THE PREPARATION OF GLUCOSE OXIDASE CHEMICALLY ATTACHED TO POLYSTYRENE AND ITS USE IN THE AUTOMATED ANALYSES OF GLUCOSE

W.E.HORNBY, H.FILIPPUSSON and Avril McDONALD
Department of Biochemistry, University of St. Andrew's, Fife, Scotland

Received 26 May 1970

1. Introduction

In recent years a great variety of water-insoluble derivatives of enzymes have been described [1]. At the same time there has been a certain degree of speculation with regard to the utilisation of these products in their capacity as specific catalysts for both analytical and preparative processes [2]. To date, however, the demonstration of their full potential has not been realised. In this paper, the preparation of a water-insoluble derivative of glucose oxidase (EC 1.1.3.4), prepared by covalently attaching the enzyme to the inner surface of a coiled polystyrene tube, is described, together with its utilisation for the automated analysis of glucose. We believe this to be one of the first reports of the application of a water-insoluble enzyme to automated analysis.

2. Materials and methods

The polystyrene tubes (a gift from BP Chemicals (U.K.) Ltd.) were prepared for attaching the enzyme in the following way. The tube (375 cm long, 0.2 cm internal diameter) in the form of a coil was nitrated on its inner surface only by perfusion for 20 min at 0° with a mixture of 47% (v/v) nitric acid (specific gravity 1.42) in sulphuric acid (specific gravity 1.84) at a flow rate of 50 ml . hr^{-1}. The nitrated polystyrene tube was washed free of the nitrating mixture by perfusion with water and then reduced to give the polyaminostyrene derivative by perfusion with 6.0% (w/v) sodium dithionite in 2.0 M sodium hydroxide at a flow rate of 100 ml . hr^{-1}. The reduction was performed at 70° and continued for a period of 5 hr, after which the tube was washed with 1.0 M hydrochloric acid. Diazotisation was then effected by perfusion at 0° with a mixture of 0.5% (w/v) sodium nitrite in 0.6 M hydrochloric acid at a flow rate of 100 ml . hr^{-1} for a period of 30 min. The tube was then washed through with ice-cold 1.0 mM hydrochloric acid until the effluent showed no detectable presence of nitrous acid by the starch-iodide test. Diazotisation was always performed immediately prior to coupling with the enzyme.

Glucose oxidase (Sigma Chemical Co., Type II from *Aspergillus niger*) was used. The enzyme was prepared for coupling by dialysing a solution of it against 0.1 M phosphate buffer, pH 7.5, after which the protein concentration was adjusted to 1.0% (w/v) and sucrose was added to a final concentration of 0.75 M. The glucose oxidase was then insolubilised by the perfusion of its solution through the diazotised polystyrene tube at 0° for a period of 1 hr at a flow rat of 50 ml . hr^{-1} in a closed loop. The amount of enzyme absorbed per unit length of tube reaches a maximum within 45–55 min. In order to remove any protein that was absorbed on the surface of the tube, it was perfused in turn with solutions of 0.5 M sodium bicarbonate, 1.0 M sodium chloride and finally with water. The tube was stored at 4° in the dry state.

Glucose oxidase activity was determined at 35° and pH 5.6 by the colorimetric estimation of the hydrogen peroxide with acid potassium iodide. The assays were automated on the Technicon principle using the flow circuit shown in fig. 1. Samples were run at a rate of 40 per hr and a Beckman DB Spectrophotometer with 1 cm light path flow-through cuvettes was used for monitoring the colour.

North-Holland Publishing Company – Amsterdam

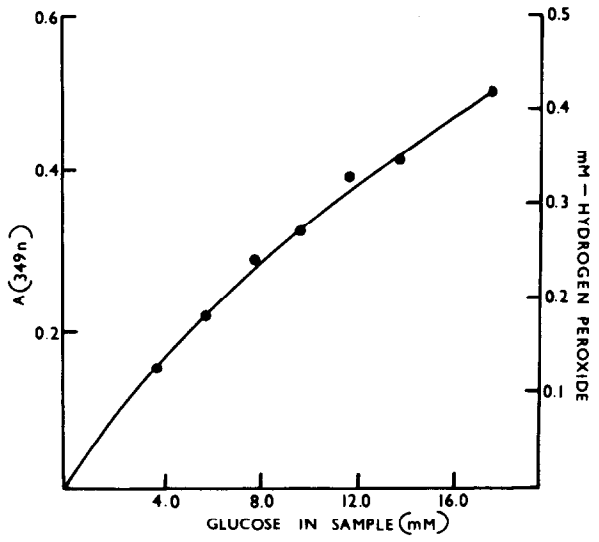

Fig. 1. Flow circuit for the automated assay of glucose oxidase-polystyrene tubes using the Technicon principle. The enzyme tube was maintained at 35° by immersion in a constant temperature water bath.

