Acta Biochimica Polonica

Vol. 51 No. 1/2004

57–66

QUARTERLY

On the peptide–antipeptide interactions in interleukin-1 receptor system*

Alicja Kluczyk[1]✉, Marek Cebrat[1], Renata Zbozień-Pacamaj[1], Marek Lisowski[1], Piotr Stefanowicz[1], Zbigniew Wieczorek[2] and Ignacy Z. Siemion[1]

[1]*Faculty of Chemistry, University of Wrocław, Wrocław, Poland;* [2]*Institute of Immunology and Experimental Therapy, Wrocław, Poland*

Received: 31 October, 2003; revised: 26 February, 2004; accepted: 08 March, 2004

Key words: interleukin-1, interleukin-1 receptor antagonist, complementary peptides, immunomodulation, vaccinia virus C10L protein

Interleukin-1 receptor antagonist (IL-1Ra) and vaccinia virus protein C10L share a VTXFYF motif, with X being Lys or Arg residue, respectively. Peptides of such sequence compete successfully with IL-1 for the cellular receptor. A pair of complementary peptides, based on the Siemion's hypothesis on the periodicity of the genetic code (QWLNIN and QWANIN), and another pair, in which, following the Root-Bernstein theory, Lys was used as complementary amino acid to Phe (QWLKIK and QWAKIK), were investigated for the peptide–antipeptide interactions using mass spectrometry (ESI-MS) and circular dichroism (CD) methods. The CD measurements indicated some conformational changes, more pronounced in the Siemion's pairs, however, no heterodimer formation was found by MS. In the region of IL-1 receptor situated close to the position of IL-1Ra in the IL-1Ra–receptor complex, a KQKL motif is present, suggesting a possibility of complementary recognition of the Root-Bernstein type in the IL-1 receptor. The biological activity of the complementary peptides is similar to that of the original ones. They efficiently compete with IL-1 and show moderate immunosuppressory activity in humoral and cellular immune response. The inhibition of the IL-1–IL-1 receptor interaction may result from the complementary peptides acting as mini-receptors with affinity for IL-1.

Interleukin-1 (IL-1) is one of the main factors of the immune response, involved in the processes of inflammation, infection and tissue damage. The importance of this cytokine

*Presented at the 17th Polish Peptide Symposium, August 31st–September 4th, 2003, Łódź, Poland.

✉The correspondence should be addressed to: A. Kluczyk, Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland; phone (48 71) 375 7218; fax (48 71) 322 2348; e-mail kluczyk@wchuwr.chem.uni.wroc.pl

Abbreviations: AcP, accessory protein; DTH, delayed type hypersensitivity; IL-1Ra, interleukin 1receptor antagonist; PFC, plaque-forming cells.

is clearly visible from the well developed regulation system, consisting of two active forms, IL-1α and IL-1β, and a unique receptor antagonist, IL-1Ra, which closely resembles the active forms as far as structure and binding properties are considered, but is devoid of biological activity. There are two types of IL-1 receptors, one of them serving only as a decoy target for IL-1, and a special IL-1 receptor accessory protein (IL-1R AcP) (Dinarello, 1996; Korherr *et al.*, 1997; Arendt *et al.*, 1998). Recently, several new members of IL-1 family have been identified on the basis of sequence homology (Sims, 2002). The effects of overproduction of IL-1 are implicated i.e. in rheumatoid arthritis, septic shock and neuronal injury (Dinarello, 1996; Rothwell, 2003). Recombinant IL-1Ra, Anakinra (Amgen), showed promising results in clinical trials for rheumatoid arthritis (Small *et al.*, 2001). It is also worth noting that IL-1 system is a target of viral mechanisms of immune evasion (Alcami & Koszinowski, 2000).

In our search for inhibitors of IL-1 action we found that the 143–148 fragment of IL-1Ra, peptide VTKFYF, competes successfully with IL-1α for the cellular receptor (Wieczorek *et al.*, 1997; Siemion *et al.*, 1998). The Lys^{145} residue and its counterpart from the agonist forms of IL-1, an Asp residue, form a molecular switch between the active and inactive IL-1 forms, without affecting the receptor affinity (Ju *et al.*, 1991). The structure of IL-1–IL-1R complex reveals that the selected fragments of IL-1 interact with the third domain of IL-1 receptor and probably also with IL-1R AcP (Greenfeder *et al.*, 1995; Schreuder *et al.*, 1997; Vigers *et al.*, 1997). We discovered that a practically identical sequence (VTRFYF, fragment 322–327) is located in a vaccinia virus protein related to the C10L vaccinia gene (Goebel *et al.*, 1990). Sequence analysis and molecular modelling studies showed that the C-terminal domain of C10L protein resembles IL-1Ra and therefore may be involved in IL-1 regulation. The peptide VTRFYF turned out to be an even more potent inhibitor of IL-1 activity than the original sequence VTKFYF from IL-1Ra (Kluczyk *et al.*, 2002).

