DEXTRAN ENZYME IMINE COMPLEXES: A PRELIMINARY STUDY

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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.

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AAA	amino acid analysis
Ab	antibody
ADH	alcohol dehydrogenase
BAPNA	N-α-benzoyl-DL-arginine-ρ-nitrolanilide HCl
BPNPG-7	blocked p-nitrophenyl maltoheptaoside
CD4	cell surface glycoprotein receptor for HIV
DMSO	dimethyl sulphoxide
DOR	double oxidised residues
FMOC	fluorenylmethoxycarbonyl
GI tract	gastro-intestinal tract
GP120	glycoprotein-120
HPLC	high performance liquid chromatography
FPLC	fast performance liquid chromatography
LLST	laser light scattering technique
met-hGH	recombinant methionyl human growth hormone
MWCO	molecular weight cut off
$NaBH_4$	sodium borohydride
$NaBH_3CN$	sodium cyanoborohydride
NAD^+	nicotinamide adenine dinucleotide (oxidised form)
mPEG	monomethoxypoly(ethylene glycol)
PEG	polyethylene glycol
PNP	purine nucleoside phosphorylase
rhGH	recombinant human growth hormone
rIGF-1	recombinant human insulin-like growth factor
rIL-2	recombinant human interleukin-2
rtPA	recombinant human tissue plasminogen activator
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electropheresis
SEC	size exclusion chromatography
SPPS	solid phase peptide synthesis
Tris	tris-(hydroxymethyl-)aminomethane
TFA	trifluoroacetic acid
TPCK	L-1-tosylamide-2-phenylethyl chloromethyl ketone

Abbreviations used for amino acids:

Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Cysteine	Cys
Glutamic acid	Glu
Glutamine	Gln
Glycine	Gly
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tyrosine	Tyr
Tryptophan	Trp
Valine	Val

Asx	asparagine and aspartic acid
Glx	glutamine and glutamic acid