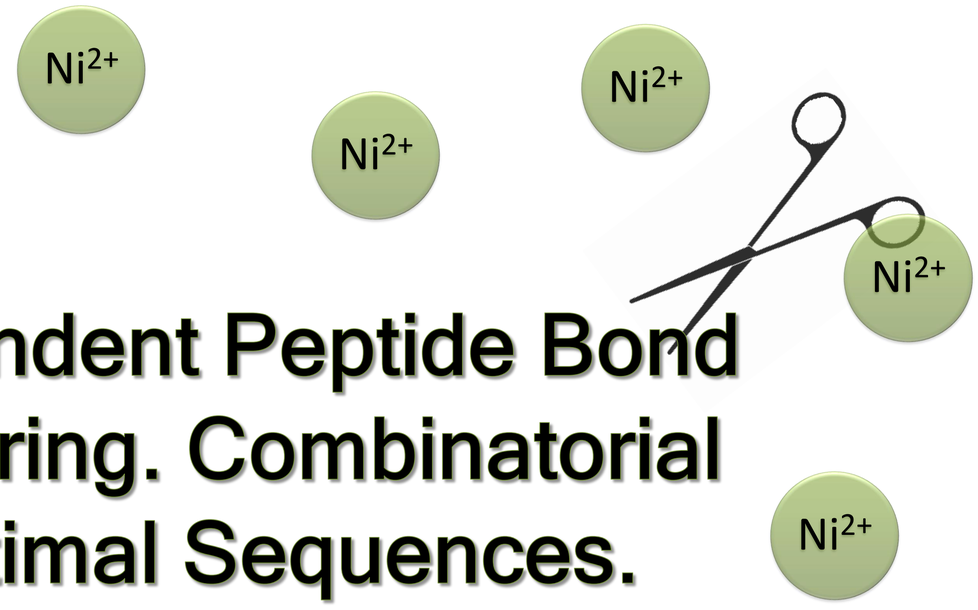
Institute of Biochemistry and Biophysics
Polish Academy of Sciences



Sequence-Specific Ni(II)-Dependent Peptide Bond Hydrolysis for Protein Engineering. Combinatorial Library Determination of Optimal Sequences.

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Introduction:

The sequence-specific cleavage of the peptide bond is a crucial procedure in protein engineering and purification. The extreme stability of this bond, with half-life for spontaneous hydrolysis estimated as 350-600 years at neutral pH and room temperature, limits the range of appropriate cleavage reagents.

Nowadays many cleavage reagents are used (proteolytic enzymes, self-cleaving intein sequences, and chemical agents, such as cyanogen bromide). Among other chemical reagents metal ions are also tested. Some of them are more or less sequence-specific. In particular, many metal ions promote the hydrolysis of Xaa-Ser peptide bond (Xaa - any amino acid). These reactions have not been demonstrated for downstream peptide bonds in longer peptides or proteins.

Results and discussion:

A combinatorial library of CH_3CO-Gly-Ala-(Ser/Thr)-Xaa-His-Zaa-Lys-Phe-Leu-NH_2 peptides was synthesized in order to study the sequence dependence of a reaction of Ni(II)-dependent peptide bond hydrolysis in a systematic manner (Xaa residues included 17 common protein binding amino acids (except Asp, Glu, and Cys) and Zaa residues included 19 common amino acids (except Cys)). The Ni(II)-dependent hydrolysis was monitored by MALDI-TOF mass spectrometry. The progress of reaction was evaluated by visual detection in mass spectra of the appearance of signals corresponding to expected products of hydrolysis, Ser/Thr-Xaa-His-Zaa-Lys-R2, at given time of incubation (t_m). The formula used for the semiquantitative evaluation of reaction progress had the form $Sc=24/t_m$ (Results are shown in tables 1-4). After statistical analysis strict relationships between the physical properties of Xaa and Zaa residues and the hydrolysis rates were found. We found that susceptibility of peptides to hydrolysis is related to the bulkiness and hydrophobicity of Xaa and Zaa substituents.

