Analytical Biochemistry 441 (2013) 218–224

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Development of a multiple-bile-ion-sensing membrane electrode

Sudeshna M. Chatterjea *, Koustubh Panda *

Department of Biotechnology and Dr. B.C. Guha Centre for Genetic Engineering and Biotechnology, University of Calcutta, Kolkata 700019, West Bengal, India

ARTICLE INFO

Article history:
Received 12 April 2013
Received in revised form 21 June 2013
Accepted 28 June 2013
Available online 16 July 2013

Keywords:
Multiple-bile-ion-sensing electrode
Changing electrode selectivity
Electrode characterization
Application in kinetics

ABSTRACT

A multiple-bile-ion-sensing polyvinyl chloride-based membrane electrode capable of monitoring any of the three common bile ions in humans, namely, cholate, deoxycholate, and chenodeoxycholate, was developed and characterized. Compared to single-bile-ion-sensing electrodes, it showed a sub-Nernstian response. All other electrode properties were, however, similar, making this a successful replacement for three individual electrodes. With appropriate conditioning, this electrode could repeatedly change selectivity without losing membrane activity. It was reproducible, was stable for 5 months, had low response time, and could be used to measure critical micelle concentrations. The lower limit of detection was 10 nM. Selectivity coefficients for various anions with respect to bile ions more or less followed the Hoffmeister series. Plots of R ((Nernst equivalent of slope in the presence of primary ion and a fixed amount of interfering ion)/(slope in the presence of only the primary ion)) vs square root of ionic strength for an interfering ion were linear. One major application of this electrode is its use in kinetics. We have tested its ability to monitor continuously changing bile ion concentrations during their interactions with a biocompatible polymer, polyethylene glycol (6000), and determined rate constants.

Bile acids constitute a large family of molecules comprising a steroid structure with four rings containing hydroxyl groups present in different orientations, and a 5- to 8-carbon side chain terminating in a carboxylic acid, making them weakly surface active [1,2]. Though these biological detergents are mainly involved in dietary lipid absorption and cholesterol homeostasis, over the past few years they have been shown to also act as signaling molecules [3–7]. To elucidate physiological roles of bile salts in molecular level and to provide a model for lipid protein interaction it is essential to study their interactions with proteins kinetically and at equilibrium.

A number of methods are currently available for determining bile salt concentrations in solution, using which bile salt protein interactions have been studied at equilibrium [8]. Reports on kinetics of these interactions, however, are scarce because of the lack of a suitable technique for estimating continuously changing bile salt concentrations in solution. This is mainly because surfactants do not have concentration-dependent sharply changing properties except for surface tension and conductance, which are not suitable for kinetic purposes especially in the presence of salts. To overcome this limitation, we have focused on the use of conventional plastic ion-selective electrodes, a methodology well established in literature that has been used for estimating synthetic surfactants in solution [9–13].

Maulik et al. used a CTAB (cetyltrimethyl ammonium bromide)-selective membrane electrode to study the kinetics of its interactions with biopolymers [10–12]. Bile salt-selective membrane electrodes could similarly be used for determining kinetic parameters of their interactions with proteins. Some papers in the past have reported preparation of bile salt-selective liquid membrane electrodes [13–16] and used them to study their interactions with bovine serum albumin at equilibrium [15].

Since it would be a more efficient use of electrodes if a single electrode could be used to monitor multiple ions in solution (selective to a single ion at a time), we have attempted to develop and characterize a polyvinyl chloride (PVC)-based multiple-bile-ion-sensing electrode. Ideally this new electrode could be used to study the interactions of three major bile salts (cholate, deoxycholate, and chenodeoxycholate) with proteins. To establish that this multiple-ion-sensing electrode could truly be a replacement for three single-ion-sensing electrodes, a comparative study with a cholate-selective, a deoxycholate-selective, and a chenodeoxycholate-selective electrode was done at each step of characterization. Electrode characterization was done in terms of response time, detection limit, drift, stability, lifetime, reproducibility, pH, temperature, and response in comparison to ideal Nernstian behavior. Interfering effects of other bile salts, synthetic surfactants, cholesterol, and inorganic ions during the monitoring of the principal bile ion were studied and selectivity coefficients calculated. In addition to estimating the free bile ion concentration in solution, including physiological fluids, the electrode's ability to monitor continuously changing bile ion concentration was tested

* Corresponding authors. Fax: +91 033 2461 4849.
E-mail addresses: sudeshnachatterjea@hotmail.com (S.M. Chatterjea), pandak66@yahoo.co.uk (K. Panda).

0003-2697/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2013.06.022

by kinetically following the reactions of each of the three bile salts with polyethylene glycol, and rate constants were calculated.

Materials and methods

Materials

The bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC) monohydrate were obtained from M.P. Biomedicals (USA), and sodium chenodeoxycholate (NaCDC) was obtained from Sigma–Aldrich (USA). The surfactants CTAB, sodium dodecyl sulfate (SDS), and Triton X-100 were from Sisco Research Laboratories (India), cholesterol and Brij 56 were from Sigma–Aldrich, and Tween 20 was from Merck (Germany). The polymer PVC (low molecular weight) was from Sigma–Aldrich, and polyethylene glycol (PEG 6000) was from BDH (UK). Dioctyl phthalate (used as plasticizer) and tetrahydrofuran (THF) were from Sisco Research Laboratories. Cyclohexanone and cholesterol were from Sigma–Aldrich. All sodium and potassium salts and all other chemicals were from Sisco Research Laboratories or Loba Chemie (India) and were of analytical-reagent grade. All the bile salts, surfactants, THF, and cyclohexanone were 99% pure and were used as received. All solutions were prepared using conductivity water.

