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MOTIFS WITH POTENTIAL PHYSIOLOGICAL ACTIVITY IN FOOD PROTEINS – BIOPEP DATABASE

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Abstract. Proteins are the multifunctional food components affecting the living organisms. One of the proteins function is the impact on the body due to the presence of motifs that show specific physiological and biological activities. Due to the worldwide growth of demand for the food containing bioactive components, increasing attention has been paid recently to the use of bioactive peptides as physiologically active food ingredients. They are important elements of the prevention and treatment of various lifestyle diseases. In addition to its primary function and according to current knowledge, each protein may be a reserve source of peptides controlling the life processes of organisms. For this reason, in this work, application of a new, additional criterion for evaluating proteins as a potential source of biologically active peptides, contributes to a more comprehensive and objective definition of their biological value. A complementary part of such research is the strategy for evaluation of the food proteins as precursors of biologically active peptides which involves the database of proteins and bioactive peptides – BIOPEP (available online at: http://www.uwm.edu.pl/biochemia). The database contains information on 2123 peptides representing 48 types of bioactivities, their EC_{50} values and source of origin. Proteins (706 sequences) are considered as bioactive peptide precursors based on newly introduced criteria: the profile of potential biological activity, the frequency of bioactive fragments occurrence and potential biological protein activity. This original and unprecedented so far approach, started to be successfully and more widely applied by other authors. BIOPEP can be interfaced with global databases such as e.g. TrEMBL, SWISS--PROT, EROP and PepBank. Recently the BIOPEP database was enlarged with the data about allergenic proteins, including information about structure of their epitopes and molecular markers.

Key words: bioactive peptides, proteins, BIOPEP database

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INTRODUCTION

Due to their nutritional value and functional properties, proteins are the major structural components of food. They are used as a source of energy, but primarily as the amino acids essential for the synthesis of body proteins. In addition, many proteins show specific biological activities which can have an influence on the functional or health-promoting attributes of foodstuffs. These proteins and the products of their hydrolysis, peptides, may affect both food properties and bodily functions. Every protein may play the role of a precursor of biologically active and functional peptides. Increasing attention has been paid recently to the use of bioactive peptides as physiologically active food ingredients which are important elements of the prevention and treatment of various lifestyle diseases. There is a growing worldwide demand for this kind of food. Moreover, research studies on protein hydrolysates and peptides are in progress. Peptides derived from proteins may lower blood pressure, inhibit the activity of proline endopeptidases, stimulate the immune system, exhibit opioid activity, act as opioid antagonists, contract smooth muscles, inhibit platelet aggregation, inhibit HIV proteinase and oxidation processes, show antibacterial and fungicidal activity, surface activity, bind ions and participate in the transport of minerals, determine the sensory properties of food products and improve their nutritional value, and support the control of body weight. According to Karelin et al. [1998], apart from its primary function, each protein may be a reserve source of peptides controlling the life processes of organisms. For this reason, the determination of a new, additional criteria for evaluating proteins as a potential source of biologically active peptides contributes to a more comprehensive and objective definition of their biological value [Dziuba and Iwaniak 2006]. Potentially biologically active protein fragments in which structural motifs resemble those of bioactive peptides remain inactive in precursor protein sequences. These fragments, released from the precursor by proteolytic enzymes, may interact with selected receptors and affect the physiological functions of the body as well as the functional properties of food products (Fig. 1).

In addition to analytical methods, computer-aided techniques are also employed to evaluate food components, including proteins. The process of modeling the physicochemical properties of proteins [Lackner et al. 1999], predicting their secondary structure [Bairoch and Apweiler 2000] or searching for a homology between proteins to identify their functions [Kriventseva et al. 2001, Bray et al. 2000] requires analyses supported by databases of protein sequences or sequence motifs [Bennett et al. 2004, Colinge and Masselot 2004]. A complementary part of such research is the strategy of analyzing of food proteins as precursors of biologically active peptides which involves the use of the BIOPEP [Dziuba et al. 2009]. It is a database of proteins and bioactive peptides, available online at http://www.uwm.edu.pl/biochemia. This database contains information on peptides which represent 48 types of bioactivities, EC_{50} values and a source of origin. Proteins are evaluated as bioactive peptide precursors based on new, additional criteria: the profile of potential biological activity, the frequency of bioactive fragments occurrence and potential biological protein activity [Dziuba et al. 2003]. This original approach, not described earlier by other authors, started to be successfully and more widely applied by other scientific groups [i.e. presented by Wang and Gonzalez de Meija in 2005]. BIOPEP can be interfaced with global databases like e.g.: TrEMBL, SWISS-PROT, EROP and PepBank. Recently the BIOPEP database was enlarged with

the data concerning the allergenic proteins, including information about structure of their epitopes and molecular markers.