Usually, the main focus of attention in search for IL-1 inhibitors is directed at the "Boraschi loop", the 47–55 fragment of IL-1β, the main point of structural differences between the agonist – IL-1β and antagonist – IL-1Ra (Antoni *et al.*, 1986; Boraschi *et al.*, 1990). One of the approaches used the antisense peptide strategy to generate IL-1 inhibitors acting as mini-receptors for IL-1 (Davids *et al.*, 1997; Heal *et al.*, 2000).

The interest in antisense peptides (for a review see Root-Bernstein & Holsworth, 1998) is constantly growing, with significant controversies concerning the design of the antisequence to the investigated peptide. The method proposed by Blalock, based on Mekler hypothesis (Mekler, 1969), recognises the antipeptide as a result of reading the complementary DNA strand in the classical 5'→3' direction (Blalock & Smith, 1984). The parallel (3'→5' direction) reading of complementary DNA strand was suggested by Root-Bernstein (1982). Another approach, developed from the periodicity of the genetic code, was presented by Siemion (for a review see Siemion, 1995). This method uses the 3'→5' direction reading, however, in the equivalent codons the first two bases of a codon are complementary, whereas the third is exactly the same. The tables of complementary amino acids according to various theories can be found in Siemion (2001).

The above procedures are compared by using the Phe codons as an example:

DNA sense strand	5' TTT TTC 3'
DNA antisense strand	3' AAA AAG 5'
Peptide sequence	Phe Phe
Antipeptides:	
Blalock	(5' GAA AAA 3') Glu Lys
Root-Bernstein	(3' AAA AAG 5') Lys Lys
Siemion	(3' AAT AAC 5') Asn Asn

There are several reports on the results of using the antisense procedures by Blalock and Root-Bernstein in the same experimental system, both in analytical studies (Madhusudanan *et al.*, 2000) and biological experiments (Holsworth *et al.*, 1994; Davids *et al.*, 1997), but no agreement on their superiority has been established. The procedures by Root-Bernstein and Siemion were evaluated by us using fragments of transforming growth factor β_2 (TGFβ_2) (Siemion *et al.*, 2001).

In this work, we applied the complementarity (antisense) strategy to fragments of IL-1Ra (VTKFYF) and C10L protein (VTRFYF), following two directions: design and synthesis of complementary peptides and search for complementary motifs in IL-1 receptor.

We synthesised two sets of complementary peptides, one designed according to Siemion's hypothesis, the other with the original Phe residues replaced not by Asn, but by Lys residue. The Phe residues in the peptides I and II have been found to be important for activity of previously investigated peptides. The theories by Root-Bernstein and Siemion differ regarding the amino acid complementary to phenylalanine, therefore both Asn and Lys residues were used in respective complementary peptides. It is also of interest that in the proximity of the investigated area of the IL-1 receptor there appear Lys residues, probably involved in IL-1 recognition.

Complementarity by:	Siemion		Root-Bernstein	
I VTKFYF	**I-S**	QWLNIN	**I-RB**	QWLKIK
II VTRFYF	**II-S**	QWANIN	**II-RB**	QWAKIK

We used mass spectrometry (MS) and circular dichroism (CD) to investigate the formation of peptide–antipeptide complexes. The biological activity of all synthesised compounds was assessed as inhibition of IL-1-induced IL-2 production, and suppression of immune response. The immunomodulatory properties were established in respect to the cellular immune response in a delayed type hypersensitivity (DTH, effector phase) experiment, whereas the influence on the humoral immune response was examined in a direct plaque forming cells (PFC) test *in vitro*.

MATERIALS AND METHODS

Peptide synthesis. All peptides were synthesised on solid support (Merrifield resin, Reanal, Hungary) using standard Boc procedure, with classical protecting groups and DCC/HOBt coupling. The indole group in tryptophan was protected by formyl group. Peptides were cleaved from the resin in a TFA:TFMSA:m-cresol solution (17:2:1, 1.5 h, 0°C) and precipitated in cold diethyl ether. The formyl group was removed by 10% piperidine solution in DMF (12 h, 0°C). The crude peptides were prepurified on a Sephadex G-10 column (1% acetic acid) and then purified using semipreparative HPLC. Purified peptides were transformed into acetate salts by lyophilization from 10% acetic acid. The purity and identity of peptides was confirmed by analytical HPLC, mass spectrometry and amino acid analysis. The analytical data of the synthesised peptides are given in Table 1.

