Design and recognition properties of a hydrophatically complementary peptide to human interleukin 1 β

Giorgio FASSINA* and Giovanni CASSANI

Protein Engineering Unit, TECNOGEN S.c.p.A., Via Ampere 56, 20131 Milano, Italy

A computer-designed hydrophatically complementary peptide to human interleukin 1 β (IL1 β) precursor sequence 204–215 recognized the 204–215 peptide as well the entire IL1 β protein with binding affinities in the micromolar range. Interaction between the complementary pair was characterized by analytical high-performance liquid affinity chromatography on columns derivatized with the computer-generated peptide. Recognition selectivity was clearly shown by the ability of the computer-generated complementary peptide columns to purify the IL1 β -(204–215)-peptide from complex synthetic mixtures with high yields, independently of the type of solid support used. Recognition specificity was demonstrated by the inability of the IL1 β -(204–215)-peptide and IL1 β molecules to interact with blank columns or columns derivatized with other non-related peptides. Furthermore, scrambling the sequence of the computer-generated peptide or the IL1 β -(204–215)-peptide in such a way as to alter their hydrophobic profiles had the effect of abolishing binding. The complementary pair failed to interact in the presence of competing peptide, thus providing further evidence of specificity. Computer-generated complementary peptide affinity columns also proved useful for purification of recombinant human IL1 β protein directly from crude *Escherichia coli* lysates.

INTRODUCTION

Several lots of experimental work support the original theory (molecular recognition theory) proposed by Blalock and colleagues [1–4] that interacting peptides can be deduced from the transcription of complementary DNA stretches. The molecular recognition theory has been applied successfully to the design *de novo* of numerous peptide ligands directed towards various peptides and proteins, such as angiotensin II [5], insulin [6], melanocyte-stimulating hormone [7], fibronectin [8], s-peptide [9,10], substance P [11], [Arg⁸]vasopressin [12], luteinizing hormone-releasing hormone [13], growth hormone-releasing hormone [14], interleukin 2 [15], c-ras protein [16], enkephalins [17] and cystatin [18], as well as in the search for complementary sequences between hormones and corresponding receptors as putative recognition sites [19], and internally in protein sequences to identify intramolecular interacting stretches [20].

Numerous sequence modelling and redesign studies [9,10,12,16] of complementary DNA-deduced peptides have been carried out in an attempt to gain preliminary understanding of the physical nature of the interaction. Complementary peptide recognition is dependent on peptide pair length, showing higher affinity for longer sequences, and occurs with higher affinity in buffer at low ionic strength and pH ranging from 5 to 7. The interaction does not depend on preferred peptide spatial conformation, since inversion of the peptide, *N*-terminus to *C*-terminus, leaves the peptide-binding properties unaltered [9], and so does incorporation of amino acids of the *D*-configuration into the peptide sequence [16]. Moreover, the recognition process is not affected by the presence of detergents and denaturants [16]. These data together suggest a multilocalized type of interaction, where many residues along the complementary pair participate in stabilizing contacts [9,10,12,21]. One of the most important features of complementary DNA-deduced interacting peptides is their hydrophobic complementarity derived from the hydrophobic anti-correlation of complementary codons [22]. On the basis of

this observation, a computer program has recently been developed which allows deduction of a hydrophatically complementary peptide sequence from the primary target sequence characterized by improved binding properties when compared with complementary DNA-deduced peptides [16,23]. To challenge the methodology further, the analysis has been extended to evaluate the binding properties of a computer-generated peptide (δ IL) hydrophatically complementary to residues 204–215 of interleukin (IL1 β) precursor. The IL1 β molecule belongs to a family of related proteins involved in a wide range of immunological and inflammatory responses to injury and infection [24]. The active circulating form is an approx. 17.5 kDa *C*-terminal fragment derived from a 31 kDa precursor, including amino acids 117–269 of the primary gene product [25]. Recognition specificity and selectivity in the interaction between the complementary peptide pair have been investigated by analytical affinity chromatography using zonal and competitive elution on δ IL peptide affinity columns, and assessing the ability of immobilized δ IL peptide to recognize IL1 β -(204–215)-peptide as well as full-length IL1 β from crude synthetic peptide mixtures or bacterial lysates.

