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RIGIN, ANOTHER PHAGOCYTOSIS-STIMULATING TETRAPEPTIDE ISOLATED FROM HUMAN IgG

Confirmations of a Hypothesis

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Structure-function and conformational studies of the molecule of phagocytosis-stimulating tetrapeptide tuftsin permitted the conclusion that among products resulting from splitting of H-chain of IgG Human EU by trypsin, besides tuftsin (sequence 289–292), tuftsin-like tetrapeptide Gly-Gln-Pro-Arg may be also present; theoretical conformational analysis shows a considerable similarity of spatial arrangement of this tetrapeptide and tuftsin which testifies in favour of potential tuftsin-like activity of the tetrapeptide. Gly-Gln-Pro-Arg was synthesized and its phagocytosis stimulating activity was found equal to that of tuftsin.

Key words: energy calculation; structure-activity studies; tuftsin-like peptides.

Splitting leukokinin (fraction PC-IV of γ-globulins obtained by separation of blood plasma proteins on phosphocellulose) with trypsin, Najjar and his co-workers isolated four active fragments able to stimulate phagocytosis – tuftsin I, II, III and IV. Only the amino acid sequence of tuftsin IV has been determined, Thr-Lys-Pro-Arg (Najjar, 1973), referred to here as tuftsin. Later tuftsin was shown to be a fragment of immunoglobulin H-chain of class G (Nishioka *et al.*, 1973; Spirer *et al.*, 1977) (for instance, sequence 289–292 in H-chain of Ig G Human EU; see Edelman, 1970).

It is probable that other leukokinin fragments (tuftsin I, II and III) either comprise a tuftsin sequence which is split off immediately upon placement on the leukocyte membrane or are tuftsin-like tetrapeptides with similar structural and conformational properties. The tuftsin sequence comprises . . .-Pro-Arg fragment whose structure coincides with the nucleus of the so-called "common" fragments (. . . Pro/Val ⟷ Arg/Lys . . . , ⟷ denotes both possible directions of peptide chain acylation), which were determined on the basis of the principles of signatures and equivocation in Chipens *et al.* (1979*a*) (Table 1).

Structure-function studies of tuftsin analogs (Fridkin *et al.*, 1977; Konopinska *et al.*, 1976; Najjar, 1973; Stabinsky *et al.*, 1978) show that the character of an amino acid in position 1 is not of great importance in providing specific binding of tuftsin molecule with cell receptors and antibodies. At the same time analogs with lengthened or shortened amino acid sequence as a rule do not display immunostimulator properties. A total theoretical conformational analysis of the tuftsin molecule in Nikiforovich (1978) showed that stable conformations are characterized by the proximity of the ε-amino

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TABLE 1

Comparison of primary structures of some "common" fragments of peptide-protein substances which are presumed to comprise equifunctional amino acid residues

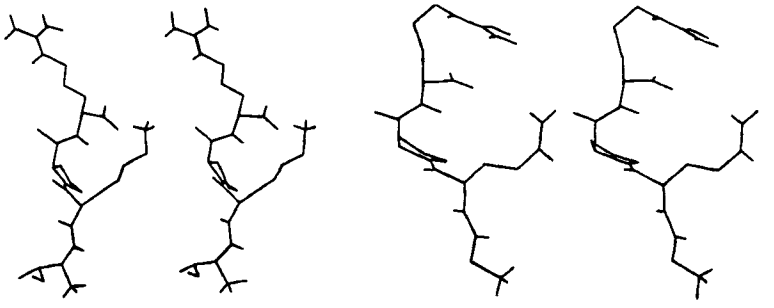
Angiotensin				Val^{3}	Arg	Asp^{1}
Fibrinopeptide B	Gly^{17}	Val	Lys	Pro	Arg	Asp ^{12}
Toxin (*D. polylepsis*)	Gly^{55}	Pro	Lys	Val	Lys^{59}	
IgG Human EU		Thr^{289}	Lys	Pro	Arg	Glu ^{293}
Toxin (*D. jamesoni*)		Pro^{47}	Lys	Val	Lys^{50}	
IgG Human EU		Gly^{341}	Gln	Pro	Arg	Glu ^{345}
BPP[a] (Agkistrodon halys blomhoffii)	Pro^{4}	Pro	Arg	Pro	Lys^{8}	
IgG Human EU	Pro^{244}	Pro	Lys	Pro	Lys^{1}	Asp ^{249}