ESI-MS investigations. A Finnigan MAT TSQ-700 spectrometer with electrospray ionisation source (ESI-MS) was used to record the spectra of peptides and 1:1 peptide/antipeptide mixtures. Droplet desolvation was effected on a capillary heated to 160°C. Peptides were dissolved in a 1:3 (v/v) water/methanol mixture.

CD spectroscopy. CD measurements were performed with a Jasco J-600 spectropolarimeter, using a pathlength of 0.1 cm. Peptides were dissolved in methanol at a concentration of 4.23×10^{-5} M. The spectra of all peptides and 1:1 peptide/antipeptide mixtures were recorded at room temperature.

Peptide-IL-1 competition. The activity of the peptides was investigated as the influence

on IL-1-dependent IL-2 production in LBRM-33-1A5 cell line. The procedure was described in details before (Wieczorek *et al.*, 1997; Siemion *et al.*, 1998; Kluczyk *et al.*, 2002). Briefly, LBRM-33-1A5 cells were incubated with the peptides in the presence of IL-1α for 24 h. The supernatant was added to CTLL-2 cells and the growth was estimated by MTT colorimetric assay after 24 h. The direct influence of the investigated peptides on the CTLL growth was tested in a separate experiment (not shown).

The influence of peptides on immune response. The peptides were dissolved in 0.1 ml of a Cremophor EL/ethanol mixture (9:41) and diluted with 0.9% NaCl to the required concentration. The DTH reaction was measured according to the procedure of Lagrange *et al.* (1974). The PFC number was determined by the Jerne test modified by Mishell & Dutton (1967). The procedures were described in details previously (Siemion *et al.*, 1998; Kluczyk *et al.*, 2002).

RESULTS

We investigated the behaviour of peptide/complementary peptide mixtures using ESI-MS method for the detection of peptide–antipeptide complex formation. Such a method was described for similar experiments (Loo *et al.*, 1994; Madhusudanan *et al.*, 2000) and was successfully used by us before (Siemion *et al.*, 2001). In the spectra of individual peptides I, I-RB, II, and II-RB the double charged ions $[M + 2H]^{+2}$ were the most abundant signals present due to the additional basic groups in the side chains of Lys and Arg, respectively; no triple-charged ions were detected in the case of peptides I-RB and II-RB (not shown). Therefore we were looking not only for the signals of single $[M_1+M_2+H]^{+1}$ and double $[M_1+M_2+2H]^{+2}$ charged heterodimer ions, but there was a possibility of triple charged ion $[M_1+M_2+3H]^{+3}$ formation.

The signals observed during the experiments result only from protonation of the individual peptides, no trace of the expected heterodimers was found. Figure 1 shows the results obtained for the 1:1 mixtures of peptides I and I-S, and II and II-RB. To clarify the possibility of homodimer formation by the investigated peptides, we analysed the satellite peaks of the $[M_1 + H]^{+1}$ ions. A representative result (Fig. 1) for the pair I–I-S confirms the monomeric structure of the ions, with a full mass unit difference between the signals. According to the presented results, the investigated peptides do not show a tendency to form homo- or heterodimeric structures.

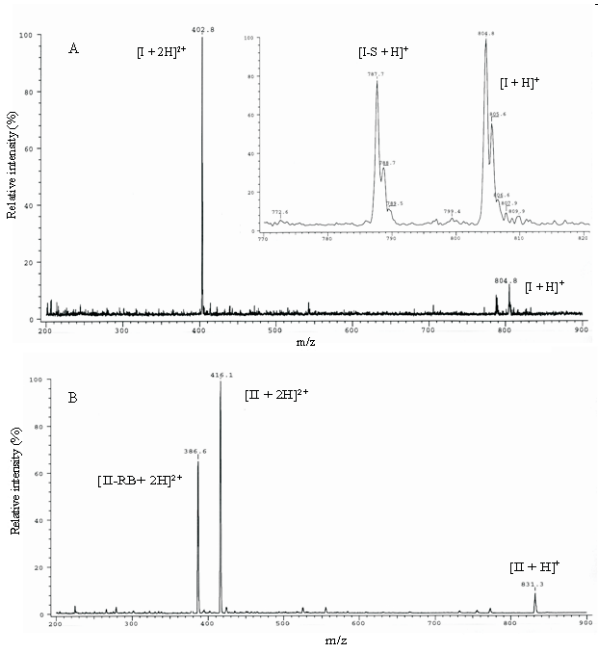

Figure 1. MS spectra of 1:1 mixture of peptide I and its complementary peptides.