3. Results and discussion

Fig. 2 shows the response of the recorded absorbance at 349 nm when glucose samples of different concentration were continuously assayed as described above. From reference to a standard curve, concurrently compiled by assaying glucose-free samples containing known amounts of hydrogen peroxide, fig. 2 also shows the activity of the tube in terms of the concentration of hydrogen peroxide formed. Since the percentage conversion of glucose at no time exceeded 10% of the original glucose concentration, the data shown in fig. 2 were used for the calculation of the Michaelis parameters, K_m and V_{max}, by the method of Lineweaver and Burk [3]; these results are shown in table 1. Table 1 also shows the corresponding values of K_m and V_{max} when the air segmentation stream was replaced by pure oxygen gas. The results show an approximate two-fold increase in the observed V_{max} of the tube when the air segmentation stream was replaced by oxygen, at the same time there was over a two-fold increase in the observed K_m of the tube with respect to glucose. For the determination of V_{max} the residence time of the substrate in the tube was calculated from a knowledge of the volume of the tube and the flow rate of

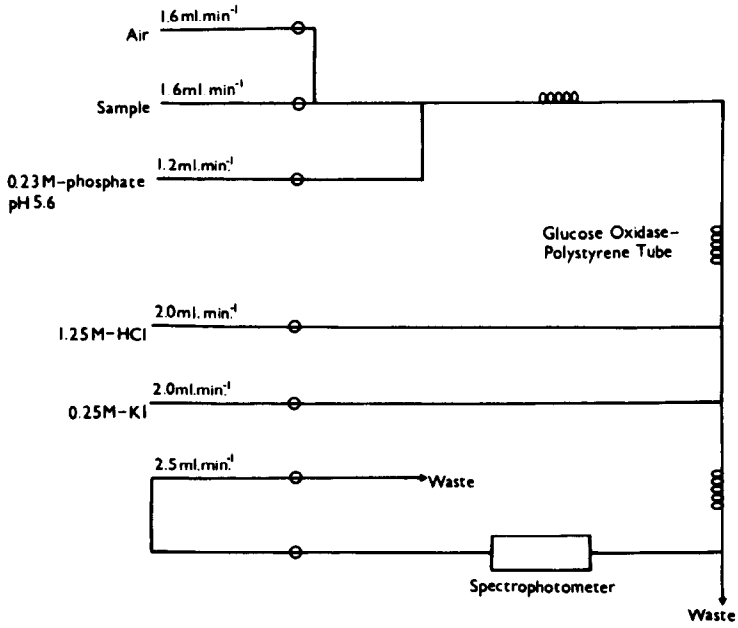

Fig. 2. The effect of glucose concentration on the production of hydrogen peroxide and the corresponding absorbance at 349 nm. The assays were performed with an air segmentation stream.

Table 1
The values for K_m and V_{max} of glucose oxidase chemically attached to polystyrene.

Conditions	K_m (mM)	V_{max}*
0.1 M phosphate, pH 5.6, with air segmentation	15.0	0.45
0.1 M phosphate, pH 5.6, with oxygen segmentation	36.0	0.88

* Expressed as mM hydrogen peroxide formed per min.

substrate through the tube. By using fig. 2 as a calibration curve, the flow circuit outlined in fig. 1 has been used for the automated determination of glucose within the concentration range 0.5 to 10 mM. The polystyrene-glucose oxidase tube used in these experiments did not show a significant loss in activity for a period of up to six weeks.

The results described above show that a water-insoluble enzyme, prepared by the covalent attachment of the enzyme to the inner surface of a tubular structure, may be employed in automated analysis. The attraction of using the enzyme in this form derives mainly from the stability of the product, which renders it possible to utilise the same enzyme repeatedly and therefore more economically. An added advantage of the particular system described here is that the enzymic determination of glucose may be performed colorimetrically without the need to use a second enzyme, peroxidase, for the determination of the hydrogen peroxide.

Acknowledgement

W.E.H. wishes to thank the Medical Reserach Council for their support.

References

[1] E.Katchalski, Ann. Rev. Biochem. 35 (1966) 873.
[2] G.Kay, Process Biochem., August (1968) 36.
[3] H.Lineweaver and D.Burk, J. Am. Chem. Soc. 56 (1934) 658.