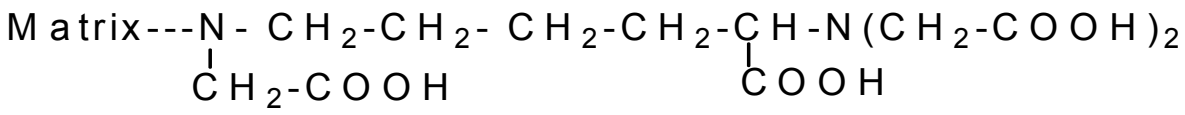
THE PATENTED
PENTADENTATE CHELATOR (PDC)
OF STRUCTURE



is the best chelator
in Immobilized Metal ion Affinity
Chromatography (IMAC)
for
6xhis-tagged protein purification
and
Zn-protein purification
and
General purification of proteins

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October, 2006

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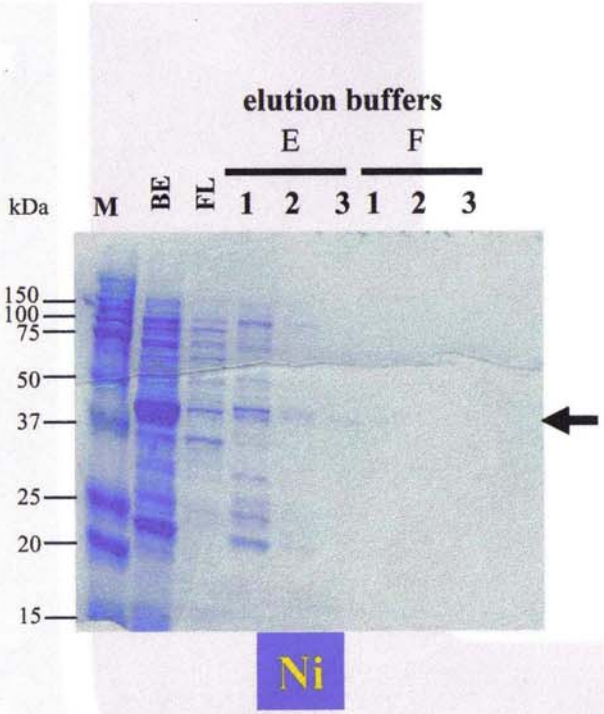
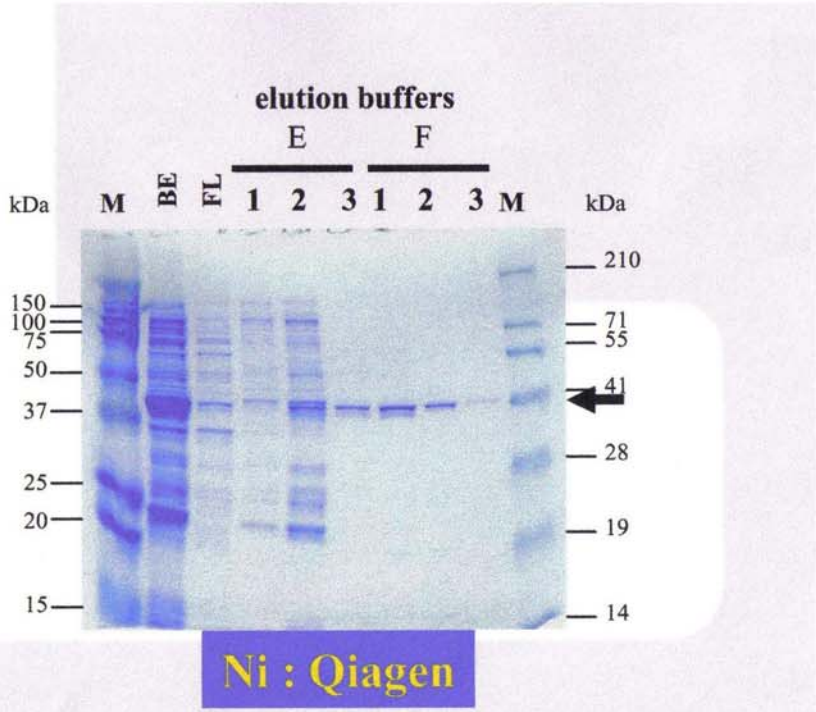
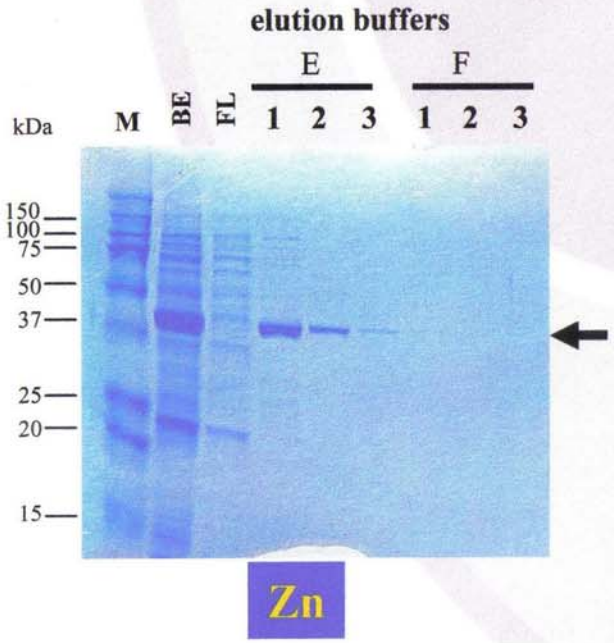
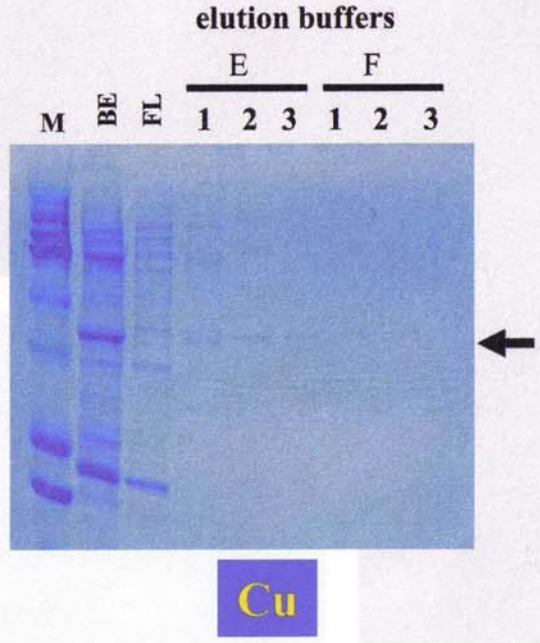
INITIAL ATTEMPTS
at
Purification of a soluble
6xhis-tagged protein
using
the patented PDC kit from AFFILAND
(Cu-PDC, Zn-PDC, Ni-PDC & Co-PDC)
in comparison with
the Ni-NTA gel from QIAGEN
by
EUROGENTEC S.A. and col.
DELPHI genetics
(Belgium)
June, 2006

