INVESTIGATIONS ON PROTEIN ADSORPTION ON *IN-VIVO* SENSOR MEMBRANE SURFACES

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SUMMARY

In order to understand the mechanism of fouling of electrode surfaces of *in vivo* electrochemical sensors used for monitoring whole blood, the investigations on protein adsorption on the membrane surfaces were undertaken. Using an oxygen sensor, the protein adsorption studies were carried out on two different polymeric surfaces - teflon (PTFE) and polyurethane (PU) - using Scanning Electron Microscopy and Fourier Transform Infrared Spectrophotometry. Investigations reveal that even a single protein like albumin or fibrinogen tend to adhere to the sensor membrane surface and influences the electrochemical characteristics of the sensor. The influence is more significant, in the case of PTFE membrane.

INTRODUCTION

For *in vitro* and *in vivo* measurements of blood using electrochemical sensor, haemocompatibility of the sensor membrane is an important consideration. In spite of the addition of anticoagulant or antiplatelet agents, the colloidal and cellular materials of whole blood tend to foul the electrode surface, rendering its functioning defective. Perhaps the most critical step in determining the biocompatibility of a surface in contact with the flowing blood is protein adsorption(1,2). The nature of these adsorbed protein layers seem to determine whether the surface is thrombogenic. The characteristics and the composition of the protein layer are believed to be important in determining the biocompatibility(3,4). The relative amount of fibrinogen and albumin adsorbed is also an important parameter(5,6). Selective protein adsorption may be dictated by the chemical and physical structures of a polymer surface(7).

The *in vivo* measurement of biological fluids with membrane electrodes continue to attract attention of various authors(8-10). Meyerhoff *et al.* have studied the factors affecting the potentiometric evaluation of various ions in protein containing solutions under various conditions(11-13).

An understanding of the mechanism by which proteins are adsorbed from blood onto polymer surfaces is likely to pave way for making more biocompatible sensors. Some data on implantable biomaterials are available but few data are available on sensor membranes. An attempt has been made by us to work out relevant information on the biocompatibility of electrochemical sensors.

This paper gives the preliminary investigations on the effects of various protein adsorption on the surface of the membranes in terms of sensor response. Two different membranes PTFE and PU, and two proteins mentioned above - albumin and fibrinogen were taken for this work. The protein adsorption has been characterized by the Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectrophotometry (FTIR).

EXPERIMENTAL

SENSOR

The investigation has been made with commercial oxygen sensor from a blood gas analyzer (Corning).

MEMBRANES

Two different membranes - PTFE and PU - were used for the present investigation. PTFE membrane was commercially obtained and the other one was prepared by controlled evaporation of a solution of Ticoflex PU tubings in tetrahydrofuran (BDH).

INSTRUMENTATION

A circulating rig was fabricated, for protein adsorption

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studies, in which a T-shaped socket was connected to silicon rubber tubings and protein solutions were circulated in the system through a peristaltic pump (Watson-Marlow). The sensor was placed in the socket and was connected to a scanning Potentiostat (EG & G PAR-362) through an electrometer tube with floating ammeter (Keithley, Model 642) for measuring the low current. The output of the electrometer tube was then connected to an X-Y recorder (Advanced Bryns).

SEM measurements were made with Jeol Scanning Electron Microscope (JSM-TICC) and FT-IR measurements were made with Matson FT-IR, Spectrophotometer (Model Alpha Centauri).

All measurements were made at room temperature, $25^{\circ}\pm 1^{\circ}\text{C}$.

MATERIALS

The protein solutions (Bovine Albumin and Fibrinogen, Sigma) were prepared in phosphate buffered saline (Oxoid) solution and concentrations were kept close to the concentration found in blood plasma (albumin 4.5 g/100 ml) and fibrinogen 0.4 g/100 ml. All solutions were prepared in double distilled water.

