A Simple Method for Estimation of Insulin Based on Changes in Membrane Permeability

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Received 14 June 2002; accepted 4 September 2002

A simple method for the estimation of insulin concentration based on changes in membrane permeability is described. The method exploits the phenomenon of formation of hydrophilic pathways by insulin in the liquid membrane bilayers generated by the mixture of lecithin and cholesterol. It works satisfactorily for the estimation of insulin in blood serum.

Keywords: insulin estimation, liquid membrane, hydrophilic pathways

Introduction

Estimation of insulin concentration in blood serum is important for diagnosis of different types of diabetes and also in the evaluation of patients with chronic pancreatitis (Pierluissi & Campbell, 1980). The radioimmunoassay technique, though widely used for the measurement of insulin level in serum (Wilson & Miles, 1977; Yalow, 1980; Dron et al, 1980), is far from simple; the experimental setting is quite complex and the use of hazardous radioactive materials further adds to the complexity. Efforts have been made to develop enzyme-based biosensor using glucose oxidase as catalyst for monitoring blood glucose level correlating the concentration of insulin in blood (Turner & Pickup, 1985; Shaw et al, 1991). Such biosensors have their usual limitation of stability, which is governed by the stability of the enzyme. Suri et al (1995) have attempted to develop a piezoelectric crystal based microgravimetric immunoassay for the determination of insulin concentration in serum. The experimental paraphernalia of the piezoelectric crystal device is also quite cumbersome.

Liquid membrane bilayers generated by the mixtures of lecithin and cholesterol on a hydrophobic supporting membrane are workable as mimetic system of biomembranes (Srivastava & Jakhar, 1981, 1982; Srivastava et al, 1984, 1985a, 1985b, 1991; Vyas et al, 1989; Srivastava, 1989; Singh et al, 1990; Rastogi et al, 1993). The action of several channel formers has been mimicked on such liquid membrane bilayer systems; for example, it has been shown that in the lecithin-cholesterol liquid membrane bilayers hydrophilic pathways are generated by the addition of prostaglandins and also by the addition of polyene antibiotics (Srivastava et al, 1985a, 1991). Since the generation of hydrophilic pathways manifests itself in the enhancement of electrical conductance of the liquid membrane bilayer separating the two electrolytic solutions, this property has been exploited in the fabrication of a cholesterol sensor (Srivastava et al, 2000).

The present communication reports a similar method for the estimation of insulin concentration based on changes in membrane permeability. It has been shown earlier (Singh et al, 1990) that if a pH gradient is created across the liquid membrane bilayer and insulin is added on the acidic side, the permeability of the liquid membrane bilayer is increased due to the formation of hydrophilic pathways. The data also have indicated that the number of hydrophilic channels varies with the dose of the insulin added to the more acidic compartment.

Materials and Methods

Lecithin (E-merck), cholesterol (Institute of Genomics & Integrative Biology, New Delhi), insulin (Sigma, Cat. No.0377) and triple distilled water were used in this study. Phosphate buffer and acetate buffer were used for maintaining pH at 6.8 and 4.6, respectively. Aqueous solutions of lecithin-cholesterol mixtures were prepared (Gershfield & Pagano, 1972; Srivastava & Jakhar, 1982; Srivastava et al, 1991;
Srivastava et al., 2000). Stock solutions of insulin (90μg/900ml) were prepared in the acetate buffer (pH 4.6).

The set-up used for the measurements includes perspex glass cell consisting of two compartments A and B (Fig. 1) with a hole in the separating wall. A Sartorius cellulose acetate membrane M (Cat. No. 11107, pore size 0.2 μm, thickness 1×10⁻⁵ m and area 2.55×10⁻⁵ m²) using araldite resin was fixed on the separating wall. The compartments A and B were filled with the aqueous solutions of lecithin (1.919×10⁻⁵ M) –cholesterol (1.175×10⁻⁶ M) mixtures and left for several hours to equilibrate. At this particular composition of lecithin-cholesterol mixtures (Srivastava & Jakhar, 1982), the liquid membrane generated by lecithin completely covers the interface and is saturated with cholesterol. The pH of the solution in compartments A and B was maintained at 4.6 and 6.8, respectively, using appropriate buffers.