These results provided a basis for further research, aimed at the elucidation of the reaction mechanism and biotechnological applications of Ni(II)-dependent peptide bond hydrolysis.

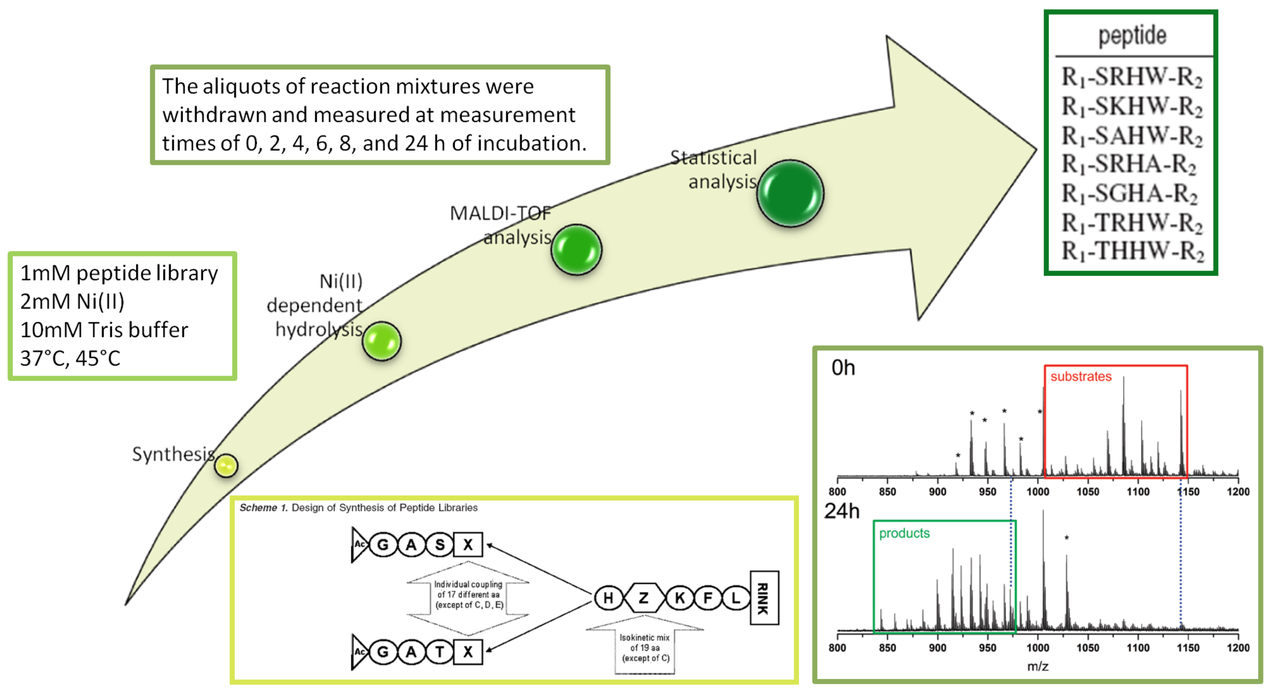

Figure 1. Scheme of our work. Phase 1: synthesis of peptide libraries. Phase 2: Reaction of library solution with Ni(II) ions. Phase 3: MALDI-TOF analysis of specific time dependent samples. Phase 4: statistical analysis of MALDI data.