MATERIALS

9-Fluorenylmethoxycarbonyl (Fmoc)-derivatized amino acids and 4-hydroxymethylphenoxycarbonate resin for solid-phase peptide synthesis were purchased from NOVABIOCHEM (Laufelfingen, Switzerland). Reagents used for peptide synthesis, cleavage and purification were from Aldrich (Milan, Italy). Eupergit C 30N (acrylic support for affinity chromatography preactivated with epoxy groups) was obtained from Rohm Co. (Weierstad, Germany). Reversed-phase h.p.l.c.-purified human IL1 β and *Escherichia coli* extracts containing recombinant human IL1 β were a gift from Dr. M. L. Melli, Centro Ricerche Sclavo (Siena, Italy).

Abbreviations used: δ , computer-generated peptide; Fmoc, 9-fluorenylmethoxycarbonyl; h.p.e.c., high-performance electrophoresis chromatography; h.p.l.a.c., high-performance liquid affinity chromatography; IL, interleukin; S δ IL, scrambled computer-generated peptide.

* To whom correspondence should be addressed.

METHODS

Computerized design of hydropathically complementary peptides

Selection of hydropathically complementary sequences to target IL1 β -(204–215)-peptide was carried out using a mathematical approach similar to that developed by Kyte & Doolittle [26] for displaying the hydropathic profile of polypeptide sequences. To each residue of the target sequence is assigned the corresponding hydropathy value obtained from the Kyte & Doolittle scale [26]. Successively, to each residue of the target sequence, a set of amino acid residues with opposite hydropathy values in the range ± 1 hydropathy unit is assigned, except for threonine, where a ± 1.1 hydropathy unit range is used. Then, the moving average hydropathy a_i [26] of the target sequence is calculated according to the formula:

$$a_k = \sum_{i=k-s}^{k+s} h_i$$

where h_i is the hydropathy value of each residue A_i of the target sequence, k is (s) to $(n-s)$, and s is $(r-1)/2$, where r is an odd numeral up to or equal to n , the number of residues in the target peptide. In a similar way, the moving average hydropathy b_i of all the complementary sequences is determined according to the expression:

$$b_k = \sum_{i=k-s}^{k+s} g_{i,j}$$

where $g_{i,j}$ corresponds to the hydropathy value of each residue in the set of residues complementary to the target residue A_i . Among all the possible complementary sequences to the target sequence, the one characterized by the lowest hydropathic score, Θ , which is inversely proportional to the degree of complementarity on a moving average base, and defined as:

$$\Theta = \sum \sqrt{(a_i + b_i)^2 / (n - 2s)}$$

is selected as the best recognition sequence. The complementary sequence characterized by the lowest hydropathic score, Θ , will have the best complementarity in the hydropathic profile with the given target sequence. This mathematical approach has been developed into a computer program for the personal computer, and called AMINOMAT.

Peptide synthesis

Peptides were prepared by solid-phase peptide synthesis following the Fmoc methodology on a fully automated model 431A Applied Biosystems Synthesizer, software version 1:1. After completion of the synthesis cycles and peptide cleavage from resin, peptide purification from site products was accomplished by reversed-phase h.p.l.c. gradient elution on a Lichrospher C₁₈ column (25 cm $\times$ 1 cm internal diameter), equilibrated at a flow rate of 3 ml/min with water/acetonitrile/trifluoroacetate (800:200:1, by vol.)

Affinity column preparation

Immobilization of APIL (GGGDGGGDDDGRDDG) (see the Results section for explanation) on Eupergit C 30N was carried out by incubating 5 mg of peptide dissolved in 10 ml of 0.1 M-NaHCO₃/0.5 M-NaCl, pH 8.5, with 1 g of support, with agitation, for 24 h. The extent of peptide incorporation was followed by reversed-phase h.p.l.c., which indicated that 70 % of initial peptide was support-bound. At the end, the resin was repeatedly washed with 0.1 M-Tris, pH 8.5, and slurry-packed on a (80 mm $\times$ 6.6 mm internal diameter) glass column. In a similar way, a blank column was prepared by incubating 1 g of Eupergit C 30N with 100 mg of Gly-NH₂. In the case of δ IL (GGGHLLFPPIIAASL) and scrambled δ IL (S δ IL; GGGLILIHPSAAIFL), owing to their poor solubility in aqueous buffers, peptides (5 mg) were dissolved in 10 ml of

Table 1. Specificity of IL1 β -(204–215)-peptide binding to immobilized δ IL

Ligand loading was determined by amino acid analysis of preweighed portions of derivatized supports. Retardation volume was determined on columns (2.3 ml total volume) equilibrated with 0.1 M-Tris, pH 6.8. Dissociation constants were determined according to eqn. (2) and assuming $P \ll M_T$.