Two bright platinum electrodes P₁ and P₂ (Elico, Hyderabad, India, Model No. EP 89) were then introduced in the compartments A and B, respectively, at a fixed distance from the membrane and also as close to the membrane as possible. To know the range of validity of Ohmic relationship, known constant currents (1 μA-30 μA) were passed using Keithley’s programmable current source Model 220 and the corresponding voltages across the electrodes P₁ and P₂ were measured using Keithley’s digital multimeter Model 195A. The V-I plot (Fig. 2) gives the domain of validity of Ohm’s law, where the curve does not pass through the origin. Since the two compartments contain solutions of different ionic composition, there will be finite potential difference across the electrodes even when no current is passed, i.e. I=0.

To construct a calibration curve, different concentrations of insulin within the physiological range (60 μg/ml-90 μg/ml) were introduced in the more acidic compartment, and magnetically stirred to ensure the uniformity of concentration. A constant current, 7 μA, which is within the domain of validity of Ohm’s law, was then passed through the system and the corresponding voltage across the electrodes P₁ and P₂ measured for each concentration of the insulin added to the more acidic compartment. The readings were taken in the intervals of 30 min; in about 30 min the system was found to stabilize to give stable values of the potential difference. The calibration plot of potential difference (V) against the logarithm of insulin concentration is shown in Fig. 3. To check the validity of the calibration curve, the concentrations of a few samples of known strength within the range of the calibration curve were estimated using the calibration curve shown in Fig. 3. All experiments were carried out at 37°C.

**Results and Discussion**

When two compartments separated by a hydrophobic supporting membrane are filled with the
aqueous solutions of lecithin (1.919×10^{-5}M)–cholesterol (1.175×10^{-6}M) mixture, a bilayer of liquid membrane consisting of lecithin and cholesterol is formed within the voids of the supporting membrane (Srivastava & Jakhar, 1982). The formation of liquid membrane is based on the hypothesis (Kesting et al., 1968), “When a surfactant is added to an aqueous phase, the surfactant layer which forms spontaneously at the interface acts as a liquid membrane”. As the concentration of the surfactant is increased, the interface gets progressively covered with the surfactant layer liquid membrane, and at the critical micelle concentration of the surfactant, it is completely covered. The earlier study (Singh et al., 1990) forms the basis of the present investigation. The pH difference between 4.6 and 6.8 across the membrane was chosen because it was found to be optimum for the generation of hydrophilic pathways.

Thus, if a fixed constant current within the domain of validity of Ohm’s law is passed, the potential difference across the electrodes P₁ and P₂ (Fig. 1) should decrease with the increase in the concentration of insulin. The concentration of insulin in the clinical sample of blood serum obtained from Lal’s Pathology Laboratory, New Delhi, was determined using the calibration curve (Fig. 3). The value of the insulin concentration in the sample provided by the Lal’s Pathology Laboratory, using MPIA (Micro Particle Immuno Assay) kit from ABBOTT, was 20.6 μU/ml. To bring this value in the range of the calibration curve, 1 ml of the clinical sample was added to 65 ml of the insulin stock solution (conc., 90μ/900 ml) and the total volume was made up to 100 ml using the solution of lecithin-cholesterol mixture (pH 4.6) so that the total concentration now becomes 65.206 μU/ml. This 100 ml solution was now put in the compartment A of the cell (Fig. 1) and the potential difference corresponding to 7μA current was measured. The value of the potential difference thus obtained was 1.235V. From the calibration curve (Fig. 3), the corresponding value of the insulin concentration works out to be 65.313 μU/ml which is in good agreement with the actual value 65.206 μU/ml. Thus the present method, which is quite simple, appears promising for the estimation of insulin.

Acknowledgement
Thanks are due to the All India Council of Technical Education, New Delhi for support and to Lal’s Pathology Laboratory, New Delhi for the supply of clinical sample along with its analysis.

References