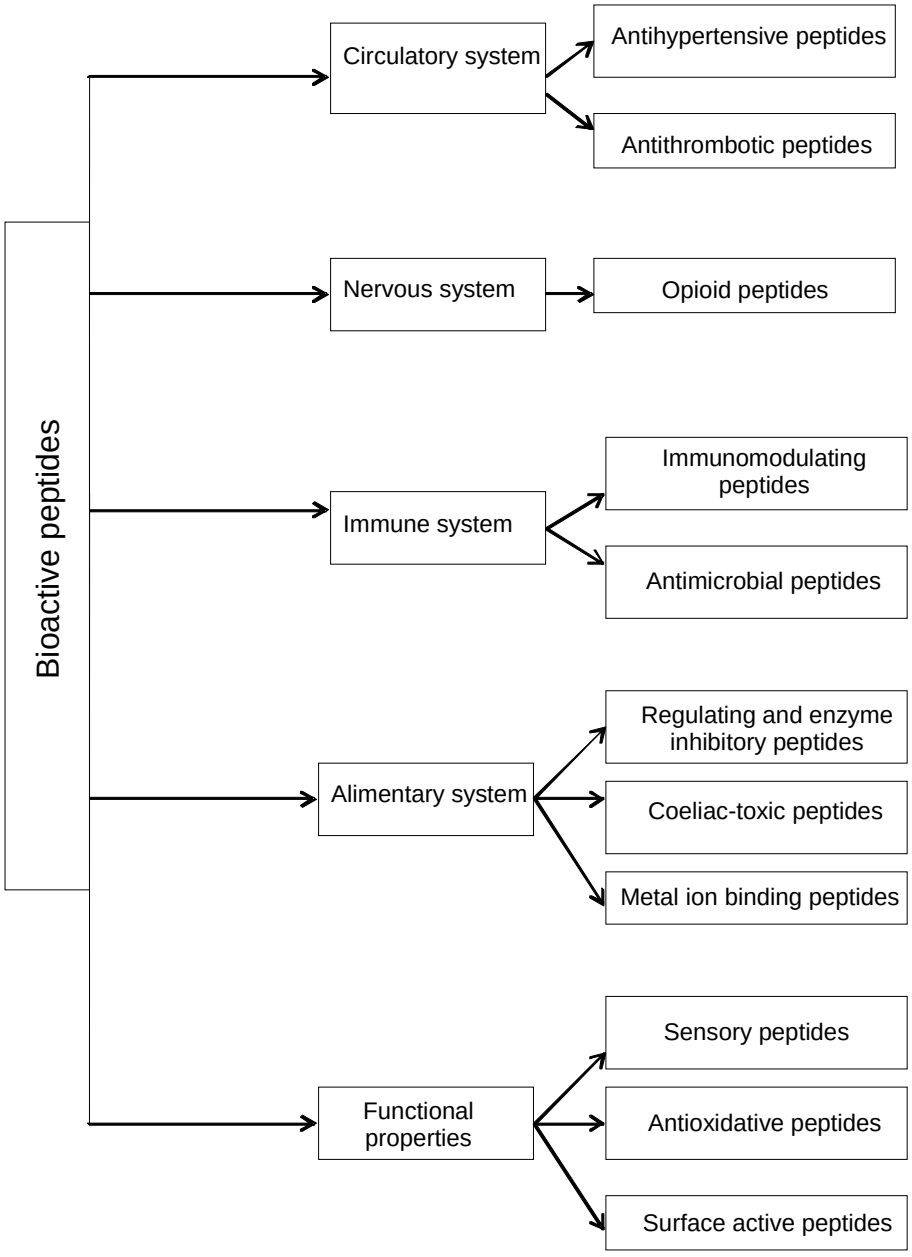

Fig. 1. Function of peptides derived from food proteins

MATERIAL AND METHODS

Database of proteins and bioactive peptides BIOPEP (www.uwm.edu.pl/biochemia) developed in the Department of Food Biochemistry) was involved to evaluate the food proteins as bioactive peptide precursors. The analysis included twenty three types of activities out of forty eight gathered in BIOPEP. They were: antiamnestic, antithrombotic, antihypertensive, immunomodulating, chemotactic, contracting, toxic, embryotoxic, antioxidative, dipeptidylpeptidase IV inhibiting, opioid and opioid antagonistic, stimulating red blood cell formation, hemolytic, binding and transporting metals and metal ions, bacterial permease ligand, anorectic, activating ubiquitin-mediated proteolysis, regulating ion flow, neuropeptide inhibiting, regulating gastric mucosa activity, antibacterial, antiviral, regulating phosphoinositol function. Peptides possessing these activities were selected by the values of the frequency of their occurrence and other health and technological properties. The experiment was carried out for 706 protein amino acid sequences available in the BIOPEP database.

FUNCTIONS OF THE BIOPEP *IN SILICO* ANALYSIS PANEL

The analytical functions are available in the "Analysis" window. This window is present after clicking on any of BIOPEP databases. These *in silico* analytical functions include: determination the type, number and the location of active fragment(s) in a protein defined as "Profiles of protein's biological activity", computation the parameters defined as A, B and Y to determine the value of a given protein as a source of bioactive peptides (toolbar "A, B, Y Calculation"), proteolytic processes design to find out what enzymes release the peptides with specific type of activity (panel "Enzymes action"). Additional functions of BIOPEP database enable to search for protein/bioactive peptide sequences (toolbar "Find") as well as contain the definitions which are suitable to understand the interpretation of results. The panel "Definitions" also includes the equations applied for protein evaluation.

In this study, the value of proteins as bioactive peptide precursors was based on the occurrence frequency of bioactive fragments in the protein chain (A) defined as:

$$A = \frac{a}{N},$$

where:

a – number of fragments with given activity in a protein chain,
N – number of amino acid residues in the polypeptide chain of a protein molecule.

RESULTS AND DISCUSSION

Physiologically active peptides form a complex and highly diversified group of compounds, with regard to their terminology, structure and functions. Many physiologically active peptides are multifunctional, as they perform regulatory functions and di-

rectly affect various developmental and metabolic processes. It should be pointed out that specific physiological effects are not always manifested following the oral administration of a peptide or as a result of peptide release by digestive enzymes; for instance, opioid and immunostimulating peptides derived from bovine milk proteins mostly and probably undergo further hydrolysis in the body [Gobbetti et al. 2002].

The richest potential source of bioactive peptides are mostly milk proteins, wheat α- and β-gliadins and collagen. Table 1 and Table 2 show the profiles of the potential biological activity of bovine lactoferrin and wheat γ-gliadin, respectively. Dipeptide and tripeptide motifs with potential antihypertensive and dipeptidylaminopeptidase IV inhibiting activity predominate in both proteins. The polypeptide chains of lactoferrin and γ-gliadin contain 97 and 40 fragments with antihypertensive activity, respectively. The number of dipeptides that act as dipeptidylaminopeptidase IV inhibitors is 13 and 11 in lactoferrin and γ-gliadin, respectively. Lactoferrin may be also a source of 10 peptides with antibacterial activity, 5 peptides with immunomodulatory activity and 4 peptides with heparin-binding activity. The above proteins contain also three fragments potentially activating ubiquitin-dependent proteolysis, two fragments with anticoagulant, opioid, antiamnestic, antioxidant and antimicrobial activity, and one fragment with bacterial permease ligand activity. In addition, γ-gliadin contains 11 potentially celiactoxic fragments.

Table 1. Profile of potential biological activity of bovine lactoferrin (*Bos taurus*) [BIOPEP ID 1212]

Lactoferrin sequence:

MKTLLILTILAMAITIGTANIQVDPSGQVQWLQQQLVPQLQQPLSQQPQQTFPQPQQTFPHQPQQQVP
QPQQPQQPFLQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQ
PFPQLQQPQQPFPQPQQQLPQPQQPQQSFPQQQRPFIQPSLQQQLNPCKNILLQQCKPASLVSSLWSII
WPQSDCQVMRQQCCQQLAQIPQQLQCAAIHSVVHSIIMQQQQQQQQQQGMHIFLPLSQQQQVGQGS
LVQGQGIIQPQQPAQLEAIRSLVLQTLPSMCNVYVPPECSIMRAPFASIVAGIGGQ

Activity	***Activating ubiquitin-mediated proteolysis***
Sequence	Location
1	2
LA	[247-248], [411-412], [434-435], [533-534], [589-590], [648-649], [39-40], [47-48], [236-237], [603-604]
RA	[39-40], [47-48], [236-237], [603-604]
WA	[560-561]
Activity	***Antihypertensive***
Sequence	Location
AA	[78-79], [604-605]
AF	[40-41], [195-196], [207-208], [241-242], [482-483], [529-530], [541-542], [685-686]
AG	[67-68], [123-124], [203-204], [402-403], [465-466], [506-507]
AH	[605-606]
AP	[1-2], [31-32], [237-238], [492-493], [592-593]
AY	[165-166]

Table 1 – cont.

1	2
DA	[55-56], [240-241], [390-391]
DG	[60-61], [201-202], [261-262], [395-396], [575-576]
EA	[66-67], [388-389], [583-584], [682-683]
EI	[80-81]
EK	[51-52], [220-221], [276-277], [521-522]
EV	[337-338]
EW	[15-16]
EY	[659-660], [664-665]
FAL	[41-43]
FG	[190-191], [278-279], [289-290], [618-619]
FGK	[278-280], [618-620]
FR	[530-531], [569-570]
GA	[30-31], [147-148], [194-195], [202-203], [494-495], [528-529]
GF	[306-307]
GGY	[396-398]
GI	[130-131]
GK	[262-263], [279-280], [403-404], [619-620], [622-623]
GL	[118-119], [406-407], [445-446], [472-473], [511-512]
GM	[62-63]
GP	[351-352]
GQ	[294-295], [366-367]
GR	[68-69], [111-112], [120-121], [653-654]
GRP	[653-655]
GS	[101-102], [290-291], [321-322], [479-480]
GT	[83-84], [576-577], [662-663]
GW	[124-125], [466-467]
GY	[191-192], [397-398], [432-433], [525-526]
HL	[246-247], [588-589]
HY	[91-92]
IA	[49-50], [669-670]
IP	[127-128], [310-311], [469-470]
IR	[46-47]
IRA	[46-48]
IW	[267-268]

Table 1 – cont.