Column	Ligand loading (μ mol)	Retardation volume (ml)	Dissociation constant $K_{M/D}$ (mM)
(APIL)-Eupergit	2.5	0.5	5
(Gly-NH ₂)-Eupergit	90.0	1.0	90
(S δ IL)-Eupergit	0.6	0.5	1.2
(δ IL)-Eupergit	0.7	350.0*	0.02
(δ IL)-Sephacrose	0.9	450.0*	0.02
(Gly-NH ₂)-Sephacrose	3.0	1.0	30

* Values determined by extrapolation at $[D] = 0$.

dimethyl sulfoxide/water (9:1, v/v) containing 500 μ l of triethylamine and incubated separately with 1 g of Eupergit C 30N for 24 h at room temperature, with agitation. Reversed-phase h.p.l.c. analysis of supernatants at the end of the incubation showed 30 % peptide coupling to the support. The derivatized supports were then washed several times first with water and then with 0.2 M-Tris, pH 8.5, and then slurry-packed in a glass column as above. Following a similar procedure, peptide δ IL (5 mg) was also immobilized on tressyl-activated highly cross-linked Sepharose (2 g). In this case, reversed-phase h.p.l.c. analysis indicated a 35 % incorporation. More accurate determinations of support-bound peptides were carried out by amino acid analysis of 0.1 M acetic acid-washed samples of derivatized support (Table 1).

Determination of binding constants

Dissociation constants for the interaction between immobilized δ IL and soluble IL1 β -(204–215)-peptide were determined by competitive elution experiments [27,28]. The extent of IL1 β -(204–215)-peptide elution volume retardation on the δ IL-Eupergit column was measured in the presence of increasing amounts of IL1 β -(208–215)-peptide dissolved in the elution buffer. Data were plotted according to:

$$\frac{1}{(V - V_0)} = \frac{K_{M/P}}{M_T} + \frac{[D]K_{M/D}}{K_{M/P}} \quad (1)$$

where V is the elution volume of IL1 β -(204–215)-peptide, V_0 the column void volume, $K_{M/P}$ the dissociation constant for the interaction between immobilized δ IL and soluble IL1 β -(204–215)-peptide, $[D]$ the concentration of IL1 β -(208–215)-peptide in the buffer, $K_{M/D}$ the dissociation constant for the interaction between immobilized δ IL and competing soluble IL1 β -(208–215)-peptide, and M_T the total amount of immobilized δ IL. In the absence of competing peptide in the buffer, eqn. (1) becomes:

$$\frac{1}{(V - V_0)} = \frac{K_{M/P}}{M_T} \quad (2)$$

for $P \ll M_T$.

High-performance electrophoresis chromatography (h.p.e.c.)

Samples of crude bacterial lysates and affinity-purified recombinant IL1 β were analysed by SDS/PAGE on a glass column (2.5 mm $\times$ 50 mm) filled with 5 % acrylamide gel/SDS connected to a model 230 h.p.e.c. system (ABI, Foster City, CA, U.S.A.). Gels were run at a constant current of 0.7 mA, and the eluate was monitored at 225 nm.

Amino acid analysis

Amino acid composition of synthetic peptides and peptide loading of derivatized supports were evaluated by reversed-phase h.p.l.c. analysis of Fmoc-derivatized acid hydrolysates [29].

RESULTS

Design of hydrophatically complementary peptides to the IL1 β -(204–215)-peptide sequence

A peptide with hydrophatic complementarity to the exposed and flexible IL1 β -(204–215)-peptide site (KNYPKKKMEKRF)

Fig. 1. Hydrophatic profiles of IL1 β -(204–215)-peptide (▲), δ IL (■) and S δ IL (●)

Profiles were determined according to the Kyte and Doolittle scale with a moving average of five residues.

Fig. 2. Binding of h.p.l.c.-purified IL1 β -(204–215)-peptide to various affinity columns

Left: binding of h.p.l.c.-purified IL1 β -(204–215)-peptide to δ IL peptide affinity column. The column was equilibrated at a flow rate of 2 ml/min with 0.1 M-Tris, pH 6.8, and the effluent was monitored at 280 nm. Samples (100 μ g) of IL1 β -(204–215)-peptide dissolved in 200 μ l of eluting buffer were injected on the column. At the point indicated by the arrows, the eluent was changed to: (a) 0.1 M-acetic acid, (b) 1 M-Tris, pH 6.8, or (c) 0.1 mM-IL1 β -(208–215)-peptide. Right: binding of h.p.l.c.-purified IL1 β -(204–215)-peptide to Gly-NH₂-Eupergit (d), S δ IL-Eupergit (e), or APIL-Eupergit (f). The columns were equilibrated at a flow rate of 2 ml/min with 50 mM-Tris, pH 6.8, and the effluent was monitored at 280 nm, with a monitor pathlength of 3 mm.

