Poster Session 3 – Analytical Chemistry

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Immobilization of oxalate oxidase from *Amaranthus* leaves on various membranes for its use in oxalate sensors

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The determination of oxalate in urine, plasma and food stuffs is very important in diagnosis and medical management of calcium oxalate urolithiasis, primary hyperoxaluria, ileal disease, steatorrhoea and fat malabsorption (Decastro 1988). Enzymic methods employing oxalate oxidase or oxalate decarboxylase being more sensitive, specific and rapid are so popular that their commercial kit for oxalate determination is available (SIGMA kit), but the cost of the enzyme makes the method very expensive. Immobilization of enzyme onto organic and inorganic supports has helped to overcome this problem. More recently, potentiometric oxalate determination has been exploited by enzyme electrode technology, utilizing barley oxalate oxidase. However, one limitation of using barley oxalate oxidase in this method is the interference by physiological concentrations of Cl^- (250 mmol mL^{-1}) and NO_3^- (8 mmol mL^{-1}) normally found in urine which needs the pretreatment of sample for removal of Cl^- prior to oxalate assay, which makes the procedure complicated and affects its reproducibility. The oxalate oxidase from *Amaranthus* leaves is not affected by the physiological concentrations of Cl^- and NO_3^- (Goyal *et al* 1999). Also the enzyme is thermo stable and remains active at high temperature. In the natural state it is membrane bound so it gets rapidly immobilized even in its partially purified form. It acts in a wider range of oxalate concentrations compared to this enzyme from other plant sources and shows no substrate inhibition at high oxalate concentration.

A membrane oxalate oxidase has been extracted from mature leaves of *Amaranthus spinosus* and solubilized using sodium deoxycholate and partially purified by 0–80% ammonium sulphate precipitation. The enzyme was purified by 10.5 fold with 55% yield. The partially purified enzyme was immobilized onto cellulose acetate, polyvinyl chloride and dialysis membrane with 92%, 89% and 20% retentions of specific activity. Immobilization conditions were standardized. The enzyme immobilized maximally onto cellulose acetate membrane in 2.5% glutaraldehyde solution in 0.01 M sodium phosphate buffer pH 7.0, when kept at 4°C for 24 h. The enzyme exhibited maximum activity at pH 4.0 and 37°C. The rate of H_2O_2 formation was linear upto 5 min. The enzyme had greater resistance towards various cations and anions found in biological fluids, compared with moss, barley, banana peel and beet stem oxalate oxidases, which make it better suited for its use in oxalate determination in biological fluids.

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