1	2
IY	[81-82], [399-400]
KA	[53-54], [221-222], [273-274], [339-340], [441-442]
KE	[85-86], [210-211], [243-244], [263-264], [520-521]
KF	[151-152], [277-278], [628-629]
KG	[100-101], [174-175], [386-387]
KL	[28-29], [73-74], [269-270], [650-651]
KP	[579-580]
KY	[522-523]
KYY	[522-524]
LA	[247-248], [411-412], [434-435], [533-534], [589-590], [648-649]
LF	[288-289], [299-300], [617-618], [631-632], [640-641]
LG	[29-30], [119-120], [305-306], [320-321], [651-652], [661-662]
LN	[232-233], [392-393], [564-565]
LQ	[109-110], [145-146], [199-200]
LRP	[74-76], [132-134], [427-429]
LVL	[383-385]
LY	[318-319]
MG	[129-130], [471-472]
NF	[103-104]
NG	[553-554], [621-622]
PG	[293-294], [493-494]
PL	[144-145], [679-680]
PR	[2-3]
PP	[292-293]
PT	[429-430], [655-656]
PQ	[88-89]
QK	[355-356]
RA	[39-40], [47-48], [236-237], [603-604]
RL	[500-501], [570-571]
RP	[75-76], [133-134], [428-429], [654-655]
RR	[20-21], [38-39]
RW	[7-8], [21-22]
RY	[323-324], [341-342]
SF	[285-286]

Table 1 – cont.

1	2
TE	[139-140], [430-431], [582-583], [645-646], [663-664]
VAA	[77-79]
VAP	[591-593]
VAV	[95-97], [436-438]
VG	[350-351], [537-538]
VK	[98-99], [209-210], [338-339], [439-440], [543-544], [607-608]
VP	[158-159], [250-251], [408-409], [516-517]
VR	[6-7], [37-38]
VW	[346-347], [548-549]
YA	[93-94]
YG	[82-83], [524-525]
YK	[72-73]
YL	[135-136], [319-320], [324-325], [433-434], [660-661]
YP	[166-167]
Activity	***Antibacterial***
Sequence	Location
APRKNVRW	[1-8]
APRKNVRWCTI	[1-11]
APRKNVRWCT-ISQPEW	[1-16]
CIRA	[45-48]
CRRWQWRMK-KLGAPSITCV	[19-37]
FKCRRWQWR-MKKLG	[17-30]
FKCRRWQWR-MKKLGAPSIT-CVRRAF	[17-41]
FKCRRWQWR-MKKLGAPSITCVRRAFA	[17-42]
FKCRRWQWR-MKKLGAPSIT-CVRRAFAL	[17-43]
FKCRRWQWR-MKKLGAPSIT-CVRRAFALECIR	[17-47]
Activity	***Anticancer***
Sequence	Location
FKCRRWQWR-MKKLGAPSIT-CVRRAF	[17-41]
RRWQWR	[20-25]

Table 1 – cont.

1	2
Activity	***Antioxidative***
Sequence	Location
HL	[246-247], [588-589]
LH	[612-613]
Activity	***Antithrombotic***
Sequence	Location
GP	[351-352]
PG	[293-294], [493-494]
Activity	***Antiviral***
Sequence	Location
ADRDQYELL	[222-230]
EDLIWK	[264-269]
Activity	***Bacterial premease ligand***
Sequence	Location
KK	[27-28], [52-53], [99-100], [356-357], [440-441], [454-455], [673-674]
Activity	***Dipeptidyl-aminopeptidase IV inhibitor***
Sequence	Location
AP	[1-2], [31-32], [237-238], [492-493], [592-593]
FA	[41-42]
GP	[351-352]
GQ	[294-295], [366-367]
HA	[253-254], [595-596]
KA	[53-54], [221-222], [273-274], [339-340], [441-442]
LA	[247-248], [411-412], [434-435], [533-534], [589-590], [648-649]
LL	[229-230], [270-271], [298-299], [571-572], [611-612], [639-640], [680-681]
LP	[218-219]
PP	[292-293]
VA	[77-78], [95-96], [149-150], [206-207], [256-257], [436-437], [540-541], [591-592]
VP	[158-159], [250-251], [408-409], [516-517]
VV	[97-98], [255-256], [345-346], [438-439], [597-598]
Activity	***Heparin-binding***
Sequence	Location
APRKNVRWCT	[1-10]
FKCRRWQWR-MKKLGA	[17-31]

Table 1 – cont.

1	2
PSITCVRRAF	[32-41]
WQWRMKKLGA	[22-31]
Activity	***Immunomodulating***
Sequence	Location
GFL	[306-308]
RKP	[578-580]
RKSSK	[415-419]
TRKP	[577-580]
YG	[82-83], [524-525]
Activity	***Opioid***
Sequence	Location
YG	[82-83], [524-525]
YL	[135-136], [319-320], [324-325], [433-434], [660-661]
Activity	***Regulating phosphoinositole mechanism in a bovine alpha-$_s$ casein***
Sequence	Location
GFL	[306-308]
Activity	***Regulating the stomach gastric mucosa***
Sequence	Location
GP	[351-352]

Table 2. Profile of protein potential biological activity of gamma-gliadin from wheat (*Triticum aestivum*) [BIOPEP ID 1397]

Gamma-gliadin sequence:

MKTLLILTILAMAITIGTANIQVDPSGQVQWLQQQLVPQLQQPLSQQPQQTFPQPQQTFPHQPQQQVP
QPQQPQQPFLQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQ
PFPQLQQPQQPFPQPQQQLPQPQQPQQSFPQQQRPFIQPSLQQQLNPCKNILLQQCKPASLVSSLWSII
WPQSDCQVMRQQCCQQLAQIPQQLQCAAIHSVVHSIIMQQQQQQQQQQGMHIFLPLSQQQQVGQGS
LVQGQGIIQPQQPAQLEAIRSLVLQTLPSMCNVYVPPECSIMRAPFASIVAGIGGQ

Activity	***Antihypertensive***
Sequence	Location
1	2
AA	[232-233]
AG	[322-323]
AP	[315-316]
EA	[288-289]

FP	[52-53], [59-60], [84-85], [92-93], [102-103], [110-111], [120-121], [128-129], [138-139], [148-149], [165-166]
GG	[325-326]
GI	[277-278], [323-324]
GM	[254-255]
GQ	[27-28], [268-269], [275-276], [326-327]
GT	[17-18]
GS	[270-271]
IF	[257-258]
IG	[16-17], [324-325]
IP	[225-226]
IR	[290-291]
IW	[205-206]
KP	[193-194]
LA	[10-11], [222-223]
LAMA	[10-13]
LN	[181-182]
LNP	[181-183]
LQ	[32-33], [40-41], [78-79], [141-142], [177-178], [189-190], [229-230], [295-296]
LQQ	[32-34], [40-42], [141-143], [177-179], [189-191]
LW	[201-202]
LVL	[293-295]
PH	[60-61]
PL	[43-44], [260-261]
PP	[307-308]
PQ	[38-39], [48-49], [53-54], [55-56], [63-64], [68-69], [70-71], [73-74], [80-81], [85-86], [88-89], [93-94], [98-99], [103-104], [106-107], [111-112], [116-117], [121-122], [124-125], [129-130], [134-135], [139-140], [144-145], [149-150], [151-152], [156-157], [158-159], [161-162], [166-167], [207-208], [226-227], [281-282]
QG	[253-254], [269-270], [274-275], [276-277]
RA	[314-315]
RP	[170-171]
SF	[164-165]
SG	[26-27]
TQ	[95-96], [113-114], [131-132]
VG	[267-268]
VP	[37-38], [67-68], [306-307]

Table 2 – cont.