[a]BPP, bradykinin-potentiating peptide.

group of Lys^{2} residue and *C*-terminal carboxyl (Fig. 1a). Such a structure of the molecule is confirmed by the presence of high biological activity of cyclotuftsin Thr-Lys-Pro-Arg as well as by similarity of CD-spectra of tuftsin and its cyclic analog in aqueous solution (Chipens *et al.*, 1979*b*). In other words, it can be presumed that during stimulation of phagocytosis the side chain of the residue in position 2 is important, mainly as a factor of additional stabilization of characteristic bend of the peptide backbone in the region of Pro^{3}, and not as a factor of direct receptor activation. The same peptide chain bend is present even in the absence of side chains in positions 1, 2 and 4: energy calculation of the space structure of tetrapeptide Ala-Ala-Pro-Ala showed that its most stable conformation, BBRB, coincides with the most stable conformation of tuftsin molecule backbone (Nikiforovich, 1978).

This does not necessarily mean that the presence of Pro residue in position 3 is crucial for the manifestation of phagocytosis-stimulating activity (cf. Konopinska *et al.*, 1976), although this particular factor is largely responsible for the realization of the "correct" tetrapeptide conformation during binding to the receptor.

Considering the above an attempt can be made to formulate requirements to tetrapeptide fragments obtained as a result of trypsin proteolysis of leukokinin and, possibly, possessing tuftsin-like activity: 1) they must be located on the external surfaces of immunoglobulin globules to be accessible to trypsin action; 2) there must be a "common sequence" of the tetrapeptides in question: X – Y – Pro-Arg/Lys where X – any residue except proline, Y – except proline and glycine; 3) the fragments must be preceded either by arginine or lysine



a b

FIGURE 1

Stereo-picture of the structure with BBRB type conformation of peptide backbone of a, tuftsin molecule (Nikiforovich, 1978); b, Gly-Gln-Pro-Arg tetrapeptide molecule (structure 3 from Table 3).

TABLE 2

Analysis of amino acid sequence of H-chain of immunoglobulin IgG Human EU

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          5         10        15        20        25        30
Z V Q L V Q S G A E V K K P G S S V K↓V S C K A S G G T F S
         35        40        45        50        55        60
R S A I I W V R Q A P Q Q G L E W M G G I V P M F G P P N Y
         65        70        75        80        85        90
A Q K F Q G R V T I T A D E S T N T A Y M E L S S L R S E D
         95       100       105       110       115       120
T A F Y F C A G G Y G I Y S P E E Y N G G L V T V S S A S T
        125       130       135       140       145       150
K↓G P S V F P L A P S S K↓S T S G G T A A L G C L V K D Y F
        155       160       165       170       175       180
P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y
        185       190       195       200       205       210
S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K↓
        215       220       225       230       235       240
V D K↓R↓V E P K S C D K↓T H T C P P C P A P E L L G G P S V
        245       250       255       260       265       270
F L F P P K P K D T L M I S R↓T P E V T C V V V D V S H E D
        275       280       285       290       295       300
P Q V K↓F N W Y V D G V Q V H N A K↓[T K P R]↓E Q Q Y B S T Y
        305       310       315       320       325       330
R V V S V L T V L H Q N W L D G K↓E Y K C K↓V S N K↓A L P A
        335       340       345       350       355       360
P I E K↓T I S K A K↓[G Q P R]↓E P Q V Y T L P P S R↓E E M T K↓
        365       370       375       380       385       390
N Q V S L T C L V K G F Y P S D I A V E W E S N D G E P E N
        395       400       405       410       415       420
Y K↓T T P P V L D S D G S F F L Y S K L T V D K↓S R W Q E G
        425       430       435       440       445
N V F S C S V M H E A L H N H Y T Q K↓S L S L S P G
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In amino acid sequence (one-letter designations of amino acids) are underlined sites which are located on external surfaces of domains of IgG Human EU H-chain globule and sites connecting separate domains. The arrows show the location of trypsin action. Tetrapeptides enclosed in a frame meet the requirements specified in the text.

