First European Conference on Chemistry for Life Sciences

Rimini (Italy), October 3-8, 2005

A.D. MDLXII

Uniwersytet Wrocławski

Nickel Binding to Cap43 protein

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INTRODUCTION

Cap43 has been reported to be specifically induced by nickel compounds in a variety of cell lines [1,2]. Although the function of the Cap43 protein (MW 43,000) is not clear, it does appear to be induced in response to an increase in intracellular concentration of Ca^{2+}, caused by nickel ion exposure in cultured human cells [2], for this reason is named Cap43: *Ca*lcium *p*rotein *43*,000. Cap43 protein is expressed at low levels in normal tissues however, in a variety of cancers, it is overexpressed in cancer cells. The high level of expression in cancerous status combined with the elevated stability of Cap43 protein makes it an excellent cancer marker.

A possible way to better understand the molecular mechanisms implicated in toxicity and carcinogenicity of nickel compounds is to study the characteristics of the proteins expressed by the genes specifically induced by these carcinogens. For this reason we focused our attention to investigate the interaction ability of nickel to Cap43 protein[3,4]. The peculiarity of protein Cap43 is its new mono-histidinic motif consisting of ten amino acids (***TRSRSHTSEG***) repeated three times in the C-terminus.

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  1 MSREMQDVDL AEVKPLVEKG ETITGLLQEF DVQEQDIETL HGSVHVTLCG TPKGNRPVIL
 61 TYHDIGMNHK TCYNPLFNYE DMQEITQHFA VCHVDAPGQQ DGAASFPAGY MYPSMDQLAE
121 MLPGVLQQFG LKSIIGMGTG AGAYILTRFA LNNPEMVEGL VLINVNPCAE GWMDWAASKI
181 SGWTQALPDM VVSHLFGKEE MQSNVEVVHT YRQHIVNDMN PGNLHLFINA YNSRRDLEIE
241 RPMPGTHTVT LQCPALLVVG DSSPAVDAVV ECNSKLDPTK TTLLKMADCG GLPQISQPAK
301 LAEAFKYFVQ GMGYMPSASM TRLMRSRTAS GSSVTSLDG
340 TRSRSH345TSEG TRSRSH355TSEG TRSRSH365TSEG
370 AHLDITPNSGA AGNSAGPKSM EVSC
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Sequence of Cap43 protein

We have analyzed, for Ni(II) binding, the 30-amino acid C-terminal sequence of the protein, *TRSRSHTSEG-TRSRSHTSEG-TRSRSHTSEG,* by a combined pH-metric and spectroscopic (UV-VIS, CD, NMR) study.

The imidazole nitrogen atom of the histidine residue is the essential bonding site for Ni(II) ion, and the 30-amino acid sequence contain three histidine residues. Therefore, this study was also performed in order to evaluate the bonding ability of the fragment to more than one metal ion.

It is important to point out that each of the 10-amino acid fragments (TRSRSHTSEG) may coordinate one metal ion. The coordination of the metal ion starts from the imidazole nitrogen atom of the histidine residue, and with increasing the pH, Ni(II) ions are able to deprotonate successive peptide nitrogen atoms, $NiH_{-3}L$ $Ni_2H_{-6}L$ and $Ni_3H_{-9}L$ complexes for the 30-amino acid fragment, are formed (above pH 8).

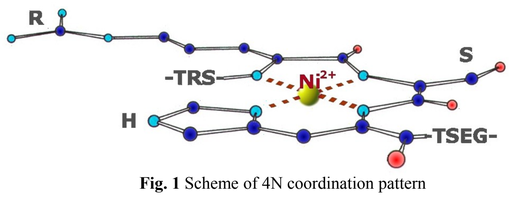


Fig. 1 Scheme of 4N coordination pattern

The formation of stable five membered chelate rings by consecutive nitrogen atoms is the driving force of the coordination process. At physiological pH (7.4) and mM concentration of Ni(II), dependently from metal to ligand molar ratio the 30-amino acid fragment forms the NiL (1:1), Ni_2L (2:1) and Ni_3L (3:1) complexes where each metal ion is coordinated by the imidazole nitrogen atom of the histidine residue of each ten-amino acid fragment.