1	2
VPP	[306-308]
VY	[304-305]
YVP	[305-307]
Activity	***Activating ubiquitin-mediated proteolysis***
Sequence	Location
LA	[10-11], [222-223]
RA	[314-315]
VV	[237-238]
Activity	***Celiac toxic***
Sequence	Location
GQ	[27-28], [268-269], [275-276], [326-327]
PFPQPQQQL	[147-155]
PFPQTQQPQ	[91-99], [109-117], [127-135]
PP	[307-308]
PQPQQQLPQ	[149-157]
PQQPQQSFPQQQRPF	[158-172]
PQQQLPQPQ	[151-159]
QPQQSFPQQQ	[160-169]
QQPFPQQPQQPFPQ	[81-94], [99-112], [117-130]
QTQQPQQPF	[94-102], [112-120], [130-138]
VQGQGIIQPQQPAQL	[273-287]
Activity	***Dipeptidyl-aminopeptidase IV inhibitor***
Sequence	Location
AP	[315-316]
FA	[317-318]
FP	[52-53], [59-60], [84-85], [92-93], [102-103], [110-111], [120-121], [128-129], [138-139], [148-149], [165-166]
LA	[10-11], [222-223]
LL	[4-5], [188-189]
LP	[155-156], [259-260], [298-299]
MA	[12-13]
PA	[194-195], [284-285]
VA	[321-322]
VP	[37-38], [67-68], [306-307]
VV	[237-238]

Motifs corresponding to peptides with antihypertensive activity are the best represented group occurring in the sequences of food proteins [Dziuba and Darewicz 2007]. The majority of known peptides showing antihypertensive activity are inhibitors of the angiotensin I-converting enzyme (ACE), peptidyl dipeptide hydrolase (EC 3.4.15.1). Peptide inhibitors of ACE are present in the amino acid sequences of many food proteins, and represent their fragments upon release (Table 3). The relationship between the structure and functions of these peptides has not been fully elucidated. However, they share certain common features. Investigations into the effect of the structure of antihypertensive peptides on their inhibitory activity have shown that enzyme-inhibitor interactions are strongly dependent on the C-terminal tripeptide sequence [Meisel 2005]. Antihypertensive peptides are competitive inhibitors of peptidyl dipeptide hydrolase, and contain hydrophobic (aromatic or branch-chained) amino acids residues in at least one of the three C-terminal positions. Many peptides contain Pro as the C-terminal amino acid. Short-chain peptides, dipeptides and tripeptides having Tyr, Phe, Trp or Pro as a C-terminal residue are particularly effective. Trp exerts the strongest influence on the inhibitory activity of peptides. In peptides with longer chains, the efficiency of ACE-inhibitor interactions is also affected by their conformation.

Table 3. Structure and frequency of motifs with potential biological activity occurrence in animal and plant proteins (BIOPEP; http://www.uwm.edu.pl/biochemia)

Activity/Structure	Protein	A value
1	2	3
Antihypertensive motifs: KYPVQPFTESQSLTL; LPP; VY; LA; FP; RY; YG; LQQ; LF; PAP; LLYQQPVLGPVRGPFPIIV; PYP; AVPYPQR; VG; GINYWLAHK; AP; AA	bovine α_{s1}-casein, genetic variant A	0.134
	bovine α_{s1}-casein, genetic variant B	0.146
	bovine α_{s1}-casein, genetic variant C	0.146
	bovine α_{s1}-casein, genetic variant D	0.146
	bovine α_{s2}-casein, genetic variant A	0.086
	bovine β-casein, genetic variant A^1	0.196
	bovine β-casein, genetic variant A^2	0.230
	bovine β-casein, genetic variant A^3	0.196
	bovine β-casein, genetic variant B	0.191
	bovine β-casein, genetic variant C	0.196
	bovine β-casein, genetic variant E	0.191
	bovine β-casein, genetic variant F	0.220
	bovine κ-casein, genetic variant A	0.059
	human κ-casein	0.101
	bovine β-lactoglobulin, genetic variant A	0.092
	bovine α-lactalbumin, genetic variant A	0.032
	human lactoferrin	0.061
	human lysozyme C	0.081

Table 3 – cont.

1	2	3
	bovine elastin	0.037
	bovine retinol binding protein	0.045
	chicken connectin	0.086
	rice prolamin, clone PPROL 7	0.170
	rice 13 kDa prolamin	0.124
	rice prolamin, clone PPROL 14	0.147
	wheat α/β-gliadin, clone PW 1215	0.090
	wheat α/β-gliadin, class a-V	0.077
	wheat γ-gliadin	0.042
	barley γ-hordein	0.066
	pumpkin 11S globulin	0.083
	soybean 7S globulin	0.049
	sunflower 11S globulin	0.051
	pea legumin A	0.048
	pea legumin B	0.047
	pea 11S legumin	0.107
	faba bean legumin – B chain	0.071
	oat 12S globulin	0.042
	serendipity berry monellin – A chain	0.111
	serendipity berry monellin – B chain	0.120
Antithrombotic motifs: MAIPPK; NQDK; GPRG; GP; PG; DGEA	bovine β-casein, genetic wariant E	0.003
	bovine κ-casein, genetic variant A	0.003
	bovine hemoglobin α chain	0.007
	bovine collagen α1 chain	0.274
	bovine tropoelastin	0.067
	chicken connectin	0.043
	chicken myosin	0.043
	wheat α/β-gliadin (clone PW 8142)	0.022
	serendipity berry monellin A chain	0.003
Opioid motifs: RYLGYLE; YPSF; YPWTQRF; GYYPT	bovine α_{s1}-casein, genetic variant A	0.032
	bovine β-casein, genetic variant A^2	0.029
	bovine β-casein, genetic variant F	0.019

Table 3 – cont.

