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These advantages were exploited in the purification of Human Serum Albumin (HSA) from plasma. HSA is a bulk protein product which is produced in 300–500 t per year, the major part of it being purified by the traditional *Cohn* fractionation method, but a well-described two-step chromatographic procedure is used as well [7]. Based on this process, we designed a membrane method employing a sequence of anion- and cation-exchange membranes (Sartobind Q and S, Sartorius, Göttingen, Germany), which is shown in Fig. 3. Since essentially the same raw material was used in both chromatographic modes (pooled human plasma), a valid comparison of the methods is possible. HSA isolation using modified membranes was operated successfully on the laboratory scale showing a ninefold increase in HSA productivity compared to the process based on porous adsorbent particles (220 g HSA/l of membrane adsorbent and hour compared to 26 g HSA/l of Sepharose FastFlow and hour as described by Berglöf *et al.* [8]). A scale-up to a total membrane volume of 110 ml showed a decline in productivity, the values reached, however, still were fourfold higher compared to the process mentioned above (79 g HSA/l of membrane adsorbent and hour). The reduced performance on the large scale could be attributed to the construc-

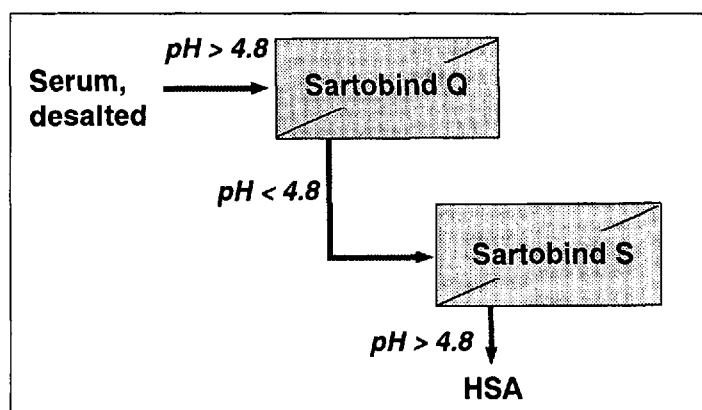


Fig. 3. Principle of the tandem ion-exchange HSA purification employing membrane adsorbents

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Summarising, membrane adsorbents may be an alternative to traditional chromatographic matrices especially for large-scale applications. By introducing a sphere of polymer-grafted ligands to the internal surface of the membrane, capacities similar to porous particles can be obtained. The continuity of the phase allows the use of thin stacks of membrane sheets at optimised column length, thus permitting high linear flow rates and short cycle times without a loss in capacity and resolution.

This work was part of the BMBF-project 'Stoffumwandlung mit Biokatalysatoren' (Ministry of Science and Education, Federal Republic of Germany). The support by Sartorius AG (Göttingen, Germany) is gratefully acknowledged.

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Cross-Linked Enzyme Crystals as Novel Materials for Catalysis and Chromatography

Alexey L. Margolin*

Cross-Linked Enzyme Crystals (CLEC®) [1] offer a unique combination of features normally associated with both enzymes (high activity and selectivity, ability to function under mild reaction conditions, ease of disposal) and hetero-

geneous catalysts (stability in different environments, recycling). This set of properties makes CLEC catalysts extremely useful in organic synthesis. Until recently, the use of crystalline enzymes was limited due to widely held misconceptions. These

include 1) the perceived difficulties of large-scale protein crystal preparation, 2) the perceived mechanical fragility of protein crystals, and 3) the expected reduction in reaction rates over solution state reactions. Our recent research has demonstrated that these misconceptions are ill founded.

Indeed, five CLEC catalysts of lipases from *Candida rugosa*, *Pseudomonas cepacia*, thermolysin, subtilisin, and penicillin acylase have been manufactured on a multi-kilogram scale and commercialized by *Altus Biologics Inc.*, and many

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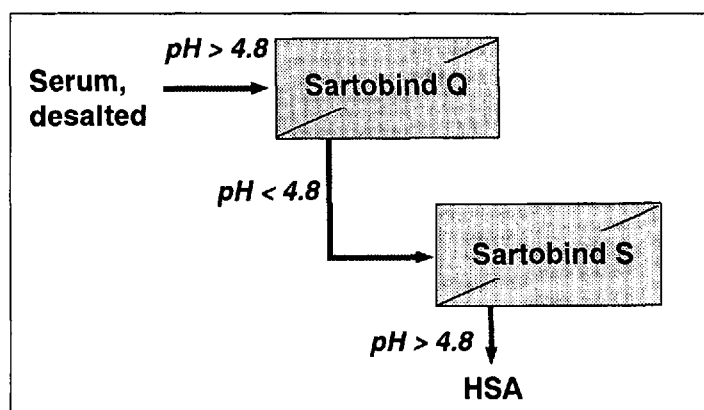


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include 1) the perceived difficulties of large-scale protein crystal preparation, 2) the perceived mechanical fragility of protein crystals, and 3) the expected reduction in reaction rates over solution state reactions. Our recent research has demonstrated that these misconceptions are ill founded.