TABLE 3

Description of the peptide backbone stable conformation types of Gly-Gln-Pro-Arg molecule

No.	Backbone structure	Gly		Gln					Pro			Arg				U-U_{min} kcal/mol
		φ	ψ	φ	ψ	χ_1	χ_2	χ_3	ψ	φ	ψ	χ_1	χ_2	χ_3	χ_4	
1	RBRB*	−106	−155	−126	121	−159	176	−95	−45	−150	131	−158	−108	72	−108	0.00
2	BBBB*	−109	117	−124	131	−70	90	−93	138	−140	134	−158	−109	73	−106	0.26
3	BBRB*	−128	137	−133	133	−85	177	−100	−37	−144	135	−158	−109	72	−105	1.86
4	BLRB*	−108	163	57	114	−158	180	−93	−43	−148	131	−158	−108	72	−108	4.28
5	BBBL*	−112	120	−125	132	−70	88	−96	124	63	142	−163	−112	72	−100	6.08
6	BBRL	−111	119	−124	131	−71	89	−94	−38	63	143	−165	−112	73	−100	6.42
7	RLBB	−89	−149	50	97	−67	88	−88	136	−141	133	−158	−108	73	−107	7.62
8	BLBB	−104	160	53	105	−157	−64	106	117	−146	132	−156	−108	73	−107	7.89

Dihedral angle values according to IUPAC-IUB Commission, 1970; designation of peptide backbone conformations corresponds to main local minima of potential maps of *N*-acetylaminoacid methylamides, located in the following areas: B $-\varphi = 0°/-180°$, $\psi = 0°/180°$; R $-\varphi = 0°/-180°$, $\psi = 0°/-180°$; L $-\varphi = 0°/180°$, $\psi = 0°/180°$

*Backbone conformation types common with those of tuftsin molecule.

(consequence of trypsin substrate specificity shown in Mosolov, 1971).

Table 2 presents the results of an analysis of amino acid sequence of IgG Human EU H-chain (X-ray data on the tertiary structure of the globule of IgG Human EU H-chain are presented in Deisenhofer *et al.*, 1976). It is obvious that besides tuftsin only tetrapeptide Gly^{341}-Gln^{342}-Pro^{343}-Arg^{344} meets the above requirements; the same sequence is made up by H-chains of other human immunoglobulins (see Dayhoff, 1974).

However, similarity of amino acid sequences is not necessarily accompanied by similarity of conformations: meanwhile, it was assumed that a set of stable conformations of "tuftsin-like" fragment 341–344 must comprise space structure characteristic of tuftsin molecule.

Total theoretical conformational analysis of Gly-Gln-Pro-Arg molecule confirmed the above assumption (basic principles of energy calculation methods and the system of potential functions of atom-atomic interactions had been described in Nikiforovich, 1978). Table 3 lists eight types of the most stable ($\Delta U < 8$ kcal/mol) structures of the tetrapeptide backbone with the optimal (from the energetic point of view) spatial arrangement of side chains of Gln and Arg residues; among them all four types of structures of tuftsin peptide backbone meet the same energy criterion. In particular, it is obvious that the spatial organization of the most stable tuftsin structure bears a close resemblance to the most stable tetrapeptide structure (Fig. 1a and b). Greater compactness of tuftsin structure is due to electrostatic interaction of oppositely charged lysine ϵ-amino group and *C*-terminal carboxyl, which is stronger than non-bonded interactions of the side chains of Gln residue and peptide backbone in the region of Arg residue in the case of the tetrapeptide in question.

Thus, the results of the theoretical conformational analysis also suggest that the tetrapeptide Gly-Gln-Pro-Arg, found in amino acid sequence of IgG Human H-chain as a result of structure-function studies involving the principles of signatures and equivocation, will possess tuftsin-like activity.

This tetrapeptide (named rigin) was synthesized by us using the classical methods of peptide chemistry and obtained as a homogeneous substance with correct amino acid and element analysis data, $[\alpha]_D^{22} -77.0°$ (*c* 0.6 in 5% CH_3COOH). CD-spectrum of rigin in water solution is similar to that of tuftsin. The results of biological experiments demonstrated that rigin exerts the same phagocytosis-stimulating activity toward blood leukocytes of the rat and *Staphylococcus aureus in vitro* as tuftsin: phagocytosis index 33.3% (for tuftsin – 32.8%), phagocytic stimulation 63% (for tuftsin, 104%) at concentration 10^{-7} M.

A detailed description of its synthesis and biological properties will be published later. The obtained results provide confirmation of the presence of an immunostimulatory tetrapeptide other than tuftsin in the products of trypsin hydrolysis of IgG Human.

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