1	2	3
	bovine κ-casein, genetic variant A	0.018
	bovine α-lactalbumin, genetic variant B	0.033
	bovine β-lactoglobulin, genetic variant A	0.012
	rabbit α-lactalbumin	0.082
	bovine α-hemoglobin	0.014
	horse α-hemoglobin	0.014
	goat α-hemoglobin	0.071
	bovine troponin C	0.004
	chicken myosin, subunit 1	0.005
	wheat glutenin, HMW subunit	0.121
	wheat α/β-gliadin, clone PW 1215	0.003
	wheat α/β-gliadin, clone PW 8142	0.007
	wheat α/β-gliadin, class I	0.011
	pea legumin A2	0.002
Opioid antagonistic motifs: YPSYGLN; YIPIQYVLSR; YPYY	bovine α_{s1}-casein, genetic variant A	0.005
	bovine α_{s1}-casein, genetic variant B	0.005
	bovine α_{s1}-casein, genetic variant C	0.005
	bovine κ-casein, genetic variant A	0.011
	caprine κ-casein, genetic variant A	0.010
Immunomodulating motifs: LLY; HCQRPR; YKPR; YGG	bovine β-casein, genetic variant A^2	0.024
	bovine β-casein, genetic variant A^3	0.019
	bovine β-casein, genetic variant B	0.019
	bovine β-casein, genetic variant E	0.024
	bovine α-lactalbumin	0.025
	human α-lactalbumin	0.024
	caprine α-lactalbumin	0.024
	horse myoglobin	0.022
	chicken tropomyosin, β chain	0.011
	rice prolamin, clone PPROL 14	0.020
	rice prolamin, clone PPROL 7	0.020
	rice prolamin, clone PPROL 4A	0.020
	faba bean HMW legumin	0.015
	serendipity berry monellin chain B	0.020

Table 3 – cont.

1	2	3
Peptidase inhibitory motifs: IHPFAQTQ; IHPFAQTQ; GP; FP; LLSPWNINA; VP; LPPV	bovine α_{s1}-casein, genetic variant B	0.085
	bovine α_{s2}-casein, genetic variant A	0.048
	bovine β-casein, genetic variant A[1]	0.148
	ovine β-lactoglobulin	0.085
	bovine tropoelastin	0.146
	bovine α-1 (I) collagen	0.258
	chicken α-1 (I) collagen	0.205
	wheat α/β-gliadin, clone PTO	0.054
	wheat α/β-gliadin, clone PW8142	0.089
	wheat γ-gliadin	0.066
	barley γ-hordein	0.105
	rice prolamin, clone PPROL 17	0.040
	rice prolamin, 10 kDa fraction	0.097
	oat 12S globulin	0.060
	soybean albumin 1	0.077
	pea legumin A2	0.040
	serendipity berry monellin chain A	0.111
Coeliac-toxic motifs: QPYP; QQPY; PSQQ; QQQP; PFPPQQPYPQPQPF; QPFRPQQPYPQPQP	wheat α/β-gliadin, class A-I	0.042
	wheat α/β-gliadin, class A-II	0.027
	wheat α/β-gliadin, class A-III	0.025
	wheat α/β-gliadin, class A-IV	0.020
	wheat α/β-gliadin, class A-V	0.019
	wheat α/β-gliadin, MM1	0.029
	wheat α/β-gliadin, clone PW 1215	0.020
	wheat α/β-gliadin, clone PW 8142	0.030
	wheat α-gliadin fragment	0.040
	wheat α-gliadin	0.040
	wheat γ-gliadin, class b-1	0.020
	wheat ω-gliadin	0.040
	wheat ω-gliadin B	0.034
	wheat glutenin, type II, 3 group subunit	0.020
	oat avenin	0.038
	oat avenin N9	0.038

Table 3 – cont.

1	2	3
	barley γ3-hordein	0.031
	barley B1 hordein	0.017
	barley B3 hordein	0.015
Antibacterial and antiviral motifs: RPKHPIKHQGLPEQVLNENLLRF; LKKISQRYQKFALPQY; CKDDQNPHISCDKF; AASDISLLDAQSAPLR	bovine α_{s2}-casein, genetic variant A	0.019
	bovine α-lactalbumin	0.025
	ovine α-lactalbumin	0.032
	arabian camel α-lactalbumin	0.008
	bovine lactoferrin	0.017
	human lactoferrin	0.004
	caprine lactoferrin	0.006
	bovine hemoglobin α-chain	0.007
Antioxidative motifs: LH; HHPLL; HL	horse hemoglobin β-chain	0.034
	bovine κ-casein, genetic variant A	0.030
Chemotactic motifs: VGAPG; PGAIPG; LGTIPG	bovine tropoelastin	0.024
	bovine α-1 (I) collagen	0.001
Embryotoxic motifs: RGD; YIGSR	chicken α-1 (I) collagen	0.002
	bovine α-1 (I) collagen	0.001
	bovine α-1 (III) collagen	0.001
	white lupine PR-10 protein	0.006
	gingko 11S globulin	0.002
Ion flow regulating motifs: DY; TSLYR	vetch narbonin	0.010
	narbon bean narbonin	0.010
	serendipity berry monellin chain A	0.022
Gastric mucosa function regulating motifs: GP; PG; GPGG; PGP	chicken α-1 (I) collagen	0.210
	bovine α-1 (I) collagen	0.267
	bovine α-1 (III) collagen	0.272
Ubiquitin-mediated proteolysis activating motifs: RA; LA; WA	human lysozyme C	0.038
	chicken connectin (titin)	0.046
	sorghum kafirin PSKR2	0.041
	sorghum kafirin PSK8	0.037

Table 3 – cont.

1	2	3
Phosphoinositol action regulating motifs: GFW; GFL; LGY; GLY	human α-lactalbumin	0.008
	bovine α-lactalbumin	0.008
	caprine α-lactalbumin	0.008
	ovine α-lactalbumin	0.008
	arabian camel α-lactalbumin	0.008
Bacterial permease ligand motifs: KK; KKKA	bovine troponin C	0.052
	horse myoglobin	0.037
Anorectic motifs: PGP; APGPR	bovine β-casein, all genetic variants	0.005
	chicken α-1 (I) collagen	0.036
	bovine α-1 (I) collagen	0.042
	bovine α-1 (III) collagen	0.045
	bovine elastin	0.001
	gingko biloba, 11S globulin	0.002

Some tripeptides and peptides with longer chains have Arg or Lys as a C-terminal residue. As demonstrated by FitzGerald and Meisel [2000], the activities of the above peptides are also determined by the positive charge of guanidine or ε-amine residues of amino acids. The replacement of Arg residue at the C-terminus reduces the activity of the analogue. This suggests that the interaction between the inhibitor and the anion group situated beyond the active site of the enzyme may affect the mechanism of ACE inhibition. Some peptide inhibitors have glutamic acid at the C-terminal position.

Animal proteins provide a potentially richer source of antihypertensive peptides than plant proteins [Dziuba et al. 2009]. The average frequency of occurrence of motifs with antihypertensive activity in the protein sequences of chicken meat, β-lactoglobulin, β-casein, soybean globulins and wheat gliadins is 0.086, 0.092, 0.194, 0.051 and 0.090, respectively (Table 3). However, differences in the frequency of occurrence of potentially active sequences in plant and animal proteins are usually small. There is no simple relationship between the frequency of occurrence of bioactive fragments in a given protein and its potential activity. β-casein is the richest source of antihypertensive peptides, and the frequency of their occurrence in this protein is almost twofold higher than in rice prolamins. However, the potential antihypertensive activity of β-casein is approximately threefold lower, compared to rice prolamins. This results from the fact that fragments showing antihypertensive activity found in rice prolamins are more potent inhibitors of ACE than the corresponding fragments of β-casein.

