PREPARATION OF CHEMICALLY MODIFIED BEAD CELLULOSE RESINS AND THEIR APPLICATION TO PROTEIN PURIFICATION

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ERRATA

Page 39 : the legend for Table 2.7 (line 7) should refer to Table 2.6, not 2.7.

Page 55 : insert "+ 1 ml ethanol" (in paragraph 5), to dissolve ligands.

Page 56 : line 5 of paragraph 2 should read "0.7-1.0 ml/g".

Page 61 : the activation level should be "0.093 mMoles/g" in line 1, paragraph 2.

Page 72 : swap titration figures of AGE cysteamine (rows 6 and 7).

Page 118 : the figure caption should be "...PPA (59%)".

Page 120 : delete "for", line 7, paragraph 1.

Page 129 : "mMoles/g dry" should read "0.043 mMoles/g", line 1, paragraph 1.

Page 139 : change the legend labels for AMP to "(D)" and APIMID to "(C)".

Page 153 : change Figure 6.10 to Figure 6.11, lines 5 and 9, paragraph 2.

Page 161 : change "Figure 5.1" to "Figure 5.3", paragraph 1, line 4.

Page 164 : delete "and eluted", paragraph 1, line 4.

Page 169 : the legend's second sentence should read "pH 5.2 and 7.5 respectively".

Page 171 : the percentage should be 50-70% paragraph 2, line 3.

Page 171 : change "Figure 7.6" to to "Figure 7.5", paragraphs 1 and 2.

Page 180 : the vinyl pyridine figure should be "$7.60".

ABSTRACT

A bead cellulose matrix, Perloza™, was chemically modified to prepare inexpensive resins for chromatography. Conventional and novel resins were produced. Adsorption and elution methods suitable for industrial chromatography were developed. An agarose matrix, Sepharose™, was used for comparison.

Matrix activation with carbonyldiimidazole (CDI) was optimised for Sepharose and Perloza. Improved, reliable performance was obtained using column solvent exchange, with an imidazole tracer. Substitution efficiency of 75-98% was obtained for aminoacyl ligands/spacer arms by minimising water content. The aqueous carboxymethylation level obtained for Perloza was 0.3-0.4 mMoles/g dry. This was increased to 1.3-2 mMoles/g dry, using 75-80% DMSO solvated Perloza. Epichlorohydrin and bisepoxide activation levels (+/- organic solvents) were low.

Etherification of Perloza with allyl bromide or allyl glycidyl ether resulted in high **allylation** levels (> 1.50 mMoles/g), even in aqueous media. Matrix allyl groups were reacted with bromine water or aqueous N-bromosuccinimide, to produce (predominantly) bromohydroxypropyl groups. Subsequent attachment of amine and thiol ligands, by nucleophilic **substitution**, was simple and efficient.

Allyl matrices were also used for free radical **addition** of sulphite and various thiols (mercaptoethanol, mercaptoacids, glutathione). Efficient addition was found without thermal or chemical catalysis. Addition of mercaptoacetic acid followed by carboxylate titration was the preferred measure of (allyl) activation level. Addition of several other thiols occurred at 60°C.

The usefulness of allyl chemistries was exemplified by preparation of **ion exchange** resins. Their physical and chromatographic properties compared favourably with commercial resins. They combined good laboratory performance with high flow rates and simple, cheap preparation suited to large scale use.

Mixed mode resins were prepared from CDI and allyl matrices. These contained charged (secondary amine or carboxylate) and hydrophobic (alkyl spacer arm and/or ligand) groups. The milk clotting enzyme **chymosin**, was adsorbed to these resins at high and low ionic strength. Near homogeneous chymosin was eluted by a pH change, which induced electrostatic repulsion. Alkyl carboxylate resins were preferred. They were simple to prepare, use and regenerate, despite the use of crude broths.

The presence of charged groups could cause non-specific adsorption, interference with target protein adsorption and greater fouling. Weak acid and base hydrophobic groups (e.g. pyridyl) were attached to matrices and titration confirmed that uncharged and charged forms were obtained in a pH range (5-9) suitable for protein chromatography. At low ligand density, the salt promoted hydrophobic adsorption properties of these resins (uncharged from) were similar to those of Phenyl Sepharose. At higher ligand density, retention was longer, eventually leading to adsorption independent of ionic strength. Complete elution was obtained by pH adjustment (to the partially ionised resin form). Chymosin was strongly adsorbed to uncharged pyridyl (**hydrophobic ionisable**) resins and rapidly eluted by a small pH change. High ligand density (strong adsorption) is favourable for large scale use because the ionic strength of feedstreams does not need to be adjusted prior to loading.