Preparation of membrane

To compare the performance of the multiple-bile-ion-sensing electrode with single-ion-sensing electrodes, three individually responsive membrane electrodes selective to cholate (El_C), deoxycholate (El_{DC}), and chenodeoxycholate (El_{CDC}) and a multiple-ion-sensing electrode ($El_{C/DC/CDC}$) were prepared.

Carrier complexes were prepared according to the methods given by Hayakawa and Kwak [9] and Maulik et al. [10]. CTA–NaC carrier complex was prepared by mixing 25 ml each of 50 mM CTAB and 50 mM NaC. The complex coacervate formed was extracted with equal volumes of a 1:1 mixture of methanol and chloroform. The solvent was evaporated, and the complex obtained was dried and stored under vacuum. Following this method, the other two carrier complexes CTA–DC and CTA–CDC were also prepared. To prepare the multiple bile ion carrier complex, a mixed coacervate (CTA–(C + DC + CDC)) was prepared following the procedure used by Panda et al. [11]. NaC, NaDC, and NaCDC (25 mM each) were mixed with 75 mM CTAB and the water-insoluble coacervate was extracted with the methanol:chloroform mixture as usual. To prepare membranes, 0.35 mg of PVC and 15 mg of carrier complex were dissolved in 1.1 ml of dioctyl phthalate and 10 ml of cyclohexanone by gentle heating. The mixture was then spread on a flat petri dish (10 cm diameter) and allowed to evaporate at room temperature overnight. Disks of the plastic membrane formed were cut and fixed at ends of PVC tubes using PVC dissolved in THF as glue. These tubes were then placed in conditioning solution (1 mM relevant bile salt solution and 10 mM NaCl) for 3–4 h or overnight [10–13].

El_C and $El_{C/DC/CDC}$ conditioned in NaC became cholate ion selective, El_{DC} and $El_{C/DC/CDC}$ conditioned in NaDC became deoxycholate ion selective, and El_{CDC} and $El_{C/DC/CDC}$ conditioned in NaCDC became chenodeoxycholate ion selective. Since $El_{C/DC/CDC}$ could be used to determine any of the three bile salts, for the purpose of distinction it is denoted as $El_{\mathbf{C}/DC/CDC}$ when used for measuring cholate, as $El_{C/\mathbf{DC}/CDC}$ when measuring deoxycholate, and $El_{C/DC/\mathbf{CDC}}$ when measuring chenodeoxycholate ions. After being reversible to one bile salt, it could be made to be solely reversible to another after washing and conditioning in the appropriate bile salt solution. The response of an electrode reversible to one bile salt toward the other two and the interference effect of one bile salt in the determination of another in a solution containing a mixture are discussed later (Results and discussion).

Storage of membrane electrodes

For daily use, electrodes were stored air-dried. For long-term storage, they were air-dried and stored in sealed plastic bags at room temperature. Storage in conditioning solution or water deactivated them. Electrodes were ready for use after conditioning for 15–30 min.

Electrometric measurements

For electrochemical measurements, the following assembly was used: calomel//reference solution/CTA + selective membrane/test solution//calomel. The two calomel electrodes were connected in opposition with salt bridges nullifying all effects of reference electrode, junction potentials, Donnan effects, etc., so that the resulting potential depended only on the membrane potential. The reference solution contained a 1 mM bile salt solution in 10 mM NaCl (conditioning solution) and the test solution contained varying concentrations of bile salt. The cell electromotive force (emf) was measured using a Kusam-Meco digital multimeter with an accuracy of 0.1 mV. The entire assembly was thermostated in a water bath (0.2 °C uncertainty) and the water bath was placed on a magnetic stirrer to measure stable cell potentials under constant stirring condition [10–12].

To test performance of the membrane electrode system, plots of cell emf with gradually increasing bile salt concentrations in the test solution were constructed. Electrode characterizations were done according to IUPAC recommendations [17]. Electrode response slopes for El_{NaC} (55 mV decade^{-1}) and El_{NaCDC} (50 mV decade^{-1}) were near Nernstian and for El_{DC} and $El_{C/DC/CDC}$ were sub-Nernstian (35 mV decade^{-1}) at 298 K. All these electrodes were also reversible to the glycine and taurine conjugates of their corresponding bile salts. In this paper characterization was done using the unconjugated forms of the bile salts.

Effects of temperature, pH, and interfering ions on electrode performance

Since emf depended only on membrane potential, we neglected the effects of temperature, pH, and interfering ions on the reference electrode.

For estimating the effects of temperature on electrode function, emf vs log[bile salt] plots were constructed at 298, 303, 310, and 318 K. The break in the plots corresponded to the critical micelle concentration (cmc) of the bile salt. From each plot, electrode response slope and cmc were calculated. Cmc values obtained for a bile salt using the multiple-ion-sensing and the individually responsive electrodes were compared.

To determine the working pH range for $El_{C/DC/CDC}$, the Nernst equivalents of slope were determined and electrode behavior was compared with El_C, El_{DC}, and El_{CDC} at pH 6.8, 7.8, 8.8, 9.8, and 10.8. To emphasize the suitability of electrode use at blood pH (7.4), and in the pH range (7.4–8.5) of hepatic bile [18], the electrodes were further tested at pH 7.4, 7.6, 8.0, 8.2, and 8.6. Interference from salts limited the choice of buffers. Because of low interference, 10 mM KH_2PO_4/K_2HPO_4 buffer was selected for maintaining pH 6.8–8.0, 10 mM Hepes buffer for 8.2, and 50 mM Glycine/NaOH for pH 8.6–10.8. Bile acids precipitate at pH lower than 6.5, while at higher pH electrode slopes were low because of interference from buffers.

Selectivity coefficients of the electrodes for various interfering bile ions and anions were calculated using a modified version of the fixed interference method [19,20] as described using practical