Fig. 3. Binding of h.p.l.c.-purified recombinant IL1 β to δ IL-Eupergit (a), δ IL-Sephacrose (b) and Gly-NH₂-Eupergit (c)

The columns (2.3 ml) were equilibrated at a flow rate of 2 ml/min with 0.1 M-Tris, pH 6.8, and the effluent was monitored at 280 nm. Samples (100 μ g) of IL1 β dissolved in 200 μ l of elution buffer were injected on the column. At the point indicated by the arrows, the eluent was changed to 0.1 M-acetic acid.

Fig. 4. Determination of hydrophatically complementary peptide dissociation constants

The δ IL-Eupergit column was equilibrated at a flow rate of 2 ml/min with 0.1 M-Tris, pH 6.8 containing IL1 β -(208–215)-peptide at concentrations of 0.005 mM (---), 0.01 mM (---) and 0.05 mM (—), the effluent being monitored at 280 nm. Samples (100 μ g) of IL1 β -(204–215)-peptide dissolved in 200 μ l of eluting buffer were injected on the column. Extent of IL1 β -(204–215)-peptide retardation on IL1 β -(208–215)-peptide competitor concentration is plotted in the inset according to eqn. (2).

[30] was designed using a computer program able to generate peptide sequences hydrophatically complementary on a moving average basis to a target sequence using only the amino acid sequence of the target. The computer-generated complementary peptide (HLLFPPIIAASL) (δ IL) to the IL1 β -(204–215)-peptide

Fig. 5. Affinity purification of IL1 β -(204-215)-peptide from crude mixture

(a) High-performance liquid affinity chromatography (h.p.l.a.c.) profile of crude peptide mixture containing trace amounts of IL1 β -(204-215)-peptide eluted from the δ IL-Eupergit column equilibrated with 0.1 M-Tris, pH 6.8, at a flow rate of 1 ml/min. At the point indicated by the arrow, the eluent was changed to 0.1 M-acetic acid. Reversed-phase h.p.l.c. analysis of (b) crude peptide mixture, (c) crude peptide mixture with trace amount of IL1 β -(204-215)-peptide, (d) unbound h.p.l.a.c. fraction peak 1, and (e) bound h.p.l.a.c. fraction peak 2.

sequence was designed using the software AMINOMAT (see the Methods section), and its hydrophatic profile is reported in Fig. 1, and compared with the IL1 β -(204-215)-peptide profile. The complementary peptide (δ IL) was synthesized by solid-phase synthesis introducing a triglycine spacer at the *N*-terminus, to provide augmented accessibility after immobilization on the solid support for the interaction with IL1 β and its fragment 204-215. In order to evaluate the sequence specificity of the interaction, and the role of complementarity of hydrophatic profiles, the δ IL sequence was scrambled in such a way as to abolish the hydrophatic complementarity (Fig. 1). This peptide, denoted scrambled δ IL (S δ IL) (LILIHPSAAIFL), was also synthesized with a triglycine spacer at the *N*-terminus for subsequent immobilization on the solid phase. In a similar way, the IL1 β -(204-215)-peptide sequence was scrambled (MFYKKENRKKKP). A comparison was also made between the binding properties of the computer-generated hydrophatically complementary peptide and a peptide obtained by complementing the charge of IL1 β -(204-215)-peptide. This peptide was designed by replacing each negatively charged residue in the IL1 β -(204-215)-peptide sequence with arginine, each positively charged residue with aspartate, and the remaining residues with glycine. Denoted APIL (DGGGDDDDGRDDG), it was synthesized with a triglycine spacer at the *N*-terminus, and purified and immobilized on Eupergit C 30N to prepare an affinity column.

Fig. 6. Affinity purification of IL1 β -(204-215)-peptide on δ IL-Sepharose from crude peptide mixture

(a) H.p.l.a.c. profile of crude peptide mixture containing trace amounts of IL1 β -(204-215)-peptide eluted from the δ IL-Sepharose column equilibrated with 0.1 M-Tris, pH 6.8, at a flow rate of 1 ml/min. At the point indicated by the arrow, the eluent was changed to 1.0 M-Tris, pH 6.8. Reversed-phase h.p.l.c. analysis of (b) crude peptide mixture containing trace amounts of IL1 β -(204-215)-peptide, (c) unbound h.p.l.a.c. fraction peak 1 and (d) purified h.p.l.a.c. fraction peak 2.