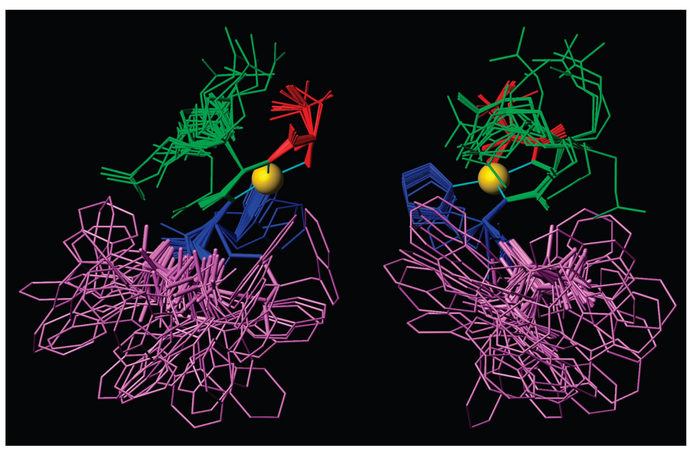

Figure 2. Orthographic projections of the ensemble of 30 lowest-energy structures of the proposed hydrolytically reactive NiH-2L complex of the R1-SRHW-R2 peptide. The Ser residue is in red, Arg in green, His in blue, and Trp in violet, while the Ni(II) is in gold and the four N-Ni(II) bonds are in cyan. Other residues are invisible for the sake of clarity.

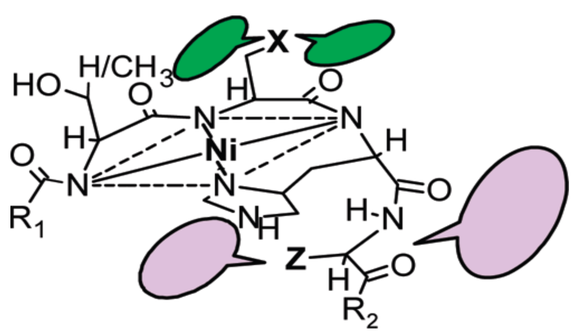

Figure 3. Structural sketch of the proposed hydrolytically reactive $NiH_{-2}L$ complex of a library peptide L. Green and violet clouds indicate areas of influence of Xaa (X) and Zaa (Z) residues, respectively.

Table 1. Hydrolysis Scores and Xaa and Zaa Residue Rankings, Obtained by MALDI-TOF Screening of the Library of 1 mM R_1-Ser-Xaa-His-Zaa-Lys-R_2 Peptides Incubated with 2 mM Ni(II) at 45 °C for 8 h in 10 mM Tris Buffer, pH 8.2

X \ Z	E	D	N	Q	S	A	P	H	T	G	V	Y	M	I	L	K	F	W	R	Sc
G	0	0	0	0	0	0	0	0	0	0	3	0	0	3	3	3	0	4	3	19
A	0	0	0	0	0	3	0	4	0	0	3	3	0	4	4	6	3	6	6	42
S	3	3	0	0	3	3	0	3	0	3	4	3	3	6	6	6	3	12	6	67
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	3	0	0	6	3	0	6	6	6	3	6	6	45
N	0	0	0	0	0	0	0	3	0	0	3	3	0	4	4	4	3	6	6	36
V	0	0	0	0	0	0	0	3	0	0	3	3	0	3	3	4	3	4	3	29
Q	0	0	0	0	0	3	0	3	0	0	4	4	3	6	6	6	4	6	6	51
L	0	0	0	0	0	0	0	0	0	0	3	3	0	3	3	4	3	4	4	27
I	0	0	0	0	0	0	0	3	0	0	3	0	0	3	3	6	0	4	3	25
H	0	0	0	0	0	0	0	4	0	0	6	4	0	6	6	12	3	12	6	59
M	0	0	0	0	3	3	0	3	0	0	6	4	3	12	12	6	4	6	6	68
K	3	0	0	0	0	4	0	3	0	0	6	4	4	12	12	12	4	12	6	82
F	3	0	0	0	0	3	0	6	0	0	6	6	3	6	6	6	6	6	6	63
Y	0	0	0	0	0	0	0	4	0	0	4	4	0	6	6	6	4	6	6	46
R	3	0	0	0	4	6	0	4	3	4	12	6	3	12	12	12	4	12	12	109
W	0	0	0	0	0	3	0	3	0	3	12	6	3	12	12	12	4	6	6	82
Sc	12	3	0	0	10	28	0	49	3	10	84	56	22	104	104	111	51	112	91	850

Table 2. Hydrolysis Scores and Xaa and Zaa Residue Rankings, Obtained by MALDI-TOF Screening of the Library of 1 mM R_1-Ser-Xaa-His-Zaa-Lys-R_2 Peptides Incubated with 2 mM Ni(II) at 37 °C for 8 h in 10 mM Tris Buffer, pH 8.2