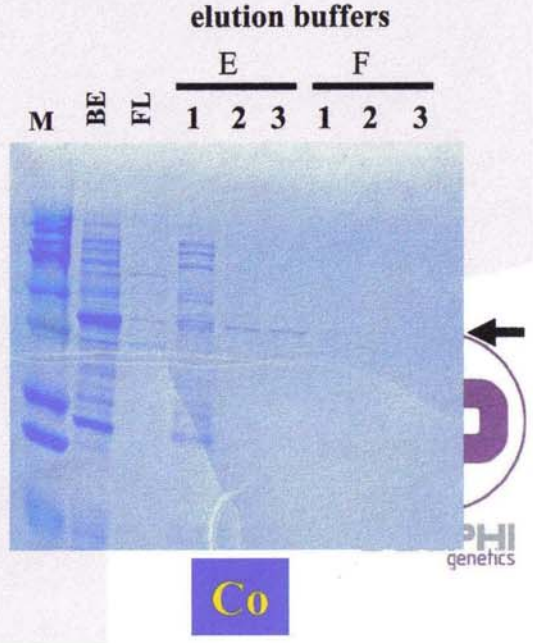
M : Markers expressed in Dalton
BE : Crude extract = lysate
FL : Flow through
Buffer E : 100 mM Imidazole pH 7.5
Buffer F : 300 mM Imidazole pH 7.5