ACE peptide inhibitors are present in many food raw materials and products, including fish [Fujita et al. 2000], sake [Saito et al. 2005], casein [Silva and Malcata 2005], whey [Abukabar et al. 1998] and cereal proteins [Fujita et al. 2000]. Maruyama and Suzuki [1982] isolated four peptides from a tryptic hydrolysate of casein. These pep-

tides, with the structure of dodecapeptide EFVAPFPEVFGK, pentapeptide FFVAP, hexapeptide TTMPLW and heptapeptide AVPYPQR, strongly inhibited ACE activity *in vitro*. Their sequences corresponded to the following fragments of α_{s1}-casein: 23-34, 23-27, 194-199, and to fragment 177-183 of β-casein. Oral administration of a tryptic hydrolysate of casein lowered blood pressure in spontaneously hypertensive rats, but had no effect on the electrocardiogram, heart histopathology and serum lipid concentrations. A similar response was observed in rats given sour milk [Xu 1998]. Clinical examinations involving hypertensive human subjects also showed that the administration of 95 ml of sour milk daily significantly reduced systolic blood pressure [Yamamoto 1997]. In a group of prehypertensive human subjects, four weeks of repeated daily intake of 3.8 g C12-peptide (containing a bovine casein hydrolysate) resulted in a substantial decrease in systolic and diastolic pressure (in comparison with the placebo group) as well as in plasma angiotensin II levels [Cadée et al. 2007]. In another study three strong ACE inhibitors with the LRP, LSP and LQP sequences were isolated from α-zein hydrolyzed with thermolysin. Six hours after the oral administration of these peptides (30 mg kg^{-1} body weight), systolic blood pressure was found to decrease up to a maximum of 15 mmHg [Kim et al. 2001, Meyer et al. 2001].

Examples of food originated peptides with anticoagulant activity are presented in Table 3. The sequences of these peptides are dominated by glycine and proline. This is natural, because both glycine and proline occur in the N-terminal, tripeptide sequence of fibrin (α-chain), which is important for the process of fibrin polymerization. The richest source of peptides with anticoagulant activity is collagen. The collagen sequence consists of a number of repeating motifs containing glycine and proline residues. The peptide profile of chicken collagen contains 303 fragments with potential anticoagulant activity, dominated by dipeptides (126 PG and 123 GP fragments) and tripeptides (52 PGP fragments). Two DGEA fragments and one KDGEA fragment are also present. The secondary structure of motifs containing anticoagulant peptides in collagen and in other proteins is unordered in > 60%, with α helices accounting for around 3%. The strongly hydrophilic character of the above motifs (mean hydrophobicity of approx. –1.2 for proteins in the BIOPEP database) indicates that they are available to the action of proteolytic enzymes and can easily release anticoagulant peptides.

Nonaka et al. [1995, 1997] examined the effect of peptides isolated from enzymatic hydrolysates of collagen and the corresponding of synthetic analogues on fibrin polymerization and platelet aggregation. The GPR peptide was isolated from a bacterial collagenase hydrolysate of porcine skin collagen by ultrafiltration, reverse osmosis and reversed-phase liquid chromatography. The above-mentioned authors demonstrated that the GPR tripeptide and its synthetic analogues corresponding to the motifs in the collagen sequence and to the peptides in thermolysin hydrolysates of collagen inhibited ADP-induced platelet aggregation. Such peptides as GPRG, GPRGP, GPRPP and GPRPPP at a concentration of 0.3 mM suppressed human platelet aggregation in more than 50%. This suppression was not directly related to thrombin inhibition [Nonaka et al. 1997]. Synthetic peptides, GPR analogues extended at their C-terminus and thermolysin hydrolysates of collagen did not inhibit thrombin. The peptide analogues of GPR extended at their N-terminus, such as AGPR and GPAGPR, as well as those with single amino acid substitution, including SarPR (Sar = sarcosine), GPK, GAR and AGPR, at a concentration from 0.1 to 0.8 mM, had no inhibitory effect on platelet aggregation.

Many peptides derived from κ-casein possess anticoagulant activity. According to the interesting hypothesis, κ-casein and fibrin γ chain have evolved from a common ancestor 450 million years ago [Jolles et al. 1978]. Indeed, there is a homology between the mechanism of chymosin-induced milk coagulation and thrombin-induced blood coagulation [Fiat et al. 1993]. At the first stage of enzymatic coagulation of milk, chymosin specifically hydrolyzes κ-casein. As a result of hydrolysis of the Phe^{105}-Met^{106} bond, the N-terminal fragment, para κ-casein, combines with other casein fractions to form an insoluble curd, and casein macropeptide (CMP), a C-terminal soluble fragment of κ-casein, is released to whey. Its tryptic hydrolysate contains anticoagulant peptides [Fosset and Tome 2000]. The main isolated anticoagulant peptide corresponds to a motif of κ-casein (fr. 106-116) with the MAIPPKKNQDK sequence. This peptide and the fragments with the sequences KNQDK (112-116) and NQDK (113-116) are known as casoplatelins. They are both structurally and functionally similar to the C-terminal dodecapeptide of the γ chain of human fibrin with the HHLGGAKQAGDV sequence. The amino acid residues, Ile^{108}, Lys^{112} and Asp^{115}, in a κ-casein occupy homologous positions, compared to the γ chain of human fibrin. The above amino acid residues present in anticoagulant peptides derived from κ-casein are responsible for competitive inhibition of the binding of fibrin γ chains with receptor sites on the surface of blood platelets [Schlimme and Meisel 1995]. There is also a homology between the fibrin α-chain tetrapeptide RGDX and the KRDS sequence corresponding to lactoferrin fragment 39-42. The KRDS peptide displays anticoagulant activity [Rutherford and Gill 2000].

The precursor proteins of opioid peptides, similarly as opioid receptors, are synthesized primarily in the central and peripheral nervous system as well as in the immune system and in the endocrine system. A characteristic feature of the structure of typical opioid peptides is an identical N-terminal amino acid sequence – YGGF, responsible for interactions with opioid receptors [Chaturvedi et al. 2000].

The endogenous opioidergic system is supplemented with opioid peptides originating from food proteins. Opioid peptides whose precursors are food proteins show affinity for opioid receptors and a similar type of activity as endogenous opiates. They are formed in the digestive tract as a result of the digestion of precursor proteins, and exert a direct effect on specific receptors in the alimentary tract. Moreover, following their absorption into the bloodstream, they may interact with endogenous opioid receptors. The opioid peptides found in the hydrolysates of food proteins or produced during digestion have been defined as exorphins, due to their exogenous origin and morphine-like activity. The structure of exorphins resembles that of atypical endogenous opioid peptides, with the N-terminal tyrosine residue, important for the ligand-receptor interactions. In some cases a tyrosine residue at the N-terminus is replaced with some other amino acid residue (e.g. R, G, V, L).