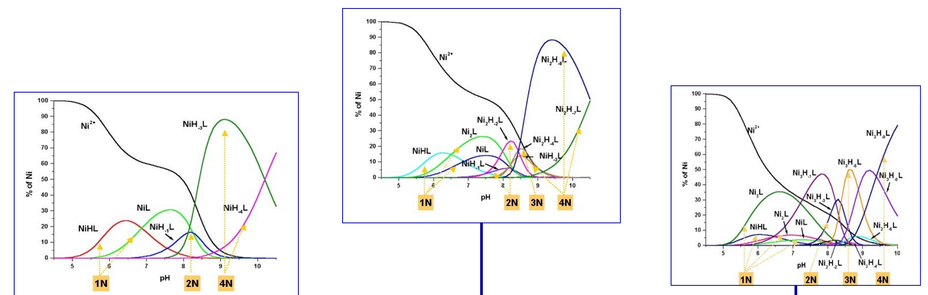
From NMR experiment, the diamagnetic shifts induced by Ni(II) were consistent with strong binding to a square-planar site formed by four nitrogen atoms derived from His ($Nd1,N_H$) and from N_H of Ser and Arg, in the same coordination pattern and ability of Ni(II) to all three identical repeated region. Strong shifts in the aliphatic proton resonances of arginine suggest an active involvement of the its side-chain in the complex stability.

Both spectroscopic and potentiometric studies performed on the 30-amino acids peptide, support the existence of relatively effective metal binding site in the C-terminal region of Cap43 protein. Our results suggest that the entire Cap43 protein could be one interesting target for Ni(II) ions.

REFERENCE

- 1. D. Zhou, K. Salnikow, M. Costa, Cancer Res. 58, 1998, pp. 2182-2189.
- 2. K. Salnikow, D. Zhou , T. Kluz, C. Wang, M. Costa, in: Metal and Genetics , (Sarkar B ed), New York, 1999, pp. 131-144
- 3. M.A. Zoroddu, T. Kowalik-Jankowska, H. Kozlowski, K. Salnikow, M. Costa, J Inorg Biochem.,84(1-2), 2001, pp 47-54.
- 4. M.A. Zoroddu, M. Peana, T. Kowalik-Jankowska, H. Kozlowski, M. Costa, J Inorg Biochem., 98(6), 2004, pp. 931-939

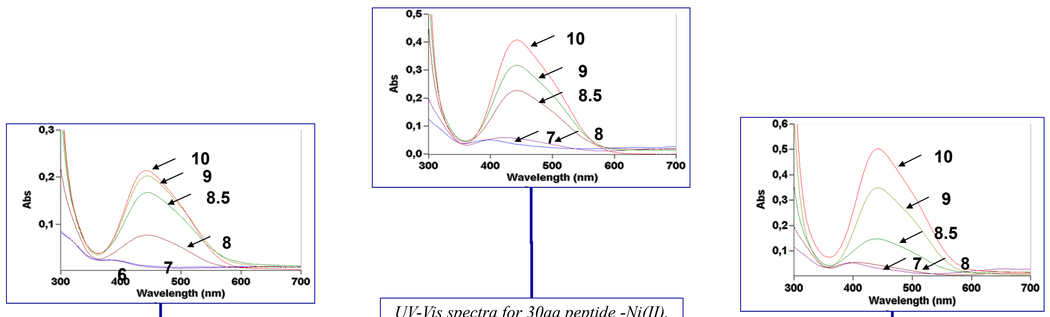
pH-METRIC STUDY



Species distribution curves for 30aa peptide -Ni(II), molar ratio 1:1, 1:2 and 1:3 respectively