A. The mixture of peptide I and the complementary peptide (I-S) according to Siemion's theory. The range of *m/z* values of potential heterodimer signals is enlarged. B. The mixture of peptide I and the complementary peptide (I-RB) according to Root-Bernstein's theory.

It was found before that in CD experiments it is possible to observe conformational changes of interacting peptides by comparing the experimental spectrum of a mixture of peptides with that calculated from the spectra of individual components. We established

earlier that the positive changes in the spectra at about 200 nm indicate the formation of a complex through a shift towards β-structure (Siemion *et al.*, 2001). It is in agreement with the Root-Bernstein suggestion that the β-structure is needed for complex formation.

We examined the spectra recorded for the mixtures of peptides with complementary peptides. In all cases there were some changes indicating a shift in conformational equilibria, but the effect was stronger for the pairs containing the complementary peptides created according to Siemion's theory. The results for the pairs I and I-S, and II and II-S are presented in Fig. 2. In the case of the peptide pair I and I-S, the measured spectrum reveals a distinct increase in β-structure, represented by a positive difference in the range of 195–200 nm, thus indicating the possibility of a peptide–antipeptide interaction. The spectrum of the mixture of peptide II and its complementary peptide II-S also evidences some conformational changes, however, the strong negative band suggests a shift into a more open conformation, an effect not expected in peptide complex formation. The changes are clearly visible despite the fact that the presence of aromatic side chains in the peptides strongly affects the spectra, partially masking the indicative region. It should be noted that no such significant differences were observed in the 200 nm range in the case of the mixtures containing the complementary peptides created according to the Root-Bernstein theory.

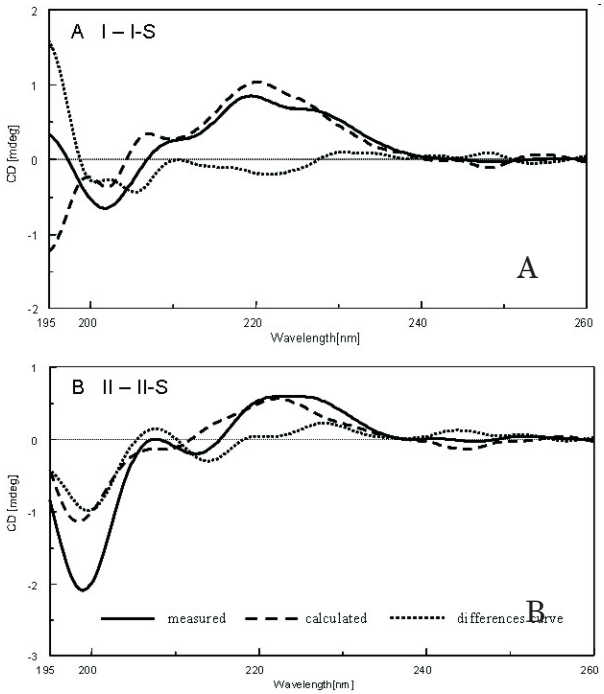

Figure 2. CD spectra of 1:1 mixture of peptides I (A) and II (B) and their complementary peptides in methanol (both antipeptides designed according to Siemion's theory).

The complementary peptides may interact with their counterparts at physiological conditions by recognition of the respective protein of origin and interference with its functions. Our complementary peptides were directed at IL-1Ra and therefore they could actually increase the IL-1 activity by reducing the number of available antagonist molecules. However, the results show that all the complementary peptides strongly inhibit IL-1-dependent IL-2 production (see Fig. 3). The most active compounds, peptides I-S, II and II-RB preserved their activity even after a 20-fold increase in IL-1α concentration. The effects observed for the complementary peptides in the PFC (*in vitro*) and DTH (*in vivo*) experiments were similar to those of peptides I and II (see Fig. 4). In the presented experiments all the compounds show a moderate immunosuppressory activity.

