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Enzyme Chemical Engineering and Its Application to Biosensors

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Chemistry at Massey University, Turitea, New Zealand.

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2001
ABSTRACT

Enzyme chemical engineering is a fast growing area in biotechnology. It has been used to change the stability, solubility, activity and other properties of enzymes for more control over and wider application of enzymes.

In this thesis, this technology is applied to another new and fast growing area of research: biosensors. Over the last decade, biosensors are gaining increasing awareness as a highly attractive analytical tool. One of the current challenges in this area is to identify a universal and scaleable way to produce sensitive, stable, instantaneous, and easy to prepare biosensors for mass production.

In this study, enzyme chemical engineering is adopted as a new approach and glucose oxidase is served as a model to build a biosensor system in attempting to address the above challenge.

In the study, glucose oxidase was used as the catalyst to chemically amplify the redox reaction of glucose. Haemin was employed as the bifunctional promoter to act as a "bridge" to connect glucose oxidase (GOD) and electrode. Haemin, similar to ferrocene also acts as a mediator to transfer electrons between the active center of the enzyme and the electrode.

In the construction of a haemin-glucose oxidase biosensor, haemin was covalently bound with glucose oxidase. The haemin-glucose oxidase conjugate was then chemisorbed on to the platinum electrode to modify the electrode surface and form an "enzymatic redox center-bridge-electrode" system. The modification of the glucose oxidase with haemin comprised of two steps: converting the haemin carboxyl group to the reactive enol ester and then covalently bonding to an amino group of glucose oxidase. For chemisorption, the electrode was soaked in a solution of the haemin-glucose oxidase conjugate in phosphate buffer solution (pH 7.0) at 4°C for 16 hours. The same experiment was carried out by using unmodified glucose oxidase as a blank.
The following facts proved that the covalently bound haemin-glucose oxidase system was formed successfully: 1) The large molecule fractions eluted from the Sephadex G-10 gel column had the enzyme activity and other characteristics of glucose oxidase. 2) The same fractions retained about 2/3 to 3/4 of the specific activity of original glucose oxidase. 3) The absorbance spectra of these fractions showed the peaks corresponding to both haemin and glucose oxidase.

The following evidence suggests that the haemin-glucose oxidase conjugate was successfully chemisorbed on to the electrode surface: 1) The cyclic voltammogram of the electrode chemisorbed with conjugate was completely different from that adsorbed with glucose oxidase alone. 2) The cyclic voltammogram of the conjugate chemisorbed electrode in the solution with glucose was quite distinct from that without glucose. Thus a different species from either glucose oxidase or haemin was chemiabsorbed on to the electrode.

Furthermore, the conjugate chemisorbed electrode showed linearity between current response and glucose concentration at a range from 0mM to 10mM. The ratio of the current response to glucose concentration was about 1.6µA/mM. However, the platinum electrode adsorbed by GOD alone had a poor response to glucose. The response time of the system of platinum electrode-haemin-glucose oxidase was very rapid at less than one minute, and the response fell initially but then remained stable over a period of 14 days.

Thus the experimental data proved that the system of platinum electrode-haemin-glucose oxidase met the requirements for a glucose sensor in the factors of the sensitivity, linear response range, lifetime, ease of preparation, convenience of operation, non-toxicity and low cost. In other words, it demonstrated the characteristics of a glucose biosensor.

Finally, using the preparation of this glucose biosensor as a model, the electrochemical mechanism of the biosensor system was proposed. The model was also used to suggest a systematic approach for constructing amperometric biosensors. The extension of this approach and the potential applications of
this type of biosensor are also discussed.
ACKNOWLEDGEMENTS

The author would like to thank the following for their advice and assistance in the compilation of this thesis:

First of all, I would like to express my very sincere thanks to my supervisor Associate Professor David R. K. Harding, for his supervision, advice, encouragement and support during my study and the completion of this thesis and beyond.

My especial thanks go to AgResearch (Palmerston North), in particular Dr. Alan Hart and Dr. Jim Dunlop for their assistance throughout this study.

My thanks also go to Associate Professor Len Blackwell and Associate Professor Kathy Kitson for their guidance, advice and encouragement.

I would like to acknowledge the other members from the Separation Science Unit for their assistance and support. Thanks to all my colleagues at Massey University who lent me a hand when help was needed.

Also, I would like to thank my family, my parents and my brother, for their love and support.
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<tr>
<td>DCC</td>
<td>Dicyclohexycarbondi-imide</td>
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<td>DEC</td>
<td>1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride</td>
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<td>EHG</td>
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