Immobilized PDC

patented since 1998 by Affiland

Matrix---N(CH_2COOH)-CH_2-CH_2- CH_2-CH_2-CH(COOH)N(CH_2-$COOH)_2$

See details at http://www.affiland.com/imac/pdc.htm

CHARACTERISTICS

- **At basic pHs : Immobilized PDC is a pentadentate chelator (Figure 1).**
- **At physiological pHs : Immobilized PDC is a mixture of tetradentate and pentadentate chelator (Figure 2 + Figure 1)**

RESULTS

1) **Immobilized PDC can form stable octahedral complexes M^{2+}- PDC with metal ions M^{2+} such as Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+}, etc…**

2) **Such octahedral complexes possess only one free site for interaction with electron donor group of DNA, proteins… (Figure 1 & Figure 2).**

3) **As histidine and histidine residue are known as the ONLY α–amino-acid and its corresponding residue capable of forming stable octahedral complexes with M^{2+}; the M^{2+}- PDC with one free site cited in point 1) and 2) results so in high selective binding for histidine residues of 6xhis-tagged proteins and/or histidine containing proteins.**

4) **The presence of only one free site of the M^{2+}- PDC complexes for interaction also results in low concentration of imidazole (50-100 mM) at physiological pH being sufficient for the elution of 6xhis-tagged proteins from a M^{2+}- PDC column.**

5) **Due to steric effects of the interaction between a histidine residue and the free site of the M^{2+}- PDC complexes, the patented PDC kit composed of 4 columns of Cu-PDC, Zn-PDC, Ni-PDC and Co-PDC is the ONLY WAY of saving time and money during initial attempts at purification of a his-tagged protein.**

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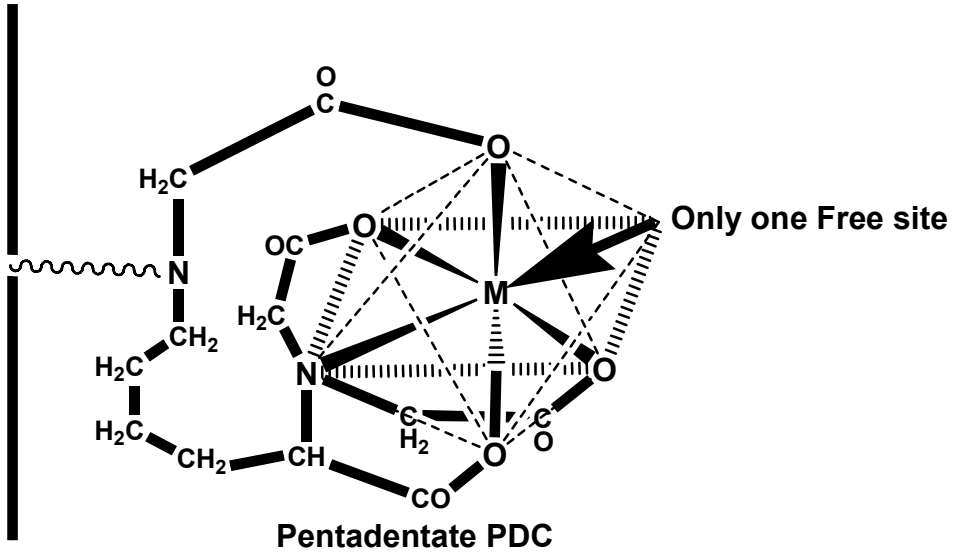