DISCUSSION

The interest in the possible peptide–complementary peptide interactions in IL-1 receptor system was prompted by the fact that the 143–148 fragment of IL-1Ra competes with IL-1 cellular receptor, but shows only limited immunosuppressory activity. The area of IL-1 receptor involved in IL-1 recognition is discontinuous, moreover, the receptor undergoes distinct structural changes adapting to the active forms of IL-1 during recognition process (Schreuder *et al.*, 1997). According to the structure of the IL-1–IL-1R complex, the

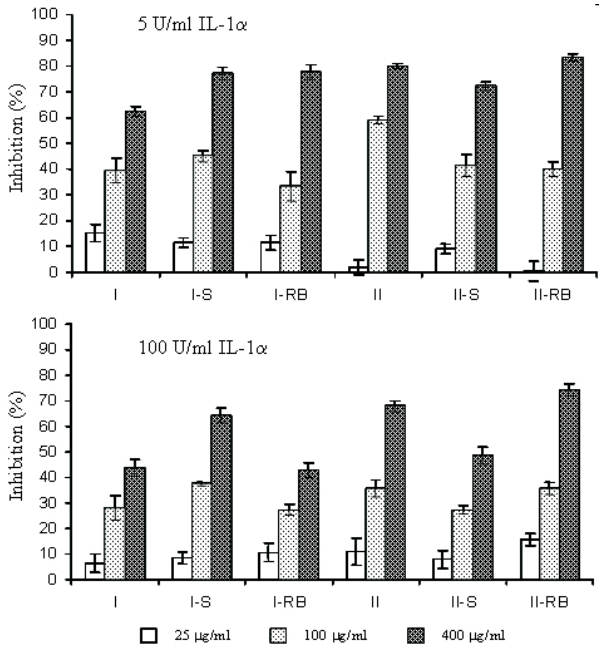

Figure 3. Competition between the investigated peptides and IL-1 for cellular receptor.

The influence of the investigated peptides on IL-1-dependent IL-2 production was analysed as CTLL-2 cell line growth in the presence of supernatant of LBRM-33-1A5 cells treated with different doses of the peptides and IL-1α. The data represent means ±S.D. from at least 4 independent experiments.

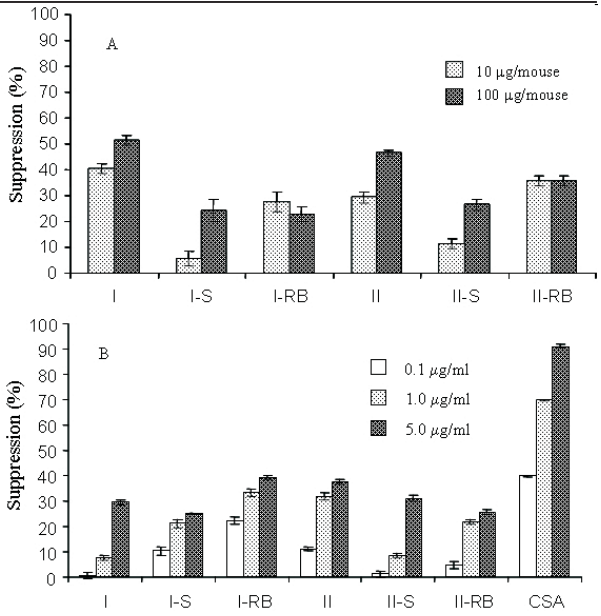

Figure 4. Immunosuppressory activity of the investigated peptides.

A. Delayed type hypersensitivity (DTH) reaction (effector phase of foot-pad test) in 129/Iiw mice sensitized with sheep red blood cells (SRBC) and treated i.p. with the peptides 1 h after the challenging dose of the antigen. CSA — cyclosporine. The data represent means ±S.D. from at least 10 independent experiments. B. Direct plaque-forming cells (PFC) numbers in mouse spleen cell cultures of CBA/Iiw mice immunized with SRBC and treated i.p. with the peptides. The data represent means ±S.D. from at least 4 independent experiments.

selected region of IL-1Ra molecule is involved rather in the recognition process, potentially with IL-1R AcP (Casadio *et al.*, 2001), than in the direct generation of a biological effect (Ju *et al.*, 1991). Moreover, we have found a very similar motif in a protein of vaccinia virus from the *Poxviridae* family. These viruses are known to inhibit IL-1 activity by producing a soluble IL-1 receptor and blocking IL-1β maturation (Smith *et al.*, 1997).

Therefore the efficient inhibition of IL-1 by IL-1Ra and the presence of the VTXFYF sequence in the viral protein suggest that this motif could be important for structural recognition and design of potential IL-1 inhibitors. It is also possible that an antipeptide to the VTXFYF fragment may act as a mini-receptor for IL-1, recognising the structural characteristic of the interacting area.