RESULTS AND DISCUSSION

In order to see whether the rate of flow of liquid in the circulating rig has any influence on the performance of the electrode, the water was circulated in the rig at different speeds in the range 100 ml - 500 ml per minute. It was observed that there is no change in the cyclic voltamogramme (c.v). The observation was made with PU as well as PTFE membrane.

In order to find out the relative permeability coefficient⁽¹³⁾ of the two membranes, PU and PTFE, the steady state current was measured in identical conditions. The value 1.4 thus obtained suggests that the sensor with PU membrane is more sensitive.

The c.v. with water and albumin for PU membrane has been shown in Figure 1. The two c.vs are almost identical except for the fact that with albumin in the circulating ring, there is an upward shift. Similar observations were made with fibrinogen. However, prolonged contact of membrane surface with albumin solution brings noticeable change in the c.v. (Figure 2) when PTFE was used.

The SEM studies clearly show that protein molecules get adsorbed at the surface. The scanning of adsorbed surfaces at various places by Electron Microscopy reveal that the adsorption on PU surfaces is comparatively lower than on PTFE surface. This trend is followed by proteins albumin and fibrinogen.

The FTIR spectra of fresh membrane surface and after the membrane was immersed in the protein solution were recorded. These two adsorption spectra were then subtracted. The appearance of amide I and amide II bands⁽¹⁴⁾, in the subtracted spectra, in the regions 1640 cm^{-1} and 1550 cm^{-1} reveal that the protein is adsorbed at the surface and confirms the findings of the SEM studies. The ATR spectra also reveal the same result.

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Figure-1

Fig. 1. Current-Voltage Curve for PU membrane.

Figure-2

Fig. 2. Current-Voltage Curve for P.T.F.E. membrane after prolonged contact.

Figure-3

Fig. 3. Sensor Surface After Prolonged Use.

Figure-4

Fig. 4. Membrane Surface under SEM.

REFERENCES

1. Jakobson, R.J. and Grendreau, R.M. Artif (1978) Organs, 2, 183.
2. Ivarson, B. and Lundstrom, I. (1986) in "Critical Rev. in Biocompatibility", (ed.) D.F. Williams, CRC Press, Florida.
3. Lyman, D.J., Knutson, K., McNeil, B. and Shibata, K. (1975) Trans. Am. Soc. Artif. Intern. Organs 21, 49.
4. Roohk, H.V., Rick, J., Hill, R., Hung, E. and Bartlett, R.H. (1976) Trans. Am. Soc. Artif. Intern. Organs 22, 1.
5. Lee, R.G. and Kim, I.W. (1974) J. Biomed. Mater. Res. 8, 251.
6. Grosse-Seistrup, G., Olsen, D.B., Lemm, W., Unger, D., Affeld, K., Krautzberger, W. and Buckerl, F.I. (1978) Trans. Soc. Biomater., 2, 102.
7. Lyman, D.J., Metcalf, L.E., Albo, D., Richards, K.F. and Lamb, J. (1974) Trans. Am. Soc. Artif. Inst. Organs. 20, 474-479.
8. Hartland, A.J., Neary, R.H. (1999) Clin. Chem. 45, 1091 .
9. Ye, Q.S. Keresztes, Z., Hovvai, G. (1999) Electroanal. 11, 729.
10. Katsu, T., Nakashima, K. (1999) Analyst 124, 883 (1999).
11. Olivina, C.H., Rebelo, R.R., Cameo, M.J.F. (1999) Electroanal. 11, 825.
12. Lutze, D., Mernva, R.K., Frielich, A., Meyerhoff, M.E. (1999) Freieniurs, J. Anal. Chem. 364, 41.
13. Ramamurthy, N., Baliga, N., Wakofield, T.W., Andrews, P.C., Yang, V.C., Meyerhoff, H.E. (1999) Anal. Biochem. 266, 16.
14. Hitchman, M.L. (1978) "Measurement of Dissolved Oxygen", John Wiley & Sons, New York.