The main sources of opioid peptides derived from food proteins are gluten (or gliadin), α_s-casein, β-casein and hemoglobin (Table 3). Opioid peptides present in foods are resistant to further hydrolysis by small intestinal enzymes, and exert a direct influence on specific receptors in the digestive tract. When absorbed into the circulatory system, these peptides supplement the endogenous opioidergic system. Peptides with opioid activity were identified and described for the first time in β-casein hydrolysates. The ones that have been studied most intensely are β-casomorphins (1-11), i.e. fragments of the β-casein sequence 60-70 (YPFPGPIPNSL). Fragments of this motif also

show the opioid activity [Brantl et al. 1981]. β-casomorphins are biologically important active compounds present in the diet. They are resistant to the action of digestive enzymes and may affect the physiological functions of the small intestine [Hayes et al. 2007]. Apart from their opioid activity, β-casomorphins play a vital role in the gastrointestinal tract. Due to the enhancement of water and electrolyte absorption in the small intestine, β-casomorphins prolong gastrointestinal transit time, which is a component of their antidiarrheal action. They can also affect nutrient absorption and insulin secretion. Moreover, they show natural affinity for the μ-receptor and, as demonstrated in experiments on rats, exert a strong analgesic effect, induce apnea and irregular breathing, and affect sleep patterns [Meisel and FitzGerald 2000].

Casomorphins and lactorphins are present in many fermented dairy products obtained with the use of lactic acid bacteria. This results from the specific activity of proteolytic enzymes of these microorganisms. In dairy products manufactured using *Lc. lactis* strains, β-casein is hydrolyzed by extracellular PI-type proteinase into many different oligopeptides, including β-casomorphin (1-9) [Hayes et al. 2007]. Particularly high concentrations of β-casomorphin (1-11) have been reported in fermented products containing milk contaminated with proteolytic bacteria such as *Pseudomonas aeruginosa* and *Bacillus cereus* [Hamel et al. 1985]. Opioid peptides, derivatives of α_s- and β-casein, are released in fermented UHT milk by the action of proteolytic enzymes of *Lb. Rhamnosus* GG. A variety of peptides are formed during the ripening of Edam and Australian Cheddar cheeses, including β-casomorphins [Dionysius et al. 2000, Sabikhi and Matur 2001]. In fermented whey produced with the use of yeasts, *Kluyveromyces marxianus* var. *marxianus*, lactorphins are formed as a result of enzymatic hydrolysis of whey proteins [Belem et al. 1999].

Some peptides derived from α_{s1}-casein and κ-casein show antagonist activity against enkephalins and casomorphins. These are casoxins A, B, C and D which structure corresponds to the fragments of κ-casein (fr. 35-41, 58-61, 25-34) and α_{s1}-casein. Casoxin D has been isolated by Clare and Swaisgood [2000] from α_{s1}-casein hydrolysate. The metoxyl derivatives of casoxins A, B and C show substantially higher activity than unmodified casoxins.

Wheat gluten is the richest source of opioid peptides. Zioudrou et al. [1979] were the first to describe the opioid activity of gluten peptides. The peptides isolated from peptic and thermolysin hydrolysates have been defined as gluten exorphins A_4, A_5, B_4 and B_5. Opioid peptides may be also formed as a result of gluten digestion with pepsin, trypsin and chymotrypsin [Kitts and Weiler 2003].

Cases of food peptides strengthening or weakening the gastrointestinal immune system have been well documented. A rich source of such peptides are rice prolamines, casein (primarily β-casein) and α-lactalbumin present in mammalian milk. The frequency of occurrence of immunomodulatory motifs in the sequences of the above precursor proteins exceeds 0.02 (Table 3). The polypeptide chains of those proteins contain 3, 5 and 3 potentially active fragments, respectively. Motifs with potential immunomodulatory activity can be found in the hydrophilic fragments of protein molecules, as proved by their negative hydrophobicity (mean hydrophobicity of immunomodulatory motifs gathered in the BIOPEP database is around –0.4). The structure of these motifs is characterized by the presence of α-helix (approximately 20%), β-turn (27%), β-structure (25%) and unordered structure (28%). Peptides isolated from tryptic hydrolysates of rice and soybean proteins activate superoxide anions which stimulate the non-specific im-

mune response [Kitts and Weiler 2003]. The peptide isolated from a hydrolysate of the albumin fraction of rice proteins, oryzatensin (GYPMYPLPR), is multifunctional. It affects cells of the immune system and smooth muscles via a single receptor. Oryzatensin and oryzatensin-related peptides contain arginine residues at the C-terminus, leucine residues at position 3 and hydrophobic residues (leucine or tyrosine) at position 5 from the C-end. Another immunomodulatory peptide is HCQRPR, derived from a tryptic hydrolysate of soybean protein, which stimulates phagocytosis and TNF production.

Sulfated glycopeptides, formed as a result of hydrolysis of egg albumin with trypsin, activate macrophages from male mice *in vitro*. They also enhance the proliferation of macrophages, the production of interleukin-1 IL-1 and superoxide anions in leukocytes. The glycoside residues present in peptides include N-acetylgalactosamine, galactose and N-acetylneuraminic acid. Glycoside and sulfonic residues play a key role in interactions with macrophage components (Tanizaki et al. 1997).

Immunopeptides originating from α_{s1}-casein, β-casein, κ-casein and α-lactalbumin may both suppress and enhance the immune response. Jolles et al. [1981] were the first to demonstrate that tryptic hydrolysates of human β-casein display immunostimulating activity. For instance, the hexapeptide VEPIPY, corresponding to fragment 54-59 of human β-casein, induces the activity of macrophages in mice and humans, and enhances the natural resistance of young mice to infections caused by *Klebsiella pneumoniae*. Peptides formed as a result of hydrolysis of κ-casein with trypsin inhibit the immune response of mouse spleen lymphocytes and rabbit Peyer's cells [Kitts and Weiler 2003]. *Lb. helveticus*-fermented milk products show an immunostimulating effect on lymphocyte proliferation *in vitro* and the ability to stimulate the phagocytic activity of lung macrophages [Laffineur et al. 1996]. These products contain numerous oligopeptides which are formed as a result of the hydrolysis of milk proteins, mainly β-casein, by the extracellular proteolytic enzymes of *Lb. helveticus*. The above strain is characterized by high proteolytic activity. The results of experiments on mice have revealed the immunostimulating and anticarcinogenic properties of peptide fractions isolated from fermented milk in the presence of the strain *Lb. helvetius* R389. Many fermented dairy products, including yogurt, enhance the immune response when milk is inoculated with *Lb. casei* and *Lb. acidophilus.* In addition, yogurt filtrate free from microbes stimulates interferon-γ (IFN-γ) production and the activity of human natural killer (NK) cells [Hayes et al. 2007].

Interesting results concerning the properties of casein-derived phosphopeptides which are known to bind calcium have been reported. These phosphopeptides enhance IgG secretion, acting *in vitro* on human lymphocytes. The immunostimulating activity of casein phosphopeptides has been ascribed to the presence of phosphoserine residues in their sequence, and phosphorylation sites have been found to be allergenic epitopes of casein [Meisel 2005].

Some physiological properties of peptides (antihypertensive, immunostimulating, anticoagulant peptides and peptides inhibiting HIV-1 proteinase) result from their ability to block proteolytic enzymes. The processes of protein hydrolysis and the inhibition of those processes are of key physiological significance for digestion, blood clotting and fibrinolysis, blood pressure regulation, hormonal neuromodulation and phagocytosis, but they also play an important role in pathological states (such as pulmonary emphysema, carcinoma and hypertension) and in various infections (HIV infections, parasitic

invasions). A major group of food protein-derived peptides are prolyl oligopeptidase (POP) inhibitors (EC 3.4.21.26), including a peptide with the HLPPPV sequence, the product of zein hydrolysis with subtilisin [Maruyama and Suzuki 1982] and a peptide with the LLSPWNINA sequence, isolated from the by-products of sake production [Saito et al. 1997]. Typical structural motifs in most POP inhibitors and other proline proteases are proline residues.