We used the set of complementary amino acid pairs developed by Siemion according to the equivalency of codons within the genetic code and supported by analysis of several properties of the coded amino acids (for a review see Siemion, 1995). The complementary sequence QWLNIN (I-S) was then used as a starting point for the search of similar motifs in the region of IL-1 receptor in contact with the C-terminal part of IL-1.

Figure 5 presents the results of an experiment establishing one of the potential contact regions of the IL-1 molecules and IL-1 receptor. Spheres of a 15 Å radius were drawn from the C_α carbons of Tyr^{147} in IL-1Ra and Thr^{147} in IL-1β. The fragments of IL-1 ligands outside the selected hexapeptides were removed to clarify the picture. In the selected area there appears a Leu residue, which is complementary to Asp, found in active forms

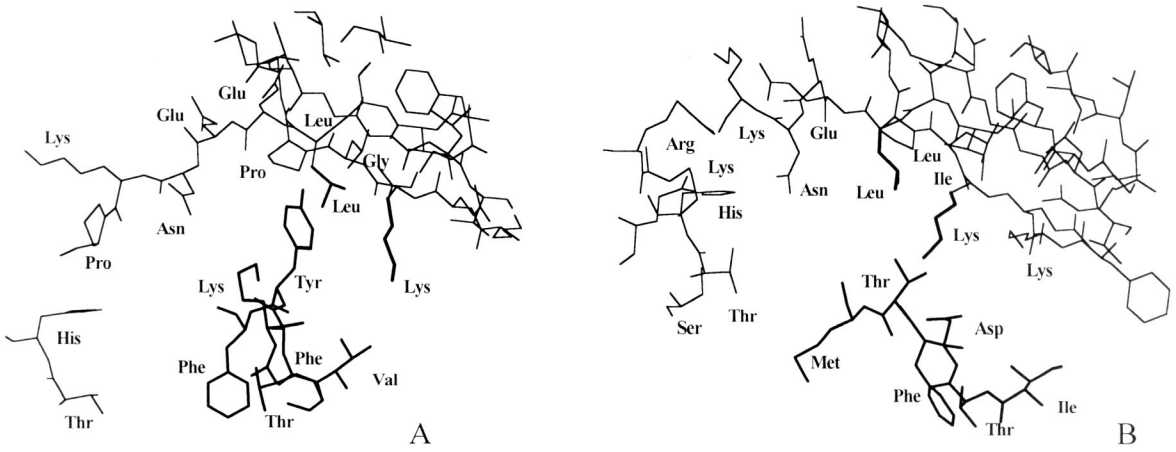

Figure 5. The orientation of peptide I (VTKFYF, fragment 143–148 of IL-1Ra) and the analogous sequence from IL-1β (ITDFTM, fragment 143–148) (thick line) within a 15 Å sphere of IL-1 receptor.

Leu^{198} and Lys^{111} residues of IL-1 receptor are highlighted (thick line). A. The complex of IL-1Ra and IL-1 receptor, PDB file 1IRA (Schreuder *et al.*, 1997). B. The complex of IL-1β and IL-1 receptor, PDB file 1ITB (Vigers *et al.*, 1997).

of IL-1, as well as complementary to Lys found in IL-1Ra (these two residues form a molecular switch between the active and inactive forms of IL-1). Our attention was also attracted by the prominent Lys residues in this region of the IL-1 receptor. On one hand these Lys residues probably interact with the Asp residue in active forms of IL-1, on the other hand, according to Root-Bernstein theory, Lys is complementary to Phe residue. In our previous investigations on C10L fragments we noticed that in the region of the VTRFYF sequence there is a FIF motif, which in our comparison of IL-1Ra and C10L sequences

Table 1. Analytical properties of investigated peptides

Peptide	Yield[1] %	Retention time[2] min	MW[3] calc/found	Amino acid analysis[4]
VTKFYF (I)	59.5	23.1	803.9/804.7	$V_{1.1}T_{1.1}K_{1.0}F_{1.9}Y_{0.9}$
QWLNIN (I-S)	20.2	24.6	786.4/787.2	$D_{2.0}E_{1.0}I_{1.0}L_{1.0}$
QWLKIK (I-RB)	32.5	16.8	814.5/815.2	$E_{0.8}I_{1.0}L_{1.0}K_{2.2}$
VTRFYF (II)	49.2	27.2	831.9/832.7	$V_{1.0}T_{1.1}R_{1.1}F_{1.9}Y_{0.9}$
QWANIN (II-S)	12.1	19.3	744.4/744.7	$D_{1.8}E_{1.0}A_{1.1}I_{1.1}$
QWAKIK (II-RB)	82.8	19.4	772.4/772.8	$E_{1.0}A_{1.1}I_{1.0}K_{1.9}$

