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Evanescent wave infrared chemical sensor possessing a sulfonated sensing phase for the selective detection of arginine in biological fluids

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Abstract

This paper describes a new infrared (IR) sensing scheme for the determination of arginine (Arg). In this method, the surface of an IR evanescent wave sensing element was modified with sulfonic acid groups to selectively interact with Arg through specific interactions with its guanidine moiety. The sulfonated sensing phase was prepared using a two-layer modification approach. To demonstrate that this assembly could be used for selective infrared sensing, a large number of amino acids were subjected to analysis. Although the sulfonate groups on the surface of the sensing element did interact selectively with the guanidine groups of Arg species, lysine and histidine units caused some interference; this problem could be minimized because of the unique IR absorption bands of the guanidine moiety of Arg. To optimize the detection conditions, we studied the effects of both the pH and the composition of the polymer. The most intense signal was obtained at pH 9. We observed different adsorption rates for the detection of Arg at different values of pH, which we attribute to changes in the accessibility of the analytes to the pore structures of the sensing phase. The composition of the base polymer was also optimized; 60% PVBC (w/w) provided a water-stable, sensitive phase for the detection of Arg in aqueous solution. Under the optimized conditions, we obtained a linear range of detection up to 0.1 mM with a detection limit of ca. 5 μM.

Keywords: Evanescent wave; Infrared; Sulfonic acid group; Guanidine moiety; Arginine

1. Introduction

Amino acids, the building blocks of peptides and proteins, play many vital roles in the metabolic processes within living bodies. Among the amino acids, arginine (Arg) is the most basic natural amino acid, having the highest proton affinity [1]. On the surfaces of proteins, Arg and two other relatively weakly basic amino acids, lysine and histidine, often play crucial roles in protein recognition [2]. Arg residues are important components for biological peptide and protein recognition and for protein folding processes. The guanidine moiety – a strongly basic functional unit responsible for the majority of this amino acid's interactive behavior – provides a site for strong electrostatic interactions and/or hydrogen bonding with anionic functional units [3]. Arg is also of considerable interest in human nutrition and health; it is present in significant amounts in many agricultural products [4]. Measuring the concentration of Arg in blood serum is a fairly effective approach toward identifying diseases, such as disorders related to amino acid metabolism, and determining the clinical states of patients having unbalanced nutrition [5]. Also, the concentration of Arg can be used to measure the maturity of peanuts and grapes, and the degree of fermentation of musts and wines. Thus, techniques for the rapid determination of the concentration of Arg in biological fluids remain in demand.

A large number of methods have been proposed to analyze the levels of Arg in biological samples, including those using HPLC [6], amino acid analyzers [7], capillary electrophoresis [8], voltammetry [9], and fluorometric methods [10]. These methods suffer from a number of disadvantages, including they being highly time-consuming and complicated in terms of the need for sample pretreatment. Although colorimetric-based analyses – e.g., the Staron–Allard and Voges–Proskauer methods [11], and Sakaguchi reaction methods [12] – are widely used because of their simplicity, such wet chemical reactions generally lack simplicity and, sometimes, specificity. The application of the

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arginase enzyme for the determination of Arg has also been reported [13,14]; this method is, however, rather slow (60 min per sample) and, furthermore, it is useless if urea or ammonia is present in the sample. The enzymatic end-point analysis method proposed by Mira de Orduna [15] is simple, but it consumes a large amount of material.

To improve upon these existing methods, we propose a new Arg sensing scheme based on an evanescent wave infrared (IR) sensor. Because of the short depth of penetration (d_p) of evanescent waves, such infrared sensors are highly suitable for the detection of organic compounds in aqueous solutions [16]. The d_p varies in the IR region from a few tenths of a micron to a few microns; these small values of d_p limit the sensitivity of this type of detection. After suitable treatment of hydrophobic thin films on the surfaces of the evanescent wave sensing elements, the sensitivity can be increased through the attraction of target compounds toward the evanescent field [17]. The ability of hydrophobic thin films to attract polar compounds is, however, limited. To improve the attraction toward polar compounds while maintaining the water stability of the sensing phase, we have devised a two-layer modification method. In this approach, a reactive hydrophobic thin film is first treated on the surface of the IRE; the desired polar functional groups are subsequently covalently bound to the surface of the hydrophobic thin film [18–22]. In this manner, water-stable sensing phases can be produced with the potential for enhanced sensitivity.

