Novel silica- and polymer-based carriers for protein separations with immobilized metal ion affinity chromatography

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Introduction

Immobilized metal ion affinity chromatography (IMAC) is a widely used separation method for purifying a broad range of proteins and peptides. It is based on the specific interaction between certain amino acid side chains exposed on the surface of proteins (mainly His, and to a lesser extent Cys and Trp) and transition metal ions, most often Zn^{2+}, Ni^{2+}, Cu^{2+} or Co^{2+}. Most commonly used supports are based on highly cross-linked spherical agarose which can be used in limited ways:

- difficult handling (storage under solvent and at 4°C),
- nickel ions are toxic so leakage could be problem in further application of enzymes.

Silica- and polymer-based carriers could solve the problems of handling and storage through their good mechanic and temperature stability and they do not lost their function when dried.
In other hand lanthanides don't show any toxic behavior in living organism so they can be bio-compatible alternatives of toxic metal ions. Other odds of these rare earth metals that in many cases their costs are comparable with nickel and cobalt salts ($LaCl_3$, $La(NO_3)_3$).

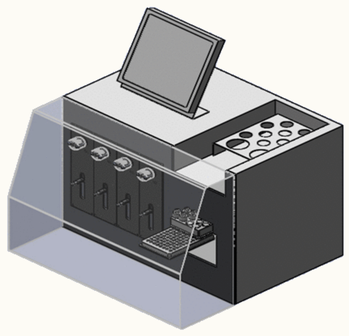
× **Our goal** is to develop novel silica- and polymer-based supports for immobilized metal ion affinity chromatography for protein purification and to investigate the effect of properties of carriers and the features of methods and reactants.

Project

Novel carriers and automated system for the rapid development of the affinity-based separations of proteins (KMR_12-1-2012-0028) [L. Poppe, Z. Boros, B.G. Vértessy, K. Kovács, A. Tóth, G. Hornyánszky, J. Nagy, B. Erdélyi, V. Bódai Erdélyiné, P. Sátorhelyi: Composition for binding and separating of proteins, *PCT Application*, **2013**]

The goal of the project is to develop a fully automated system wich can be able to separate the target protein from other compounds of the complex fermentation mixture through IMAC.

Cooperation members:
Bioorganic Research Group, BUTE (Prof. László Poppe)
Institute of Enzymology, HAS (Prof. Beáta G. Vértessy)
Fermentia Fermentation Company (Balázs Erdélyi, PhD)
BIBUS Technology Group (Róbert Vízkeleti)



Prototype of the device

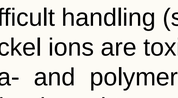
Support development

Many variable parameters of carriers, different reactants and features of technology are available to design very multifarious supports for IMAC.
If the connection between reaction-condition and its effect are known then IMAC supports could be planned for purification of suitable His-tagged protein.

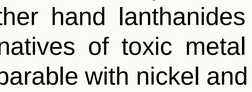
Carriers

A: Silica gels
- particle size (40-63µm; 70-200µm),
- pore size (250, 500, 1000Å).

B: Polymers

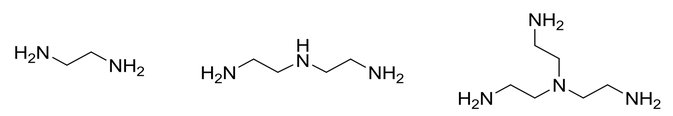


ChiralVision T2 150
Resindion EP 403/S



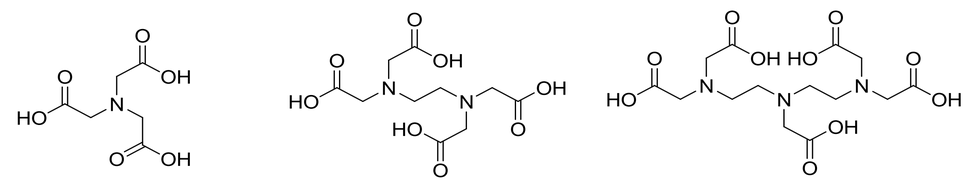
n=1 Resindion EA 403/S
n=5 Resindion HA 403/S