Figure 1

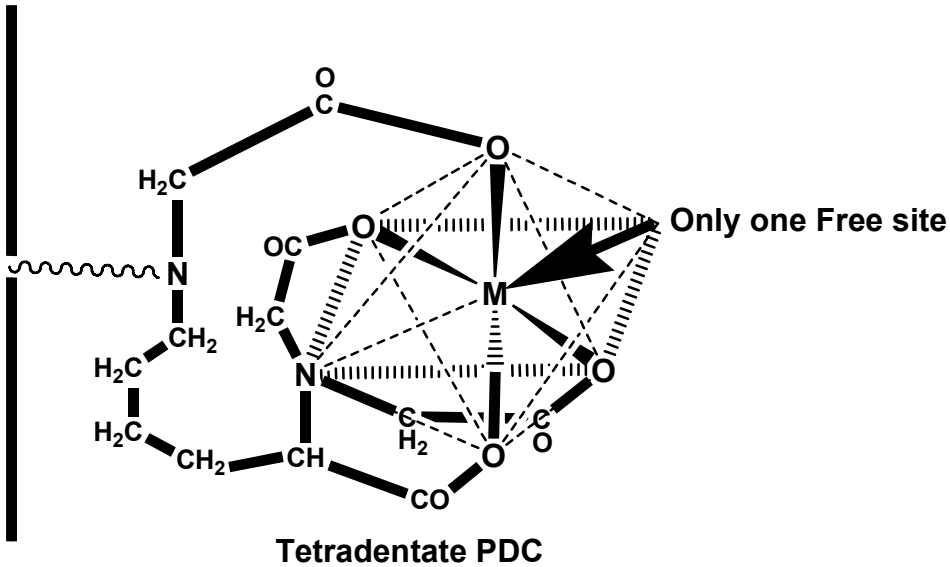

Figure 2

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Immobilized NTA (Nitrilotriacetic acid) chelator

Ref.: Hochuli et al.; J. Chromatogr. 411, 177 (1987)

Agarose---NH- $(CH_2)_n$-CH(COOH)N$(CH_2$-COOH$)_2$ (n = 2 or 4)

CHARACTERISTICS

- **At basic pHs : Immobilized NTA is a tetradentate chelator (Figure 3).**
- **At physiological pHs : Immobilized NTA is a mixture of tridentate and tetradentate chelator (Figure 3 + Figure 4)**

RESULTS

1) **Immobilized NTA can form octahedral complexes with metal ions such as Ni^{2+}, Co^{2+}…**

2) **Such complexes leav two free sites for interaction with histidine residues of the protein to be purified (Figure 3 & Figure 4). The selectivity for histidine residue binding may not be optimal because of the second free site which can be occupied by other amino-acid residues of the protein of interest such as cysteine, lysine, tryptophan, once the first free site is occupied by a histidine residue.**

3) **The presence of the tridentate NTA complex form (Figure 4) at physiological pHs may provoke an important leaching of Ni^{2+} ions during chromatography steps.**

4) **In addition, the two free sites cited above results in the need of high concentration of imidazole (250 mM or more) at physiological pH for the elution of 6xhis-tagged proteins from a Ni^{2+}- NTA or Co^{2+} - NTA column.**

5) **Due to steric effects in interaction between histidine residue and metal chelate NTA complex, the Ni-NTA and/or Co-NTA CANNOT be the UNIVERSAL WAY for general purification of his-tagged proteins (see enclosed example).**

6) **Contrary to the description given by many manufacturers, the – NH group of histidine residue is known to be incapable of coordinating with the Ni^{2+}or Co^{2+} ions of the Ni-chelator complexes.**

