surface hydrophobic interactions. Visualization by atomic force microscopy demonstrated the presence of a SlpA layer on methylated silica slides displaying a surface roughness of 0.44 ± 0.02 nm. Additionally, a protein layer was visualized by fluorescence microscopy in methylated silica capillaries coated with SlpA and fluorescein isothiocyanate-labeled. The SlpA-coating showed an outstanding stability, even after treatment with 20 mM NaOH (pH 12.3). The electroosmotic flow in coated capillaries showed a partial suppression at pH 7.50 ($3.8 \pm 0.5\ 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$) when compared with unmodified fused silica ($5.9 \pm 0.1\ 10^{-8}$ $m^2\ V^{-1}\ s^{-1}$). To demonstrate the potential of this novel coating, the SlpA-coated capillaries were applied for the first time for electrophoretic separation, and proved to be very suitable for the isotachophoretic separation of lipoproteins in human serum. The separations showed a high degree of repeatability (absolute migration times with 1.1–1.8% coefficient-of-variation (CV) within a day) and 2

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–3% CV inter-capillary reproducibility. The capillaries were stable for more than 100 runs at pH 9.40, and showed to be an exceptional alternative for challenging electrophoretic separations at long-term use.

1. Introduction

Surface layer proteins (SLPs) are present on the cell wall surface of gram-positive and gram-negative bacteria and *archaea*, forming bidimensional layers of crystalline arrays (S-layers) [1]. These layers contribute to the physico-chemical properties of the bacterial surface and determine the interaction of the cell with the environment [2]. Depending on the organism, the nature of the bacterial SLPs can vary, with masses ranging from 40 to 200 kDa and pI usually from 4 to 6 except for *Lactobacilli*, with pI between 9.4 and 10.4. Additionally, SLPs may be post-translationally modified [2–4]. S-layers are monomolecular assemblies of identical SLP subunits forming 5–20 nm thick layer lattices, with either oblique (p1, p2), square (p4) or hexagonal (p6) lattice symmetries, with identical pores in size and morphology (porosity up to 70%) [1,3,5]. In the case of the gram-positive *Lactobacillus acidophilus* ATCC 4356, which can be found in the gastrointestinal tract and in fermented dairy products, the only expressed SLP is SlpA, presenting a silent SlpB gene [6]. SlpA consists of a non-glycosylated single-chain basic protein (pI 9.5) with a molecular weight of 43.6 kDa, that assembles in a porous oblique crystal lattice (p2 symmetry) with unit cell dimensions (a = 118 A, b = 53A and $\gamma = 102^{\circ}$) [7], resulting in quite distinct and unique properties. The C-terminal domain (SAC) of the SlpA from Lactobacillus acidophilus ATCC 4356 presents a highly hydrophilic region with a high abundance of basic residues [8]. This domain proves to be involved in the cell wall anchoring, probably by non-covalent binding to the carbohydrates in the peptidoglycan layer and with electrostatic interactions with teichoic acid [7,9]. The N-terminal domain (SAN) is relatively hydrophobic and is responsible for the protein self-assembly and therefore, it is the crystallization domain [7,8].

As a rule, SLPs can be selectively extracted from bacteria by using high concentrations of salts such as LiCl or guanidine hydrochloride (GHCl). These salts disrupt the hydrogen bonding between the Slp and the secondary cell-wall polysaccharide [1,3]. After extraction and desalting, the SLPs can both reassemble in solution and recrystallize on diverse interfaces, like silicon, gold, silanized glass, cellulose, graphite, mica, lipid films or liposomes [4,10], thus presenting interesting potential (nano)biotechnological applications [11–13]. Literature has mainly focused on the characterization of the SLP of *Bacillus sphericus* and shows that the recrystallization process of this particular protein and the properties of the crystal layers generated largely depends on the experimental conditions employed, such as protein concentration, buffer composition or surface chemistry [14,15]. Their stability in presence of organic solvents, at low pH (<3) and at temperatures above 70 °C is limited, though [16].

In the case of SlpA from *Lactobacillus acidophilus* ATCC 4356, scarce literature has dealt with its surface reassembly. Treatments with 1 M and 5 M LiCl have been used for extraction and isolation of SlpA from surface layer associated proteins (SLAPs) [7,17]. SlpA was shown to be able to recrystallize as a monolayer *in vitro* on phospholipid (PL) monolayers with a net negatively charged headgroup, being the S-layer oriented with its C-terminal domain towards the PL headgroup. Interestingly, in the case of neutral phospholipids only small crystal patches were observed [7]. The SlpA from *L. acidophilus* also proved to absorb onto a moderately hydrophobic gold sensor surface displaying a defined orientation, most likely through hydrophobic interactions with the N-terminal region. The protein monolayer showed a high stability to 6 M GHCl or 25 mM NaOH exposure [18].

Capillary electrophoresis (CE) is a powerful technique for the separation of diverse types of analytes, especially biopolymers such as proteins or nucleotides [19–21]. However, some analytes such as proteins present a tendency to adsorb onto the negatively-charged wall of fused silica capillaries due to electrostatic interactions, but hydrophobic interactions can also play a role [22], [23]. This leads to the loss of separation resolution, sensitivity and in some cases, even to a lack of detection, especially for basic proteins [22]. In the past decades, many approaches have been proposed to prevent protein adsorption to fused silica capillaries, mainly by covalently-linked (permanent) or physically adsorbed (dynamic), neutral and hydrophilic coatings [22,24]. However, coating stability is usually both limited as well as restricted to mild pH working conditions usually up to pH 8, presenting a problem for certain applications [24].

Lipoproteins are an example of a family of compounds with a challenging separation by CE due to the irreversible adsorption of e.g. low density lipoproteins (LDL) to fused silica capillaries, which hampers their detection and quantification [25]. Capillary isotachophoresis (cITP) has a high interest for lipoprotein profiling in blood serum due to its high resolution in terms of sublipoprotein species (unlike conventional capillary zone electrophoresis (CZE)) mainly as a result of: low analyte concentrations compatible with peak-mode ITP; the use of spacer molecules (with intermediate mobilities with respect to the analytes) able to separate the latter into discrete peaks; and the self-sharpening character of ITP. Moreover, low μL-range sample injection volume (allowing higher sensitivity than CZE, involving nL-range volumes), relatively simple sample preparation and analysis speed are some other advantages of cITP [26]. Lipoprotein separations need to be performed in anionic ITP mode at basic pH conditions and therefore coated capillaries are needed to suppress EOF and to prevent lipoprotein adsorption to capillaries [25]. Although several approaches have been used in previous literature, long-term coating stability at their optimal separation pH above 8.8 proved to be cumbersome, also when using polyacrylamide-coated or methylated fused silica capillaries [27,28].

Considering the high interest of SLPs, and in particular the unique properties of SlpA from *L. Acidophilus* ATCC 4356 for stable surface modifications, with potential antifouling properties and possible subsequent bioconjugation chemistry, the coating of silica surfaces with this protein and their use as a stable coating material for CE was examined for the first time and demonstrated using a challenging separation as example, i.e. cITP of lipoproteins.

2. Materials and methods

The *L. acidophilus* ATCC 4356 strain was obtained from the American Type Culture Collection (LGC Standards, Wesel Germany). All reagents used were purchased from Sigma–Aldrich unless otherwise stated. A pH Meter (HI4521, Hanna Instruments) was used for buffer pH adjustment. Milli-Q water (Millipore) was used for preparation of aqueous solutions.