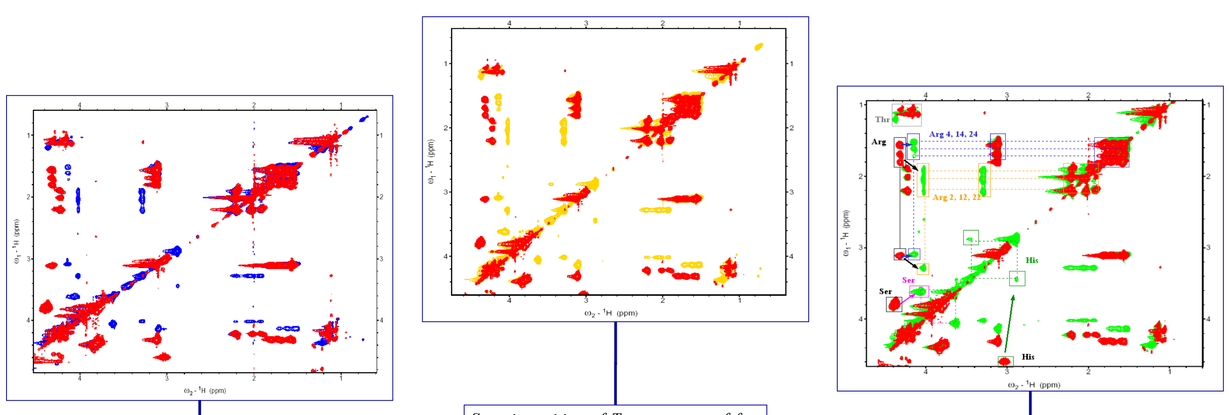
SPECTROSCOPIC STUDY

UV-Vis

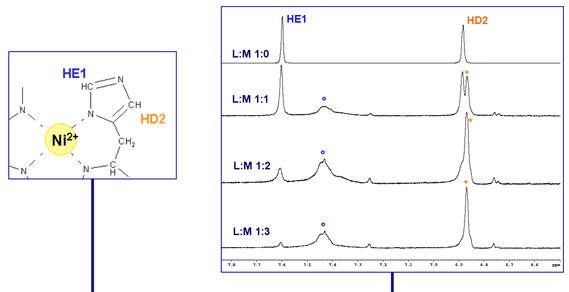


UV-Vis spectra for 30aa peptide -Ni(II), molar ratio 1:1,1:2 and 1:3 respectively

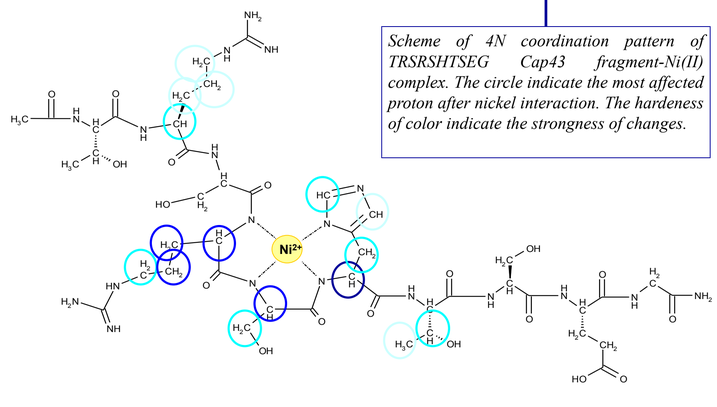
NMR



Superimposition of Tocsy spectra of free 30aa peptide (red) and the system 30aa peptide-Ni(II) molar ratio 1-1 (blu) ; 1-2 (yellow) and 1-3 (green) respectively.



Changes in intensity and chemical shift of the histidine aromatic proton (HE1 and HD2) by increasing nickel concentration. Comparison of aromatic region of 1D H NMR spectra of 30aa peptide Cap43-Ni(II) in the molar ratio 1-0, 1-1. 1-2, 1-3.



Scheme of 4N coordination pattern of TRSRSHTSEG Cap43 fragment-Ni(II) complex. The circle indicate the most affected proton after nickel interaction. The hardeness of color indicate the strongness of changes.