Poly(amine) modifiers

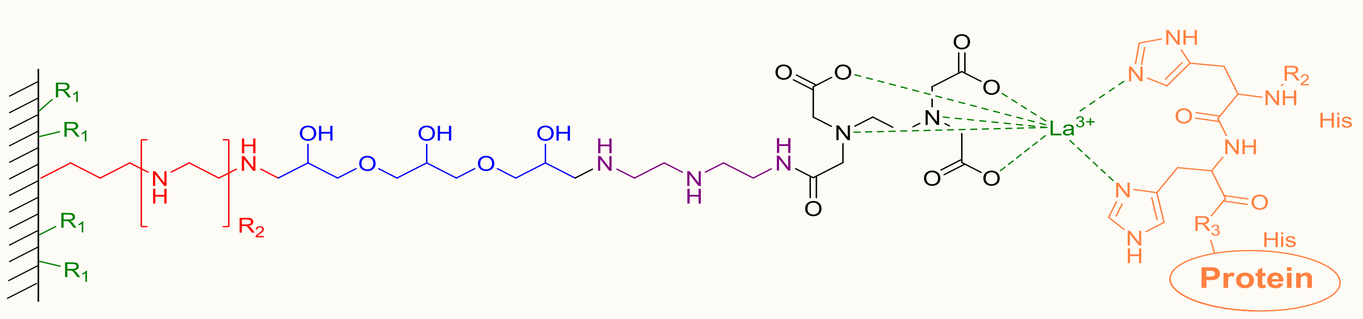


Ethylenediamine EDA | Diethylenetriamine DETA | Tris(2-aminoethyl)amine TAEA

Chelator molecules



Nitrilotriacetic acid NTA | Ethylenediaminetetraacetic acid EDTA | Diethylenetriaminepentaacetic acid DTPA



Metals:

Nickel, Cobalt

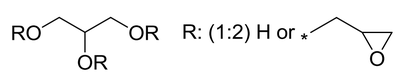
Lanthanides

Lanthanum, **Eu**ropium, **Tb**erbium, **Ce**rium, **Er**bium, Y**tterb**ium, **Th**ulium, etc.

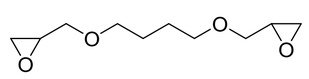
Surface modification

Inert groups (R_1) (Alkyl, aryl, heteroalkyl groups)	Active groups (R_2) (amino alkyl)
Methyl, Ethyl, Propyl, Isobutyl, Hexyl, Octyl, Decyl, Dodecyl, Octadecyl, Vinyl, Phenyl, 3-Chloropropyl, 3-Sulfanylpropyl, 3-Amino-2-hydroxypropyl, 3-Amino-2-hydroxypropyl, Perfluoroctyl, Phenyl – Methyl, Cyclohexyl – Methyl, Dimethyl, Diphenyl	Aminopropyl, 3-(2-aminoethylamino) propyl

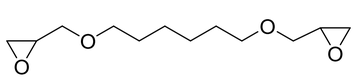
Bisepoxides



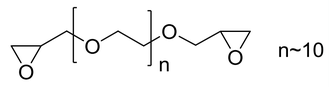
Glycerol diglycidyl ether G-DGE



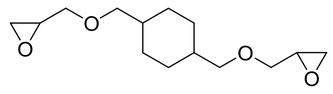
Butyl diglycidyl ether B-DGE



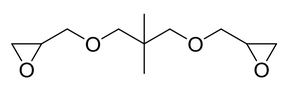
Hexyl diglycidyl ether H-DGE



Polyethylene diglycidyl ether PE-DGE



Cyclohexyl dimethyl diglycidyl ether CHDM-DGE



Neopentyl glycol diglycidyl ether NPG-DGE

Proteins

Isolated from	Enzyme type	Short name	Enzymes used
Rubrobacter xylanophilus	lipase	*Rx*Lip	biotransformation, biodiesel production
Drosophilia virilis	dUTPase	*Dv*dUTPase	could be able to track tumor diseases in medical sensors
Rubrobacter xylanophilus	phenylalanine ammonia-lyase	*Rx*PAL	medical diagnose and enzyme substitute therapy (phenylketonuria), stereoselective synthesis of α-aminoacids
Taxus canadiensis	phenylalanine 2,3-aminomutase	*Tc*PAM	stereoselective synthesis of non-natural β-aminoacids

Results

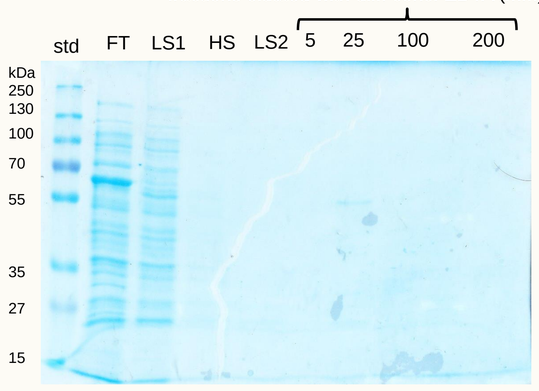
Succesfully purified enzymes in batch and continous-flow systems:

- dUTPase (human, *Drosophilia virilis*),
- phenylalanine ammonia-lyase (*Rubrobacter xylanophilus*, *Petroselinum crispum*)
- phenylalanine 2,3-aminomutase (*Pantoea agglomerans)*
- nitrilase (*Pseudomonas fluorescens*)
- ω-transaminase (*Chromobacterium violaceum*)

Benefits of these supports:
- do not loss their activity in dry and extreme temperature conditions,
- polymers are easy available and low-cost solutions of carriers,
- the rare earth metals do not show any toxic behavior in living organism so they can be used bio-compatible alternatives of nickel,
- enzymes from complex fermentation mixture could be selectively immobilized through covalent and IMAC binding („covalent-coordinated binding").