Strong adsorption to mixed mode and weakly ionisable resins was also found for **amylase**. Rapid elution (and significant purification) was again obtained by a small pH change. **Subtilisin** was adsorbed likewise by most hydrophobic ionisable resins and recovered efficiently at pH 5.2. However, subtilisin adsorption to mixed mode resins was comparatively weak, possibly reflecting the weaker hydrophobicity of subtilisin compared to amylase.

The adsorption of **catalase** on Phenyl Sepharose and (low ligand density) pyridyl Perloza was equivalent, at pH 7.5. Catalase was eluted by a pH change from the Perloza resin, whereas elution from Phenyl Sepharose required addition of ethylene glycol. This indicated that pyridyl Perloza resins would be useful for chromatography of very hydrophobic proteins.

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Finally I wish to thank Genencor for the project funding which has supported the majority of the work recorded here and my attendance at the Recovery of Biological Products VII conference at San Diego, 1994.

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LIST OF ABBREVIATIONS

AB	allyl bromide
ACA	aminocaproic acid
AEBS	aminoethylbenzenesulphonamide
AGE	allyl glycidyl ether
AMB	aminomethylbenzimidazole
AMP	(aminomethyl)pyridine (2, 3 and 4; ortho, meta and para)
APIMID	aminopropylimidazole
APM	aminopropylmorpholine
APP	aminophenylpropanediol
APS	ammonium persulphate
AUFS	full scale absorbance
AVA	aminovaleric acid
BD	butadiene diepoxide
BDE	butanediol diglycidyl ether
BHP	bromohydroxypropyl
β-lac	β-lactoglobulin
BME	mercaptoethanol
BP	benzoyl peroxide
BSA	bovine serum albumin
CD	carboxydecyl
CDI	carbonyldiimdazole
CHPA	chlorohydroxyphenylacetic acid
CM	carboxymethyl
CMC	cyclohexylmorpholinoethylcarbodiimide
CNBr	cyanogen bromide
CPAD	10-carboxypropionylaminodecyl
CYS	cysteine
DAH	diaminohexane
DCC	dicyclohexylcarbodiimide
DEA	diethylamine
DEAE	diethylaminoethyl
DEAPA	diethylaminopropylamine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DVS	divinylsulphone
ECH	epichlorohydrin

EDC	ethyldimethylaminopropylcarbodiimide
EEDQ	ethoxycarbonylethoxydihydroquinoline
GSH	glutathione
HETP	height equivalent to a theoretical plate
HIC	hydrophobic interaction chromatography
HIS	histidine
HPA	hydroxyphenylacetic acid
HPLC	high performance liquid chromatography
IDA	iminodiacetic acid
IEC	ion exchange chromatography
IgG	immunoglobulin G
IMAC	immobilised metal ion affinity chromatography
IS	ionic strength
LCC	liquid column chromatography
MAA	mercaptoacetic acid
MB	mercaptobenzimidazole
MBA	mercaptobutyric acid
MEP	mercaptoethylpyridine
MET	methionine
MHA	mercaptohexanoic acid
MIM	mercaptomethylimidazole (methimazole)
MP	(mercapto)pyridine (2 and 4; ortho and para)
MPA	mercaptopropionic acid
MSA	mercaptosuccinic acid
NBS	N-bromosuccinimide
NHBA	nitrohydroxybenzoic acid
PAGE	polyacrylamide gel electrophoresis
PALOL	phenylalaninol
PBA	phenylbutylamine
PEA	phenylethylamine
PHE	phenylalanine
PPA	phenylpropanolamine
Q	quaternary amino
QFF	Q Sepharose Fast Flow
RPC	reversed phase chromatography
SDS	sodium dodecyl sulphate
SEC	size exclusion chromatography
SHP	sulphohydroxypropyl

SP	sulphopropyl
SPPS	solid phase peptide synthesis
TBI	tetrabutylammonium iodide
TEOH	tetraethylammonium hydroxide
TMA	trimethylamine
tosyl	toluenesulphonyl
tresyl	trifluoroethanesulphonyl
TRP	tryptophan
WGAT	wheat germ aspartyl transcarbamoylase
XL	crosslinked