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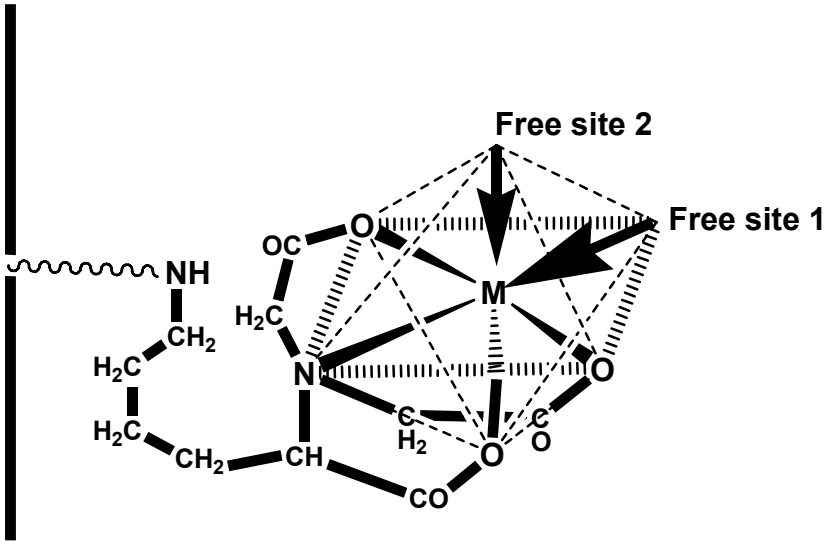
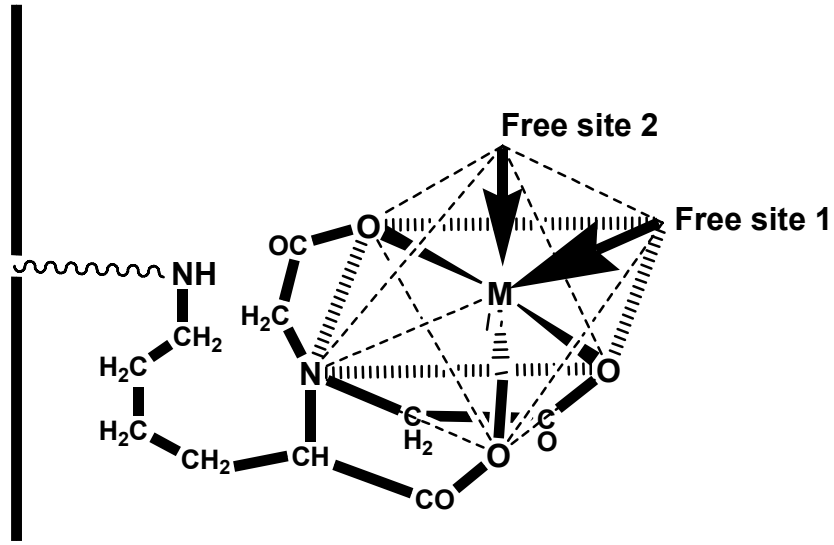


Tetradentate chelator NTA

Solid matrix

Figure 3



Tridentate chelator NTA

Solid matrix

Figure 4

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Immobilized IDA (Iminodiacetic acid) chelator

Ref.: Porath et al. - Nature, 258, 589 (1975); Biochem, 22, 1621 (1983)

Sepharose---N(CH_2-COOH)$_2$

CHARACTERISTICS

- **Immobilized IDA is normally a mixture of bidentate and tridentate chelator at physiological pH. It is therefore incapable of forming octahedral complexes with M^{2+} ions. It can ONLY form a square or tetrahedral complex with Cu^{2+}, Zn^{2+} … (Figure 5).**

- **However, in the presence of electron donor containing cross-linkers, immobilized IDA may form PSEUDO-octahedral complexes with Ni^{2+} ions, (Figure 6). These PSEUDO-octahedral complexes therefore possess three free sites for interaction with the electron donor groups of the proteins to be purified (Figure 5 & Figure 6).**

RESULTS

1) **NON-selective binding for 6xhis-tagged proteins because the Ni^{2+}- IDA is simply a PSEUDO-octahedral complex while Ni^{2+}- histidine residue is a TRUE octahedral complex. The his-tagged proteins purified from Ni^{2+}- IDA column cannot be pure and will be contaminated by a lot of other proteins.**
2) **The three free sites cited above result in the need for a very high concentration of imidazole (500 mM or more) at physiological pH for the elution of 6xhis-tagged proteins. Indeed, these CHAOTROPIC CONDITIONS of elution may result in the activity of such purified proteins being partially or totally destroyed.**

Remarks :

- **A very high affinity constant is not recommended for Affinity chromatography because the elution step of the product to be purified in this case, will necessitate CHAOTROPIC CONDITIONS i.e. very acid pHs or a very important concentration of organic compounds).**
- **The HIGH AFFINITY of a protein for an affinity matrix DOES NOT MEAN the HIGH CAPACITY and/or HIGH SELECTIVITY of the matrix for this protein.**