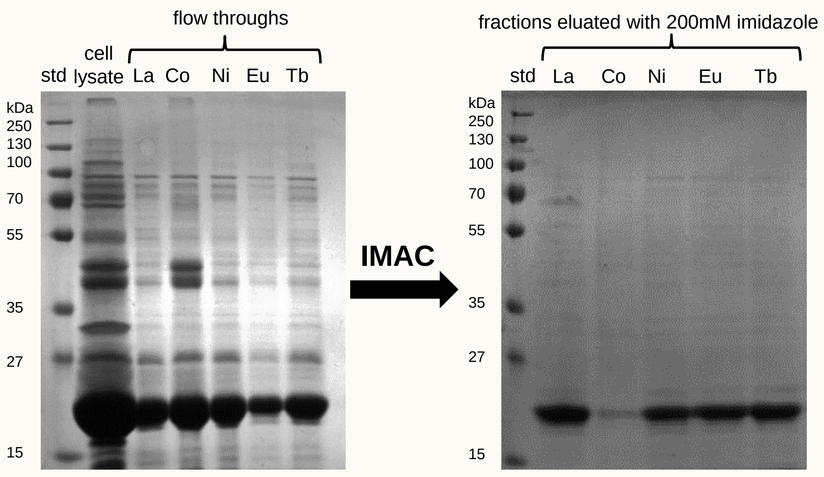
Covalent-coordinated binding of *Pa*PAM

fractions eluated with diff. conc. EDTA (mM)



Support: CV T2 150 polymer – TAEA 5mmol/g – EDTA – La^{3+}

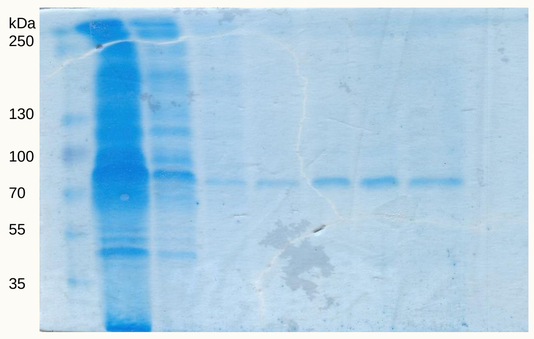
IMAC of human dUTPase with different metal ions



Support: Dv250Å silica - 3-(2-aminoethylamino)propyl and methyl (ratio 1 : 16) - EDTA

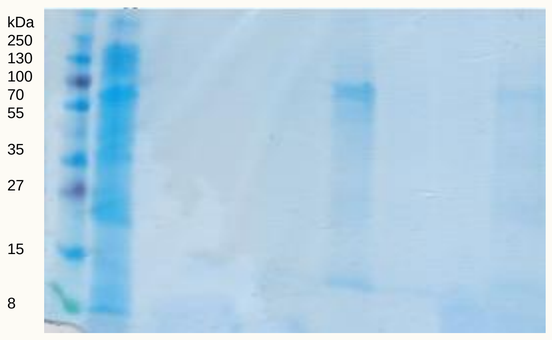
Batch IMAC of *Pc*PAL

fractions eluated with diff. conc. EDTA (mM)



Cont-flow IMAC of *Pc*PAL

fractions eluated with diff. conc. EDTA (mM)



Support: CV T2 150 polymer – TAEA 5mmol/g – EDTA – La^{3+}

Future plans

Support development:

- to preparate more mixed-function-grafted silicas and polymers,
- to define the connection between the modifier groups and the suitable proteins for selective IMAC,
- to measure the activity of primary immobilized enzymes,
- to define the exact capacity of supports,
- to investigate the recyclability, demetallization and re-metal-loading,
- to do tests with new continous-flow system.

Further enzymes:

- lipase (*Rubrobacter xylanophilus, Pseudozyma aphidis*),
- phenylalanine 2,3-aminomutase (*Taxus canadiensis*).

Std: protein standard mixture
FT: flow through
LS: low salt buffer (50 mM HEPES; 30 mM KCl; pH=7,5)
HS: high salt buffer (50 mM HEPES; 300 mM KCl; pH=7,5)
EDTA: different concentration of Na_2-EDTA in LS buffer (pH=8,0)

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