X \ Z	E	D	N	Q	S	A	P	H	T	G	V	Y	M	I	L	K	F	W	R	Sc
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	4	3	10
S	0	0	0	0	0	0	0	0	0	0	3	3	3	6	6	4	3	6	6	40
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	4	3	18
N	0	0	0	0	0	0	0	0	0	0	3	3	0	4	4	4	3	6	6	33
V	0	0	0	0	0	0	0	3	0	0	0	0	0	3	3	3	0	4	3	19
Q	0	0	0	0	0	0	0	0	0	0	0	3	0	6	6	4	3	6	6	34
L	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	0	3	3	15
I	0	0	0	0	0	0	0	3	0	0	3	0	0	3	3	3	0	3	3	21
H	0	0	0	0	0	3	0	0	0	3	6	3	0	6	6	6	3	6	4	46
M	0	0	0	0	0	0	0	0	0	0	3	3	3	4	4	3	3	4	4	31
K	0	0	0	0	0	0	0	4	0	0	4	4	3	6	6	6	4	12	4	53
F	0	0	0	0	0	0	0	3	0	0	3	3	0	4	4	4	3	4	4	32
Y	0	0	0	0	0	0	0	4	0	0	6	3	0	4	4	4	3	6	4	38
R	0	0	0	0	3	3	0	4	0	3	6	4	3	12	12	6	4	12	4	76
W	0	0	0	0	0	0	0	4	0	0	3	0	0	6	6	4	3	4	3	33
Sc	0	0	0	0	3	6	0	25	0	6	40	29	12	71	71	60	32	87	63	505

Table 3. Hydrolysis Scores and Xaa and Zaa Residue Rankings, Obtained by MALDI-TOF Screening of the Library of 1 mM R_1-Tyr-Xaa-His-Zaa-Lys-R_2 Peptides Incubated with 2 mM Ni(II) at 45 °C for 8 h in 10 mM Tris Buffer, pH 8.2

X \ Z	E	D	N	Q	S	A	P	H	T	G	V	Y	M	I	L	K	F	W	R	Sc
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6
V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	0	4	4	17
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	3	9
I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	3	9
H	0	0	0	0	4	4	0	4	4	4	6	6	4	12	12	12	6	12	6	96
M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	4	10
K	0	0	0	0	0	3	0	4	0	0	6	6	4	12	12	12	6	12	12	89
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	4	10
Y	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	4	12
R	0	0	0	0	0	3	0	4	0	0	4	6	4	12	12	12	4	12	6	79
W	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	4	0	4	4	21
Sc	0	0	0	0	4	10	0	12	4	4	16	18	15	42	42	59	16	66	59	367

Table 4. Hydrolysis Scores and Xaa and Zaa Residue Rankings, Obtained by MALDI-TOF Screening of the Library of 1 mM R_1-Tyr-Xaa-His-Zaa-Lys-R_2 Peptides Incubated with 2 mM Ni(II) at 37 °C for 8 h in 10 mM Tris Buffer, pH 8.2

X \ Z	E	D	N	Q	S	A	P	H	T	G	V	Y	M	I	L	K	F	W	R	Sc
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3
I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3
H	0	0	0	0	0	0	0	3	0	0	0	0	0	3	3	6	0	12	6	33
M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	6
K	0	0	0	0	0	0	0	0	0	0	3	3	3	4	4	3	3	6	4	33
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	6
Y	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	4	10
R	0	0	0	0	0	0	0	3	0	0	3	4	3	6	6	6	3	12	4	50
W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	3	3	10
Sc	0	0	0	0	0	0	0	6	0	0	6	7	6	13	13	25	6	48	30	160

References:

Krężel A, Kopera E, Protas AM, Poznański J, Wysłouch-Cieszyńska A, Bal W. *Sequence-specific Ni(II)-dependent peptide bond hydrolysis for protein engineering. Combinatorial library determination of optimal sequences.* J Am Chem Soc. 2010 Mar 17;132(10):3355-66.

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