One variant of immobilized IDA:

Immobilized TED {(N,N,N'-Triscarboxymethyl)ethylenediamine}

Resin---NH(CH_2COOH)-CH_2-CH_2-NH(CH_2-COOH)$_2$

At physiological pHs, the two NH groups of the TED will be partially charged - the presence of the tridentate TED (3 COO^-) complex form at physiological pHs may provoke an important leaching of Ni^{2+} ions during chromatography steps.

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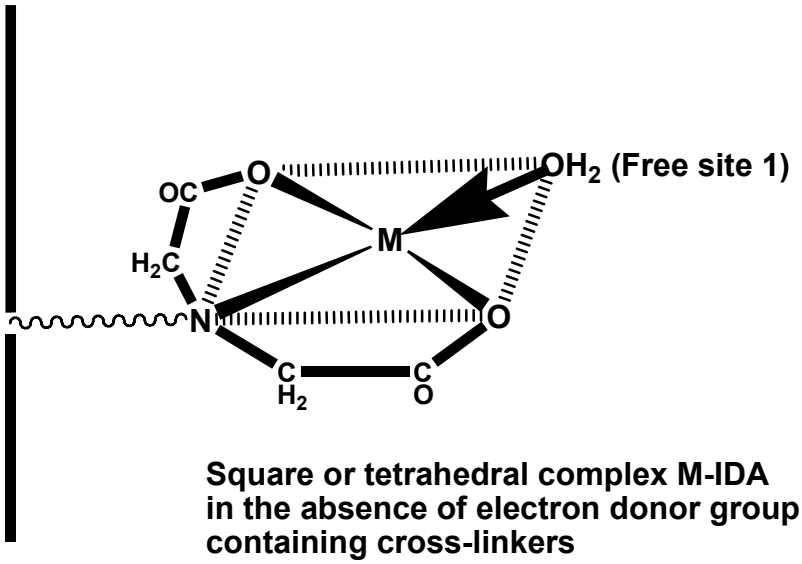
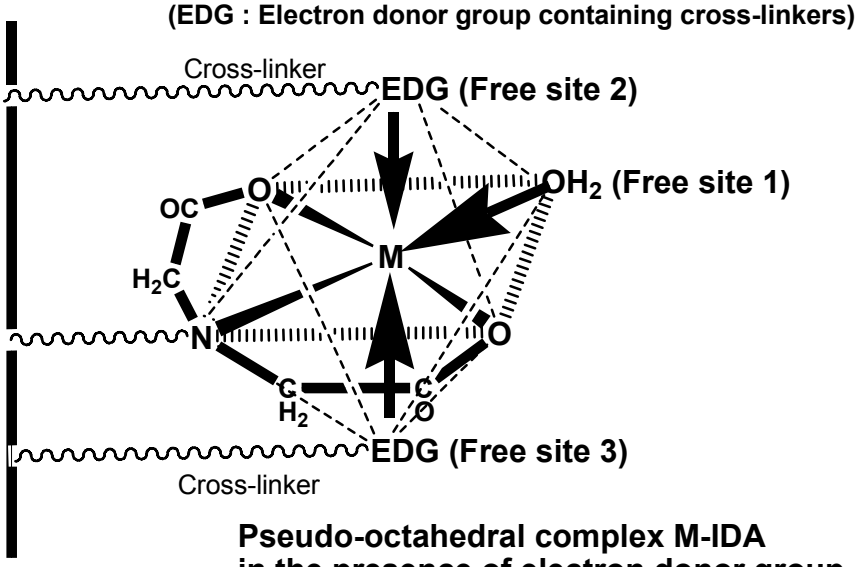


Square or tetrahedral complex M-IDA in the absence of electron donor group containing cross-linkers

Figure 5

(EDG : Electron donor group containing cross-linkers)



Pseudo-octahedral complex M-IDA in the presence of electron donor group containing cross-linkers

Figure 6

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