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IMMOBILISATION OF ENZYMES TO PERLOZA™ CELLULOSE RESIN

This thesis was presented in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry at Massey University

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ABSTRACT

The studies reported in this thesis describe the use of Perloza™ beaded cellulose resin as a solid support for enzyme immobilisation via covalent binding. The aim of the project was to extend the uses for Perloza™ and to compare the use of well known solid support activation chemistries with a recently developed one for Perloza™. Preparations such as these have potential industrial uses. Three attachment chemistries were studied. The first activation employed 1,1-carbodiimidazole (CDI) then direct attachment of enzyme. The second again used CDI activation followed by attachment of a 6-aminocaproic acid spacer arm and then the enzyme. The final method used was attachment of a diol and subsequent oxidation to an aldehyde. The diol/aldehyde method had the advantage over the CDI methods of being based on aqueous chemistries. The two CDI based methods require extensive use of dry organic solvents. The enzymes investigated in this study were trypsin, chymotrypsin, α-amylase, horseradish peroxidase (HRPO) and alcohol dehydrogenase (ADH).

Trypsin was immobilised successfully by all three chemistries. All preparations retained significant activity after immobilisation at room temperature as judged by the chromogenic substrate specific for trypsin N-α-benzoyl-DL-arginine-*p*-nitroanilide.HCl (BAPNA). Measurable activity was retained in different studies from between 2 to 7 days at 60°C. The activity of immobilised trypsin with a synthetic peptide substrate was comparable to the activity of free trypsin with the same substrate.

Chymotrypsin was also successfully immobilised using all three chemistries. Each preparation showed significant retention of activity after immobilisation as judged by the chromogentic substrate N-glutaryl-L-phenylalanine-*p*-nitroanilide (GAPNA). Stabilisation to heating at 60°C was less successful than with trypsin but significant activity was still retained for between 3 and 6 hours. The activity of immobilised preparations with a peptide substrate was comparable to free chymotrypsin.

α-Amylase, horseradish peroxidase and alcohol dehydrogenase were studied less extensively than trypsin and chymotrypsin. Nevertheless all three enzymes were

successfully immobilised onto Perloza™-CDI-ACA and Perloza™-Diol/Aldehyde. Difficulty was encountered in achieving significant levels of any enzyme immobilisation to Perloza™-CDI for all three enzymes. Subsequent activity assays showed HRPO and α-amylase retained significant activity on all three resin preparations. ADH showed no measurable activity on Perloza™-CDI and very little activity on Perloza™-CDI-ACA and Perloza™-Diol/Aldehyde.

Investigations have shown that enzymes can be immobilised on Perloza™ with retention of significant amounts of normal activity at room temperature and improved stability compared with free enzyme at high temperature. Comparisons of the CDI activations with the diol/aldeyde chemistry showed better performance by the latter in trypsin immobilisation and similar performance for chymotrypsin immobilisation. Horseradish peroxidase and α-amylase were successfully immobilised using CDI/ACA and diol/aldehyde chemistries with the CDI/ACA giving higher initial specific activities than the diol/aldehyde preparation. Alcohol dehydrogenase was also successfully immobilised but gave no measurable activity.

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LIST OF ABBREVIATIONS

AAA	amino acid analysis
ACA	6-aminocaproic acid
ADH	alcohol dehydrogenase
BAPNA	benzoyl-DL-arginine-*p*-nitroanilide.HCl
BCA	bicinchoninic acid
BPNPG-7	p-Nitrophenyl maltoheptaoside
CDI	1,1'-carbonyldiimidazole
EDC	1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride
GAPNA	N-glutaryl-L-phenylalanine-*p*-nitroanilide
HCl	hydrochloric acid
HMP	4-hydroxymethylphenoxymethyl-copolystyrene-1% divinylbenzene resin
HPLC	high performance liquid chromatography
HRPO	horseradish peroxidase
$NaCNBH_3$	sodium cyanoborohydride
NAD^+	nicotinamide adenine dinucleotide (oxidised form)
NHS	N-hydroxysuccinimide
TFA	trifluoroacetic acid