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Bio-assisted potentiometric multisensor system for purity evaluation of recombinant protein A

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ABSTRACT

Recombinant proteins became essential components of drug manufacturing. Quality control of such proteins is routine task, which usually requires a lot of time, expensive reagents, specialized equipment and highly educated personnel. In this study we propose a new concept for protein purity evaluation that is based on application of bio-assisted potentiometric multisensor system. The model object for analysis was recombinant protein A from *Staphylococcus aureus* (SpA), which is commonly used for monoclonal antibody purification. SpA solutions with different amount of host cell related impurities (*Escherichia coli*, bacterial lysate) were analyzed. Two different bio-transducers were employed: proteinase K from *Tritirachium album* and baker's yeast *Saccharomyces cerevisiae*. It was shown that both bio-transducers are able to induce changes in pure and lysate-contaminated SpA samples. Different products of yeast digestion and proteolysis with proteinase of pure SpA and lysate were detected with size exclusion high-performance liquid chromatography (SE-HPLC). The induced changes of chemical composition are detectible with potentiometric multisensor system and can be related to SpA purity through projection on latent structures (PLS) regression technique. The proposed method allows for estimation of the impurity content with 12% accuracy using proteinase K and 16% accuracy using baker's yeast. The suggested approach could be useful for early contamination warning at initial protein purification steps. The analysis requires no expensive materials and equipment, no bio-material immobilization, and its duration time is comparable with other commonly used methods like chromatography or electrophoresis though the main part of this time is related to the sample preparation.

1. Introduction

Protein production for pharmaceutical use and biochemical studies has become a highly developed technological discipline in recent years [1]. Rapid evolution of modern medicine and biotechnology significantly increases the demand for recombinant proteins [2]. Since the first recombinant protein, insulin, was introduced in early 1980s, more than 100 new recombinant proteins were approved for therapeutic use [3]. About 30% of these pharmaceuticals are produced in *Escherichia coli*, showing that it is the major source of therapeutic recombinant proteins. Well-studied genetics, fast growth dynamics and high target product yield make *E. coli* a preferred choice for non-glycosylated protein expression [4]. Besides pharmaceutical protein production, *E. coli* is widely employed in production of proteins for a broad range of biotechnological and biochemical needs. Different recombinant forms of *Staphylococcus aureus* protein A (SpA) are examples of such objects. SpA, a cell-wall protein, is extensively used for detection and purification of antibodies including therapeutic monoclonal antibodies due to its high affinity to immunoglobulins and relatively high chemical stability, comparing to other immunoglobulin-binding molecules [5–7].

Quality control comes to the foreground when the protein of interest is produced for pharmaceutical needs or as a tool for pharmaceutical manufacturing. Competitive biopharmaceutical market increases quality requirements for modern products, creating a growing demand for quality monitoring instruments.

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The proteins produced in any biological source including *E. coli* must be extremely well purified from any impurities that can potentially cause adverse side effects [9,10]. The main group of impurities in recombinant protein products is the host cell related one including host cell proteins, endotoxins (e. g. pyrogenic lipopolysaccharides derived from Gram-negative bacteria like *E. coli*), nucleic acids and cell debris [8,11]. Conventional list of methods, suitable for effective protein analysis and recommended by the International Pharmacopoeia for purity control, includes high-performance liquid chromatography (HPLC), mass spectrometry (MS), capillary electrophoresis (CE) and gel electrophoresis [12–14]. These methods provide reliable versatile data on the composition of protein solutions, thus being indispensable for pharmaceutical manufacturing. However, there are certain disadvantages of these methods such as expensive equipment, expendable supplies and a need for highly educated personnel involvement. HPLC, MS, CE and gel electrophoresis usually require sample pre-treatment, utilization of toxic reagents and/or special dyes, labels, sorbents [11,15,16]. Thus, there is always a need for novel analytical approaches that could allow solving the same tasks but at lower cost.

It was shown earlier that multisensor systems based on electrochemical methods can be used for analysis of complex and dispersed liquids, including fermentation broth [17,18], pharmaceutical solutions [19], beverages [20–22] and clinical samples [23,24]. Simultaneous detection of endotoxins and other bacterial lysis contaminating species in purified water using impedance technology was reported in [25]. Multisensor systems based on potentiometric sensors have got certain advantages compared to other methods such as rapid response, good reproducibility, the ease of construction and miniaturization [26,27]. However, potentiometric sensors are normally not sensitive to biological macromolecules and non-ionized compounds. A special transducer should be employed to analyze such substances. Low molecular weight compounds like sugars can be determined by potentiometry using enzyme- or microorganism-catalyzed reactions that would increase sample acidity according to sugar concentration [28]. Such an analytical technique usually employs immobilized bio-recognition element and it can be applied for analyte quantification in aqueous solutions [26,29,30]. In order to simplify potentiometric measurements and to avoid microorganism immobilization a new approach for sugar analysis employing a yeast-assisted pH glass electrode was recently suggested [31]. The overall idea of the approach is to pretreat a sample through yeast metabolism and to detect corresponding changes in ionic composition by potentiometric sensors. This approach seems being applicable not only for sugar quantification but also for other non-ionized substances.

