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Research Paper

Rheological and mechanical behavior of polyacrylamide hydrogels chemically crosslinked with allyl agarose for two-dimensional gel electrophoresis

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ABSTRACT

Two-dimensional (2-D) gel electrophoresis currently represents one of the most standard techniques for protein separation. In addition to the most commonly employed polyacrylamide crosslinked hydrogels, acrylamide–agarose copolymers have been proposed as promising systems for separation matrices in 2-D electrophoresis, because of the good resolution of both high and low molecular mass proteins made possible by careful control and optimization of the hydrogel pore structure. As a matter of fact, a thorough understanding of the nature of the hydrogel pore structure as well as of the parameters by which it is influenced is crucial for the design of hydrogel systems with optimal sieving properties. In this work, a series of acrylamide-based hydrogels covalently crosslinked with different concentrations of allyl agarose (0.2–1%) is prepared and characterized by creep-recovery measurements, dynamic rheology and tensile tests, in the attempt to gain a clearer understanding of structure–property relationships in crosslinked polyacrylamide-based hydrogels. The rheological and mechanical properties of crosslinked acrylamide–agarose hydrogels are found to be greatly affected by crosslinker concentration. Dynamic rheological tests show that hydrogels with a percentage of allyl agarose between 0.2% and 0.6% have a low density of elastically effective crosslinks, explaining the good separation of high molecular mass proteins in 2-D gel electrophoresis. Over the same range of crosslinker concentration, creep-recovery measurements reveal the presence of non-permanent crosslinks in the hydrogel network that justifies the good resolution of low molecular mass proteins as well. In tensile tests, the hydrogel crosslinked with 0.4% of allyl agarose exhibits the best results in terms of mechanical strength and toughness. Our results show how the control of the viscoelastic and the mechanical properties of these materials allow the design of mechanically stable hydrogels with improved sieving ability in protein electrophoresis over a wide range of molecular masses.

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1. Introduction

In the field of proteomics, the ability to detect a large number of proteins in a single analysis represents a key issue to achieve fast and efficient operation (Rabilloud et al., 2009; Miller et al., 2010). In this context, the combined use of 2-D gel electrophoresis coupled with mass spectrometry has allowed enormous advances during last few decades and has become nowadays one of the standard approaches for protein separation and identification (Rabilloud, 2002; Görg et al., 2004; Rabilloud et al., 2010; Rogowska-Wrzesinska et al., 2013).

Among the materials used for 2-D gel electrophoresis, polyacrylamide crosslinked hydrogels have been extensively investigated in the literature because their tunable mesh size porosity appears to be ideal for separating proteins and DNA samples (Calvet et al., 2004; Manns, 2005). Typically, acrylamide concentrations higher than 5% are used to form the separation matrix, with the acrylamide concentration being selected to maximize resolution of the range of proteins of interest (Gerstner et al., 2000). Lower acrylamide concentrations are necessary when resolution of large high molecular mass (HMM) proteins (>500 kDa) is sought, however at the expense of poor mechanical stability of the gel matrix that often yields difficulties in handling these media (Suh et al., 2005). Other polymeric systems alternative to polyacrylamide gels have also been proposed as separation matrices for 2-D electrophoresis, including agarose (Oh-Ishi and Maeda, 2007; Greaser and Warren, 2012), CNTs-modified polyacrylamide gels (Parthasarathy et al., 2011) and acrylamide–agarose copolymers (Roncada et al., 2005). In particular, the advantages of the acrylamide–agarose system mainly lay in the possibility of improving the resolution of large HMM proteins without compromising the resolution of low molecular mass proteins, partly due to the optimal average pore size of these materials.

Indeed, the electrophoretic migration process through the polymeric gel matrix is driven by the interactions between the protein fragments and the porous network of the gel, causing the quality of protein resolution to be highly dependent on different structural parameters characteristic of the gel matrix (Wang and Ugaz, 2006). Among these, mean gel pore size, pore size distribution and stiffness of the gel play a crucial role. In order to achieve improved separation performance in 2-D electrophoresis applications, it is therefore essential to understand the specific nature of the pore structure of the gel and the parameters through which this pore structure can be controlled and manipulated (Anseth et al., 1996; Dumitriu et al., 2011). In particular, it is of great interest to investigate structure–property relationships of these hydrogels in the attempt to optimize their functional performance.

In this work, the rheological and mechanical properties of a series of promising acrylamide-based hydrogels chemically crosslinked with allyl agarose were studied at increasing crosslinker concentrations. As previously demonstrated (Roncada et al., 2005), this class of hydrogels was found to improve protein separation in 2-D gel electrophoresis. However, no systematic investigation on the effects of chemical composition and crosslinking density on the rheological, structural and functional properties of these systems was presented. In order to obtain optimal separation performance, a thorough understanding of the relationships between chemical composition, physical structure, mechanical and functional properties of these materials is however necessary and still to be accomplished.

In the present study, the effects of chemical composition and crosslinking density on the rheological and mechanical properties of acrylamide–allyl agarose crosslinked hydrogels were thoroughly investigated. As opposed to previous work (Roncada et al., 2005), a wide range of hydrogel chemical compositions was studied and their effect on structural and functional properties of the hydrogel was elucidated. By employing dynamic rheological tests and creep-recovery tests, a correlation was found between the rheological response of these hydrogels and their sieving properties. More specifically, the evaluation of the crosslinking density by means of dynamic tests and the use of viscoelastic models for determining the resistance of non-permanent crosslinks to move in the network systems shed light on the pore structure of the hydrogel matrix and helped to clarify its influence on the electrophoretic separation performance. In addition, the mechanical stability of the crosslinked hydrogels was investigated by means of tensile tests and correlated with the crosslinking density of the gel matrix.

The aim of this study is to achieve a greater understanding of structure–property relationships in crosslinked acrylamide–agarose hydrogels and to provide useful guidelines for the design of mechanically stable hydrogels with improved protein resolution to be employed in 2-D gel electrophoresis.

2. Experimental part

2.1. *Materials and gel preparation*

Allyl agarose was prepared as described elsewhere (Chiari et al., 1995; Chiari et al., 1996; Roncada et al., 2005). Briefly, 33 mg of sodium borohydride and 1.6 mL of allylglycidyl ether were added to a suspension of 1 g of agarose in NaOH (33 mL, 0.3 M) under stirring. After slow stirring for 12 h, allyl derivative of agarose was recovered from the suspension by filtration and then washed with distilled water up to a neutral pH. Allyl agarose was finally dehydrated in methanol and dried under vacuum at 35 °C.

Acrylamide gels crosslinked with allyl agarose were prepared by dissolving different amounts of allyl agarose (from 0.2% to 1% w/v) at 95 °C, in 0.375 M Tris–HCl buffer at pH=8.8. The solution was cooled down to 40 °C and then acrylamide (10% w/v) and sodium dodecyl sulfate, SDS (0.1% w/v) were added maintaining the solution in a thermostatic bath at 40 °C. Ammonium persulfate (APS) (0.04%) and tetramethylethylenediamine (TEMED) (0.05% v/v) were also added immediately before the solution was moved to the casting mold. Polyacrylamide standard gel (PAB) was cast using a standard procedure with 10% w/v of acrylamide and 2.6% w/v of N,N′-methylenebisacrylamide. Acrylamide, N,N′-methylenebisacrylamide, APS and TEMED were purchased from Sigma-Aldrich. Allylglycidyl ether and SDS were purchased from Fluka (Buchs, Switzerland). Agarose (low EOF) was from Amersham Biosciences (Piscataway, NJ, USA).