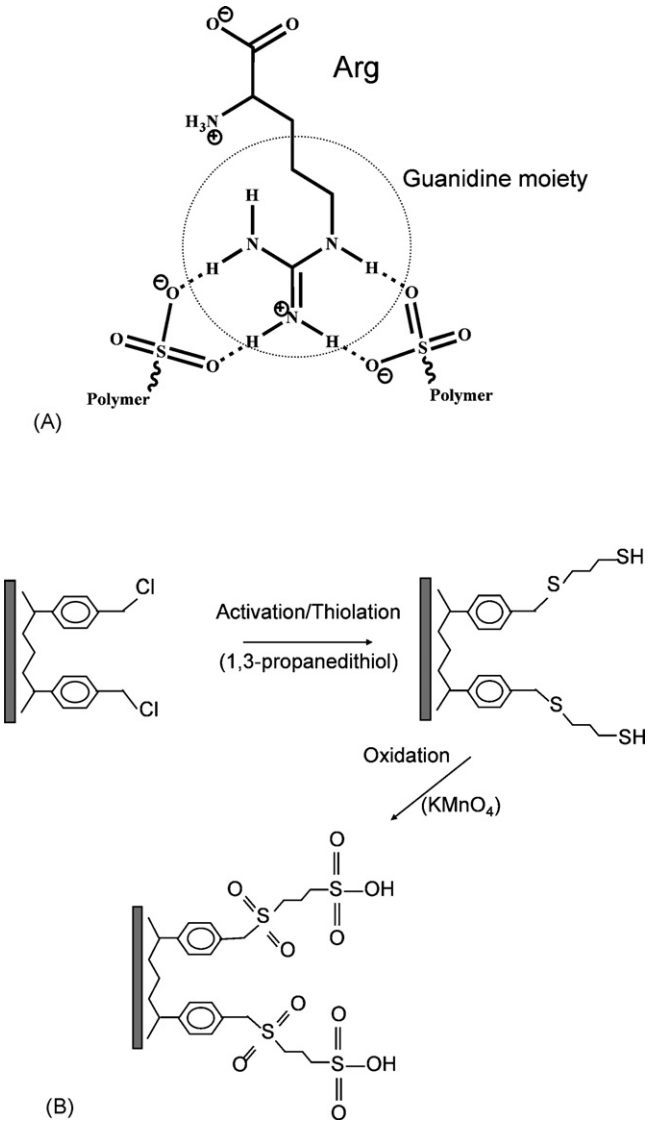

Fig. 1. (A) Schematic illustration of the interaction between two sulfonate groups and the guanidine group of Arg. (B) Procedures used in the preparation of the water-stable sulfonate sensing phase.

To produce a sensitive infrared sensor for the specific detection of Arg, we chose to modify the sensing phase with sulfonic acid groups. Several advantages exist for using sulfonic acid groups to attract Arg species. First, the sulfonic acid group is easily deprotonated; the group bears a negative charge at most values of pH. This property is highly useful in terms of attracting positively charged Arg units through electrostatic interactions. Secondly, the sulfonic acid group exhibits only a few infrared absorption bands, which are located mainly around 1000 cm^{-1}. Because these absorption bands exist far from the characteristic bands of amino acids, spectral interference is minimized. Furthermore, as demonstrated by Schug et al. [23] and Friess and Zenobi [2], sulfonic acid groups are capable of interacting selectively with amino acids possessing guanidine moieties because of their mutual forklike structures (Fig. 1A). Therefore, we believed that integrating these features with IR sensing technology would provide access to selective and sensitive IR sensors. To prepare the desired sulfonated sensing phase, we utilized the reaction scheme displayed in Fig. 1B. We used poly(vinylbenzyl chloride) (PVBC) as a reactive hydrophobic polymer with which to treat the surface of the sensing element. Through suitable reactions with thio compounds and their subsequent oxidation, a sensing phase presenting sulfonic acid groups was prepared for the direct sensing of Arg units.

2. Experimental

2.1. Chemicals

Poly(ethylene) (PE) and PVBC were obtained from Aldrich Chemical and used as received. Toluene (Acros Organics, Geel, Belgium) and *p*-xylene (Shimakyu Chemicals, Osaka, Japan) were used as solvents for the polymer solutions. Sodium iodide and potassium permanganate were obtained from Shimakyu Chemicals. Acetonitrile was obtained from TEDIA (Fairfield, Ohio). 1,3-Propanedithiol was purchased from Lancaster Chemicals. Tributylamine was obtained from Acros Organics. All reagents were of reagent grade and used as received without further purification. Deionized water was used to prepare all of the aqueous solutions.

2.2. Apparatus

The experimental setup is presented in Fig. 2A. The dimensions of the sample cell were 30 mm × 30 mm × 20 mm; 15 mL of the aqueous sample could be loaded. A 45° trapezoidal (55 mm × 4 mm × 2 mm) zinc selenide internal reflection element, purchased from International Crystal Laboratory