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DEXTRAN ENZYME IMINE COMPLEXES: A PRELIMINARY STUDY

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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1997
ABSTRACT

A model system involving the formation of protein-dextran complexes has been investigated with a view to improving existing methods of drug administration. Activation of the dextran was achieved by periodate oxidation to give levels of 7%, 21% and 56% activated glucose moieties. The protein-dextran complexes were investigated with the prospect of obtaining sustained release of proteins from the dextran in an unmodified form. Covalent conjugation of proteins to carbohydrate polymers is known to confer stability on the protein. The proteins in this study were bound to the dextran through imine bonds. The proteins investigated were lysozyme, trypsin, amylase, alcohol dehydrogenase and catalase. The selection covered a range of molecular weights and varying enzymatic activities.

As might be predicted, the speed of complex formation was shown to be greater at the 21% level of activation compared to the 7% activation of dextran in all cases studied.

Lysozyme, the smallest protein, readily formed complexes at all three levels of activation. At the 56% level the resulting complex had an extremely high MW, greater than 1MDa. The extensive binding between the dextran and lysozyme molecules resulted in a complex that was inactive and showed no signs of releasing any lysozyme, active or inactive. At the lower levels of activation, complex was formed with relative ease. Upon conjugation lysozyme exhibited only minimal activity. Release of a lysozyme-like species with normal lytic activity was observed.

Precautions were taken to minimise possible autolysis in the trypsin study. Once complexed it was postulated that autolysis would be prevented or minimised. Similarly the 56% level of activation appeared to be too high to obtain a viable complex for facile trypsin release. Sustained release of a trypsin-like protein was observed with complexes at the 7% and 21% levels. SEC and SDS-PAGE, in conjunction with a positive BAPNA assay gave support to the released species being trypsin-like. While complexed to the dextran trypsin showed no signs of activity. Released trypsin-like species and unreacted trypsin showed similar tryptic maps from a synthetic peptide, the peptide was designed to show distinctive fragments.

α-Amylase, twice the MW of trypsin and over three times the MW of lysozyme, formed complexes with ease at both 7% and 21% levels of activation. Conjugation to dextran did not effect the activity of α-amylase. Over time the release of an α-amylase-like species from the complex was observed.
Alcohol dehydrogenase and catalase are both high MW proteins. Complex formation was observed for each protein. Subsequent experiments showed that upon release the proteins appeared to dissociate, most probably into their subunits. It is also possible that the dimers and monomers bound to the dextran. The main advantage of conjugation in this case appeared to be to confer stability on the proteins. The ADH-complex exhibited enzymatic activity.

At 7% and 21% activation levels the lower MW proteins formed complexes with dextran that exhibited release of a protein species. The higher MW proteins were possibly stabilised when conjugated to dextran, but dissociated upon release. Investigations have shown that the level of activation chosen affects the extent of binding and therefore the functions of the resultant complex. Thus activation levels can be manipulated depending on the desired result. While lower dextran activation levels appeared to be more suited for smaller MW proteins, there were indications that the larger MW proteins could form beneficial complexes at higher activation levels. Results indicated that conjugation to periodate activated dextran could be extended to further proteins with the possibility of therapeutic or commercial applications.
ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisor Associate Professor David R.K. Harding for his time, input and encouragement over the last two years.

I would also like to acknowledge Debbie Frumau for running my amino acid analysis samples, and Dick Poll for his constant help with the FPLC and SMART systems. Thanks are also due to Associate Professor D.N. Pinder and Dr J. Lewis for their time and help with the LLST and Ultracentrifugation experiments respectively.

Special thanks and appreciation to Rekha Parshot and Jenny Cross for the SPPS and purification.

Thank you also to J. Battersby, Genentech Inc., South San Francisco, for assistance and suggestions with the tryptic digest studies, and for running the HPLC of the trypsin as well as for the gifted rhGH and the rough sketch that lead to Figure 1.8.2.

I would also like to thank the Departments of Biochemistry and Chemistry for their assistance along the way, especially the members of the Centre for Separation Science and Gill Norris’s lab.

Finally I would like to thank my parents and friends, in particular Suzette, Ruth, Kimberley and Morris for putting up with me especially through the last stages of my thesis.
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<td>AAA</td>
<td>amino acid analysis</td>
</tr>
<tr>
<td>Ab</td>
<td>antibody</td>
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<tr>
<td>ADH</td>
<td>alcohol dehydrogenase</td>
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<tr>
<td>BAPNA</td>
<td>N-α-benzoyl-DL-arginine-p-nitroanilide HCl</td>
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<tr>
<td>BPNPG-7</td>
<td>blocked p-nitrophenyl maltoheptaoside</td>
</tr>
<tr>
<td>CD4</td>
<td>cell surface glycoprotein receptor for HIV</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulphoxide</td>
</tr>
<tr>
<td>DOR</td>
<td>double oxidised residues</td>
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<tr>
<td>FMOC</td>
<td>fluorenlymethoxycarbonyl</td>
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<tr>
<td>GI tract</td>
<td>gastro-intestinal tract</td>
</tr>
<tr>
<td>GP120</td>
<td>glycoprotein-120</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>FPLC</td>
<td>fast performance liquid chromatography</td>
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<tr>
<td>LLST</td>
<td>laser light scattering technique</td>
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<tr>
<td>met-hGH</td>
<td>recombinant methionyl human growth hormone</td>
</tr>
<tr>
<td>MWCO</td>
<td>molecular weight cut off</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>sodium borohydride</td>
</tr>
<tr>
<td>NaBH₃CN</td>
<td>sodium cyanoborohydride</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>nicotinamide adenine dinucleotide (oxidised form)</td>
</tr>
<tr>
<td>mPEG</td>
<td>monomethoxypoly(ethylene glycol)</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PNP</td>
<td>purine nucleoside phosphorylase</td>
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<tr>
<td>rhGH</td>
<td>recombinant human growth hormone</td>
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<td>rIGF-1</td>
<td>recombinant human insulin-like growth factor</td>
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<td>rIL-2</td>
<td>recombinant human interleukin-2</td>
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<tr>
<td>rtPA</td>
<td>recombinant human tissue plasminogen activator</td>
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<td>SDS-PAGE</td>
<td>sodium dodecyl sulphate - polyacrylamide gel electrophoresis</td>
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<td>SEC</td>
<td>size exclusion chromatography</td>
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<td>SPPS</td>
<td>solid phase peptide synthesis</td>
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<tr>
<td>Tris</td>
<td>tris-(hydroxymethyl-)aminomethane</td>
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<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
<td>TPCK</td>
<td>L-1-tosylamide-2-phenylethyl chloromethyl ketone</td>
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Abbreviations used for amino acids:

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<td>Asparagine</td>
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<td>Aspartic acid</td>
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<td>Cysteine</td>
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<tr>
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<td>Pro</td>
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