Indeed, five CLEC catalysts of lipases from *Candida rugosa*, *Pseudomonas cepacia*, thermolysin, subtilisin, and penicillin acylase have been manufactured on a multi-kilogram scale and commercialized by *Altus Biologics Inc.*, and many

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more CLEC catalysts have been created on a laboratory scale [2]. These catalysts are stable and highly active in many different reaction media.

New Catalytic Applications

One of the recent developments in the CLEC-catalyzed reactions was a discovery that CLEC catalysts can be extremely active in neat organic solvents. The potential synthetic benefits of purified lipases in organic solvents, such as higher enantioselectivity, activity, and ease of downstream processing, have not been realized to date. Not only the cost of purified lipases is higher, but their stability and activity in organic solvents is lower than that of the crude counterparts. We have recently developed a novel drying procedure for CLECs of lipases and subtilisin (that, by definition, are pure enzymes) by washing them with solutions of different surfactants in organic solvents [3]. The high specific activity, purity, and stability of CLECs result in the high catalyst productivity in these media which compares favorably with the best asymmetric catalysts.

In addition to their stability against various denaturants [4], CLEC catalysts are mechanically stable for long periods of time under standard fine-chemical production conditions (agitation, pumping, and filtration) [5]. This is extremely important for industrial applications, since as a process is scaled up from the bench through pilot scale to production, there is

an increase in mechanical shear and pressure.

So far, the major applications of crystalline enzyme catalysts have been in the production of high fructose corn syrup [6], the resolution of chiral compounds [7], synthesis of peptides [8] and peptidomimetics [9], and C-C bond forming reactions [10]. Synthetic applications of CLEC catalysts will remain the major focus of research activities. In the near future, we will see many more enzymes in CLEC form, including cofactor-dependent dehydrogenases. However, since CLEC technology is broadly applicable to a wide variety of proteins, crystalline materials will undoubtedly find use in other areas.

Materials for Chromatography

One of these areas is separation of molecules *via* chromatography or simulating moving bed (SMB) technology. Unlike crystals of small molecules, protein crystals are macroporous materials and can be thought of as bioorganic zeolites. The uniform solvent-filled channels traverse the body of a crystal and, thus, facilitate the transport of ligands in and out of the crystal. The diameter of the channels depends on the nature of the protein and its crystal form, and ranges from 20 to 100 Å. Unlike the majority of current stationary phases, such as silica, zeolites, and synthetic polymers, protein crystals are asymmetric molecules made of L-amino acids and can, in principle, provide

stereoselective adsorption of chiral ligands. Protein crystals made of enzymes, receptors, or antibodies may provide yet another level of selectivity, namely specific affinity binding of small molecules to the binding sites of the stationary phases. Since proteins are weak ion-exchangers with isoelectric points from 2 to 12, one can easily manipulate binding of small molecules by changing pH and buffer content of the eluent. We will present data that demonstrate the unique properties of protein crystals in separation of mixtures of small molecules *via* several different mechanisms.

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Regeneration of Redox Enzymes for Continuous Preparative Processes

Wim A.C. Somers*, Edwin C.A. Stigter, Wim van Hartingsveldt, and Jan Pieter van der Lugt

Introduction

Oxidoreductases are valuable biocatalysts for selective conversions in industrial processes. They are used in several areas such as the pharmaceutical industry,

agricultural industry, and the food industry, *e.g.* for the enantioselective synthesis of intermediates or the production of oxidized carbohydrates. Reactions catalyzed by oxidoreductases are characterized by the need of enzyme regeneration, more

specifically by the need for cofactor and/or coenzyme regeneration [1][2]. For industrial purposes, this means that application of these enzymes on technological scale is determined by the availability of a cost-efficient process for regeneration of the enzyme.

Enzyme Electrodes for Enzyme Regeneration

Enzyme electrodes are an appropriate tool for the regeneration of redox enzymes. In these devices, oxidoreductases are immobilized together with a conducting in-

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