[1]Peptide content in crude product according to analytical HPLC (linear gradient 0–100% A/60 min, A – 80% acetonitrile+0.1% TFA in water, B – 0.1% TFA in water, RP-C18 ODS column 250 × 4 mm (Beckman), flow rate 1 ml/min). [2]Conditions as above. [3]Tandem mass spectroscopy, Finnigan Mat TQS 700 (ESI-MS). [4]Amino acid analyses were performed by using an AAA T 339 M analyser after 24 h hydrolysis in 6 M HCl. The Trp residue is not determined in this method.

forms a small bulge. All this justified the synthesis of the second set of peptides, in which Asn residues (complementary to Phe according to Siemion's theory) were replaced by Lys, the amino acid complementary to Phe according to Root-Bernstein.

The synthesised peptides were examined in respect to peptide–antipeptide complex formation and biological activity. Application of ESI-MS technique in the search for dimeric structures in the investigated pairs of peptides seemed especially promising when we considered the presence of the easily charged Lys and Arg residues in peptides I and II. Unfortunately, the complete lack of signals from potential heterodimers suggests that there is no interaction between the investigated peptides at least under the conditions of the ESI-MS experiment.

The results of CD measurements revealed some conformational changes in the peptide mixtures. The spectra of individual peptides I and II were similar and suggested the presence of a turn conformation, unfortunately, the interpretation is difficult due to the strong influence of three aromatic residues (not shown). The complementary peptides seemed to exist in methanol solution in an open conformation. As it is visible in Fig. 2, the CD spectra indicate changes in peptide conformations, which may suggest an interaction between the complementary peptides I and I-S. The differences between the results obtained by the MS and CD methods could result from the different experimental conditions (gas phase or solution), or from the extremely weak character of the peptide interactions.

The complementary peptides were generated towards IL-1Ra fragment and its viral analogue, therefore it may be expected that they should recognise IL-Ra and affect its functions. The pronounced inhibitory properties revealed in the competition experiment suggest that the peptides either interact with IL-1 receptor or with the active form of IL-1, IL-1α (no activity was detected in the absence of IL-1α). This is especially interesting, because such level of activity is, in our experience, quite unusual, particularly in peptides not sharing the VTXFYF motif. As all three IL-1 forms show only moderate sequence similarity, but high tertiary structure resemblance, it is possible that the complementary peptides form mini-receptors, and recognise not only the inactive IL-1Ra, but also the active forms of IL-1, if the recognition is based on the shape of protein domain, and not on a specific sequence.

It seems interesting to investigate longer complementary peptides to IL-1 fragments and thus attempt the modelling of the discontinuous binding sites of IL-1.

REFERENCES

Alcami A, Koszinowski UH. (2000) Viral mechanisms of immune evasion. *Trends Microbiol.*; **8**: 410–8.

Antoni G, Presentini R, Perin F, Tagliabue A, Ghiara P, Censini S, Volpini G, Villa L, Borashi D. (1986) A short synthetic peptide fragment of human interleukin 1 with immunostimulatory but not inflammatory activity. *J Immunol.*; **137**: 3201–4.

Arend WP, Malyak M, Guthridge CJ, Gabay C. (1998) Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol.*; **16**: 27–55.

Blalock JE, Smith EM. (1984) Hydropathic anti-complementarity of amino acids based on the genetic code. *Biochem Biophys Res Commun.*; **121**: 203–7.

Boraschi D, Antoni G, Perin F, Villa L, Nencioni L, Ghiara P, Presentini R, Tagliabue A. (1990) Defining the structural requirements of a biologically active domain of human IL-1. *Eur Cytokine Netw.*; **1**: 21–6.

Casadio R, Frigimelica E, Bossu P, Neumann D, Martin MU, Tagliabue A, Boraschi D. (2001) Model of interaction of the IL-1 receptor accessory protein IL-1RAcP with the IL-1β/IL-1R_1 complex. *FEBS Lett.*; **499**: 65–8.

Davids JW, El-Bakri A, Heal J, Christie G, Roberts GW, Raynes JG, Miller AD. (1997) Design of antisense (complementary) peptides as selective inhibitors of cytokine interleukin-1. *Angew Chem Int Ed.*; **36**: 962–7.

Dinarello CA. (1996) Biological basis for interleukin-1 in disease. *Blood.*; **87**: 2095–147.

Goebel SJ, Johnson GP, Perkus ME, Davis SW, Winslow JP, Paoletti E. (1990) The complete DNA sequence of vaccinia virus. *Virology.*; **179**: 247–66.

Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. (1995) Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem.*; **270**: 13757–65.

Heal JR, Bino S, Roberts GW, Raynes JG, Miller AD. (2000) Mechanistic investigation into complementary (antisense) peptide mini-receptor inhibitors of cytokine interleukin-1. *Chembiochem.*; **3**: 76–85.

Holsworth DD, Kiely JS, Root-Bernstein RS, Overhiser RW. (1994) Antisense-designed peptides: a comparative study focusing on possible complements to angiotensin II. *Pept Res.*; **7**: 185–93.

Ju G, Labriola-Tompkins E, Campen CA, Benjamin WR, Karas J, Plocinski J, Biondi D, Kaffka KL, Kilian PL, Eisenberg SP, Evans RJ. (1991) Conversion of the interleukin 1 receptor antagonist into an agonist by site-specific mutagenesis. *Proc Natl Acad Sci U S A.*; **88**: 2658–62.

Kluczyk A, Siemion IZ, Szewczuk Z, Wieczorek Z. (2002) The immunosuppressive activity of peptide fragments of vaccinia virus C10L protein and a hypothesis on the role of this protein in the viral invasion. *Peptides.*; **23**: 823–34.

Korherr C, Hofmeister R, Wesche H, Falk W. (1997) A critical role for interleukin-1 receptor accessory protein in interleukin-1 signaling. *Eur J Immunol.*; **27**: 262–7.

Lagrange PH, Mackaness GB, Miller TE, Pardon P. (1974) Influence of dose and route of antigen injection on the immunological induction of T cells. *J Exp Med.*; **139**: 528–42.

Loo JA, Holsworth DD, Root-Bernstein RS. (1994) Use of electrospray ionization mass spectroscopy to probe antisense peptide interactions. *Biol Mass Spectrom.*; **23**: 6–12.

Madhusudanan KP, Katti SB, Haq W, Misra PK. (2000) Antisense peptide interactions studied by electrospray ionization mass spectrometry. *J Mass Spectrom.*; **35**: 237–41.

Mekler LB. (1969) On specific selective interaction between amino acid residues of polypeptide chains. *Biofizika (Russ.)*; **14**: 581–4.

Mishell RI, Dutton RW. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J Exp Med.*; **126**: 423–42.

Root-Bernstein RS. (1982) Amino acid pairing. *J Theor Biol.*; **94**: 885–94.

Root-Bernstein RS, Holsworth DD. (1998) Antisense peptides: a critical mini-review. *J Theor Biol.*; **190**: 107–19.

Rothwell N. (2003) Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. *Brain Behav Immun.*; **17**: 152–7.

Schreuder H, Tardif C, Trump-Kallmeyer S, Soffientini A, Sarubbi E, Akeson A, Bowlin T, Yanofski S, Barret RW. (1997) A new cytokine-receptor binding mode revealed by the crystal structure of the IL-1 receptor with an antagonist. *Nature.*; **386**: 194–200.

Siemion IZ. (1995) The regularities of the changes of amino acid physico-chemical properties within the genetic code. *Amino Acids.*; **8**: 1–13.

Siemion IZ, Kluczyk A, Wieczorek Z. (1998) Anti-IL-1 activity of peptide fragments of IL-1 family proteins. *Peptides.*; **19**: 373–82.

Siemion IZ, Zbozień-Pacamaj R, Stefanowicz P. (2001) New hypothesis on amino acid complementarity and its evaluation on TGF-β_2-related peptides. *J Mol Recognit.*; **14**: 1–12.

Sims JE. (2002) IL-1 and IL-18 receptors, and their extended family. *Curr Opin Immunol.*; **14**: 117–22.

Small RE, Wixted MA, Roberts WN. (2001) Anakinra. An interleukin-1 receptor antagonist for treatment of rheumatoid arthritis. *Formulation.*; **36**: 191–203.

Smith GL, Symons JA, Khanna A, Vanderplasschen A, Alcami A. (1997) Vaccinia virus immune evasion. *Immunol Rev.*; **159**: 137–54.

Vigers GPA, Anderson LJ, Caffes P, Brandhuber BJ. (1997) Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1. *Nature.*; **386**: 190–4.

Wieczorek Z, Kluczyk A, Słoń-Usakiewicz JJ, Siemion IZ. (1997) A hexapeptide VTKFYF from C-terminal part of interleukin-1 receptor antagonist, an inhibitor of IL-1–IL-1 receptor interaction. *Pol J Pharmacol.*; **49**: 107–17.