In the present research we were aiming to study the applicability of bio-assisted potentiometric multisensor system for impurity detection in complex protein solutions. The model objects were purified SpA solution and SpA solutions, contaminated with *E. coli* components from the source biomaterial. In order to modify initial media sample pretreatment with two different bio-transducers was done: proteinase K from *Tritirachium album* and baker's yeast *Saccharomyces cerevisiae*. Proteinase K is an endopeptidase, which hydrolyzes a broad range of proteins yielding oligopeptides and amino acids and it is widely applied in different molecular biology processes [32]. Baker's yeast can metabolize macromolecules like proteins, oligopeptides, lipids, nucleic acids into simple compounds and ions [33]. Thus, both proteinase K and yeast can work directly as SpA sample modulators without their immobilization on sensor surface.

2. Materials and methods

2.1. Materials

Yeast extract and $CaCl_2$ (dihydrate) were from "Fluka Analytical" (Germany), peptone (Bacto™ Tryptone) was from "BD Diagnostic Systems" (United States). D(+)glucose, Na_2HPO_4, KH_2PO_4, NaCl, KCl, glycerin, and NaOH were from "Vekton" (Russia), Tween-20 was from "Helicon" (Russia) and HCl was from "Reaktiv" (Russia). Tris(hydroxymethyl)aminomethane (Tris) was from "Merck" (Germany). All chemicals were of analytical grade.

Recombinant proteinase K from *Tritirachium album* was purchased from "Thermo Scientific" (Lithuania). The activity of enzyme solution in storage buffer (10 mM Tris–HCl (pH 7.5) with 50% (v/v) of glycerol) was 60 U/ml and protein concentration was 2 mg/ml. Active dry baker's yeast *Saccharomyces cerevisiae* was from "SAF NEVA" (Russia) and was purchased from the local retail store.

Molecular weight markers for SE-HPLC: bovine thyroglobulin (670 kDa), bovine γ-globulin (158 kDa), chicken ovalbumin (44 kDa), horse myoglobin (17 kDa) and vitamin B12 (1.35 kDa) were from Bio-Rad Laboratories (USA). Other materials that were used for bacteria growth, protein purification and HPLC analysis were of analytical grade and were obtained from Sigma-Aldrich.

2.2. Samples for analysis

SpA was expressed in *E. coli* BL21 (DE3) strain (Protein Contour LLC, Russia) in the presence of 0.1 mM isopropyl-β-D-thiogalactoside for 3 h. The composition of growth media was: 1% peptone, 0.5% yeast extract and 1% NaCl and the culture was grown with shaking according to the standard protocol. Cells were harvested by centrifugation at $10{,}000 \times g$ for 30 min and re-suspended in 10 mM Tris–HCl buffer (pH 8.0), containing 1 mM EDTA, 0.1 mM PMSF and 1 mg/ml lysozyme. Cells were sonicated and frozen at −70 °C. Defrosted lysate was divided into two parts. One part was dialyzed against distilled water for preparation of the "*E. coli* lysate" sample. Another part was acidified by 85% phosphoric acid to pH=3 and centrifuged at $4000 \times g$ for 20 min Supernatant was loaded onto C10/10 column (GE Healthcare Bio-Sciences AB, Sweden), packed with 4 ml Sepharose SP (GE Healthcare Bio-Sciences AB, Sweden), and eluted with 10 mM citrate buffer pH 5.5. Elution pool containing purified SpA was dialyzed against distilled water for further use as "purified SpA" sample. Purification quality was controlled using size-exclusion high-performance liquid chromatography (SE-HPLC) (Fig. 1). The "purified SpA" sample was diluted by distilled water to match the concentration of SpA in the "*E. coli* lysate" sample (2 mg/ml).

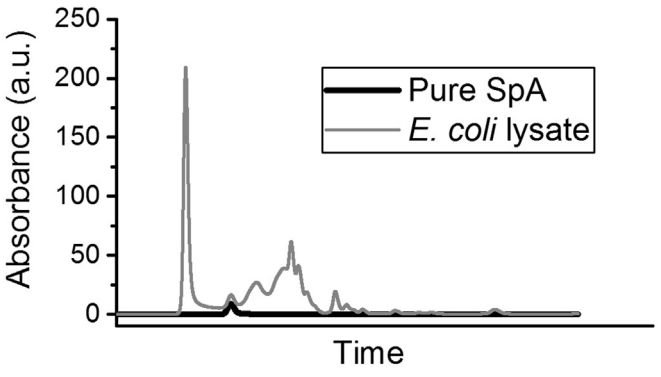

Fig. 1. SE-HPLC chromatogram of *E. coli* lysate containing SpA and purified SpA. Both samples were dialyzed against distilled water.