The richest potential source of peptides-inhibitors of proline proteases are α-chains of β-casein and collagen (Table 3). The frequency of occurrence of motifs with POP inhibitory activity in the sequences of the discussed precursor proteins ranges from 0.15 to 0.25. Motifs with potential activity of proline protease inhibitors occur in the hydrophobic fragments of protein molecules (their mean hydrophobicity in proteins listed in the BIOPEP database is approximately 0.18). The structure of these motifs is characterized by the presence of β-turn (around 23%), β-structure (53%) and unordered structure (23%), and by the complete absence of α-helix.

N-acetylpepstatin is a potent inhibitor of aspartyl proteases. To a limited extent, this peptide inhibits also HIV-1 proteinase, thus blocking the process of HIV-1 replication in the infected cell [Rival et al. 2001]. Peptides isolated from cheese and a tryptic hydrolysate of zein [Gobetti et al. 2002] inhibit the activity of thermostable proteinases and endopeptidases synthesized by psychrotrophic bacteria, *Ps. fluorescens.* This prevents the development of a bitter taste and gel formation in UHT milk during storage, and extends the shelf-life of dairy products. Control over the activity of proteolytic enzymes, including those originating from psychrotrophic bacteria, is important for improving the maintenance of foods quality.

Wheat, rye and barley grain proteins contain fragments of amino acid sequences which initiate pathophysiological processes and, in a consequence, cause intestinal epithelial damage. Protein maldigestion may lead to coma, diarrhea (the most common symptom), anemia and osteoporosis [Dewar et al. 2004]. Celiac disease is a multigenic disorder, but the mode of its inheritance remains unknown. Studies have shown that the toxicity of cereal proteins to celiac patients is related to peptides released during the digestion of these proteins. Wheat gliadin peptides have been most extensively investigated to date. According to Cornell [1996], motifs with the following sequences: QQQP, QQPY, PSQQ, QPYP are responsible for celiac disease. These motifs are also present in many non-toxic proteins, which suggests that celiac disease may be caused by extended amino acid motifs found in wheat A-gliadins and containing the above tetrapeptides [McLachlan et al. 2002]. Such assumption could provide a basis for an analysis of the potential toxicity of cereal proteins to celiac patients, involving the determination of similarities between the sequences of these proteins. The amino acid sequences of proteins of selected cereal and legume species, listed in the BIOPEP database, contain tetrapeptides responsible for celiac disease (sequences: QQQP, QQPY, PSQQ, QPYP). The most responsible for celiac disease are tetrapeptides,. Depending on the length of the protein sequences, they can appear in them in the range from 6 to 11 (Table 3). Celiac-toxic tetrapeptides can be found in the sequences of wheat α-gliadin (*Triticum aestivum*). These tetrapeptides are present primarily in the N-terminal domain of the α-gliadin chain. The number of extended amino acid motifs in this fragment ranges from 0 to 7, and it is lower than the number of tetrapeptides because some extended motifs contain two tetrapeptide sequences. For instance, the QPFPPQQPYPQPQP motif (α-gliadin, fr. 36-49) contains the following fragments: QQPY (residues 41-44) and

QPYP (residues 42-45). Extended motifs responsible for celiac disease are absent from or sporadically present in the polypeptide chains of the remaining gliadin fractions.

The sequences of many food proteins contain motifs with low occurrence frequency, corresponding to bioactive peptides that are not discussed in detail in this paper (Table 3).

CONCLUSIONS

The value of physiologically and biologically active motifs present in proteins is considered critically taking into attention many aspects including the mechanisms of action of the peptide in a body, the processes of protein hydrolysis and the inhibition of them during enzymatic digestion, the absorption of peptides into a bloodstream, the length and a structure of the peptide. Although the enormous growth on studies concerning the structure and the activity of the motives had been observed, some mechanisms of bioactivities of peptides are still not fully discovered. However, the information concerning the function and structure of bioactive proteins and peptides gathered in the various database resources interconnected with the mathematical descriptions of the biological phenomena is a powerful tool to assign the protein to a group of more potent biomacromolecules. BIOPEP is one of the tools which contributes to such approach.

The strategy of analysis of proteins as the precursors of bioactive peptides involving BIOPEP procedure enables to classify proteins to be a rich/poor source of motifs with biological and physiological functions. BIOPEP and other global datasets resources provide the useful techniques to obtain such classification in a simple and quick way. Results obtained with the use of *in silico* methodology allow for preliminary selection of the proteins which may be the most powerful precursor of bioactive motifs and then included in a further studies on potential application of such protein as the food ingredients supporting the proper body functioning. This approach can be successfully applied to introduce nutraceuticals as the valuable source of peptides with biological activities.

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MOTYWY O POTENCJALNEJ FIZJOLOGICZNEJ AKTYWNOŚCI W BIAŁKACH ŻYWNOŚCI – BAZA DANYCH BIOPEP

Streszczenie. Białka to wielofunkcyjne składniki żywności, które oddziałują na żywe organizmy. Jedną z funkcji białek jest ich wpływ na organizm wynikający z obecności motywów, które wykazują specyficzne funkcje fizjologiczne i biologiczne. Ze względu na rosnące zapotrzebowanie na produkty żywnościowe zawierające składniki bioaktywne, dużą uwagę zwraca się na zastosowanie bioaktywnych peptydów jako fizjologicznie aktywnych składników żywności, odgrywających ważną rolę w zapobieganiu i leczeniu różnych chorób wynikających ze stylu życia. Według aktualnego stanu wiedzy, poza swą podstawową funkcją, białka mogą być rezerwą peptydów kontrolujących procesy zachodzące w organizmach żywych. Dlatego zastosowanie nowego, dodatkowego kryterium oceny białek jako prekursorów bioaktywnych peptydów przyczynia się do kompleksowego oraz obiektywnego definiowania wartości biologicznej białka. Komplementarną częścią takich badań jest strategia oceny białek – prekursorów bioaktywnych peptydów oparta na wykorzystaniu bazy danych sekwencji białek i bioaktywnych peptydów BIOPEP (dostępnej pod adresem: http://www.uwm.edu.pl/biochemia). Baza zawiera informacje na temat 2123 peptydów reprezentujących 48 rodzajów aktywności biologicznych, wartości EC_{50} oraz źródła pochodzenia. Białka (706 sekwencji) są rozpatrywane jako prekursory bioaktywnych peptydów z wykorzystaniem nowo wprowadzonych kryteriów, tj.: profilu potencjalnej aktywności biologicznej, częstości występowania bioaktywnych fragmentów i potencjalnej aktywności biologicznej białka. To oryginalne i niespotykane dotychczas podejście, zaczyna być coraz częściej z powodzeniem stosowane przez innych autorów. BIOPEP może współdziałać z innymi światowymi bazami danych, np.: TrEMBL, SWISS-PROT, EROP i PepBank. Ponadto baza danych BIOPEP została powiększona o dane dotyczące białek alergennych wraz z informacjami na temat struktury ich epitopów oraz markerów molekularnych.

Słowa kluczowe: peptydy bioaktywne, białka, baza danych BIOPEP

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