Hydrophatically complementary peptide recognition: specificity

Binding characteristics of IL1 β -(204-215)-peptide and IL1 β towards immobilized δ IL were evaluated by zonal and competitive elution experiments. Affinity columns derivatized with δ IL were equilibrated with 50 mM-Tris, pH 6.8, at a flow rate of 1 ml/min. Samples (50 μ g) of IL1 β -(204-215)-peptide were applied to the column and monitored continuously by measuring u.v. absorbance of the eluate at 280 nm. After 30 min, the eluent was changed to one of the following: 0.1 M-acetic acid, 1 M-Tris, pH 6.8, or 0.1 mM-IL1 β -(204-215)-peptide (Fig. 2). In agreement with data reported previously [12,16,18], binding between hydrophatically complementary peptides is affected by variation in the pH from neutrality (Fig. 2a) and high ionic strength (Fig. 2b). Recognition is also abolished by the presence of competing IL1 β -(208-215)-peptide in the buffer, thus providing evidence of specificity in the interaction (Fig. 2c).

The fragment 208-215 was chosen as competitor because of its weak absorbance at 280 nm, allowing detection of IL1 β -(204-215)-peptide which contains a tyrosine residue. Further evidence of binding specificity is provided by the inability of IL1 β -(204-215)-peptide to bind to affinity columns derivatized with Gly-NH₂ (Fig. 2d). The hydrophatic-profile-dependence of the interaction was demonstrated by the lack of recognition properties of a scrambled δ IL peptide (Fig. 2e), where the overall amino acid composition is retained whereas the sequence is changed in such a way as to significantly modify the resulting hydrophatic profile (Fig. 1). The presence of simple ionic interactions between complementary peptide pairs as the driving force for the interaction was ruled out by the inability of IL1 β -(204-215)-peptide charge-complementary peptide (APIL) to bind

Fig. 7. Affinity purification of recombinant IL1 β from crude *E. coli* lysate

(a) H.p.l.c. profile of crude bacterial lysate containing recombinant human IL1 β eluted from the δ IL-Eupergit column equilibrated with 50 mM-Tris, pH 6.8. Bound material, denoted as peak 2, was collected for reversed-phase h.p.l.c. and h.p.e.c. analysis. (b) Reversed-phase h.p.l.c. analysis of crude *E. coli* lysate (top) and h.p.l.c.-purified fraction peak 2 (bottom). IL1 β is indicated by the arrows. (c) h.p.e.c. analysis of crude bacterial lysate (top) and purified h.p.l.c. fraction peak 2 (bottom).

IL1 β -(204–215)-peptide (Fig. 2f). This peptide was optimized in terms of charge complementarity, opposing a negative/positive residue to each positive/negative residue of IL1 β -(204–215)-peptide. Further evidence of sequence specificity was clearly shown by the inability of the scrambled IL1 β -(204–215)-peptide to bind to the δ IL-Eupergit column. All the material applied was eluted at the column void volume, providing a chromatogram very similar to those shown in Figs. 2(d), 2(e) and 2(f). The δ IL peptide is also able to recognize the complementary sequence in the full-length IL1 β molecule (Fig. 3a), whereas IL1 β is not retained by Eupergit column derivatized with Gly-NH₂ (Fig. 3c). The interaction between complementary peptides is not dependent only on a particular type of support. δ IL can also be immobilized on Sepharose 4B, which differs enormously in chemical nature from Eupergit C 30N, and still retains full binding properties for the IL1 β molecule (Fig. 3b). In Table 1 are reported the observed retention volumes of IL1 β -(204–215)-peptide on all the columns tested, and, from the M_T values determined by amino acid analysis of measured amounts of derivatized supports, the derived dissociation constants $K_{M/P}$ are calculated according to eqn. (1) or (2).

Determination of dissociation constants for hydrophatically complementary peptide recognition

Dissociation constants for the interaction between Eupergit-immobilized δ IL and soluble IL1 β -(204–215)-peptide were determined by zonal elution of fixed amounts of IL1 β -(204–215)-peptide (100 μ g) in the presence of various amounts of competing IL1 β -(208–215)-peptide dissolved in the elution buffer, 50 mM-Tris, pH 6.8 (Fig. 4). The extent of IL1 β -(204–215)-peptide retardation on the δ IL-Eupergit column was plotted against IL1 β -(208–215)-peptide concentration according to eqn. (1) (Fig. 4, inset). As predicted by eqn. (1), the elution volume of IL1 β -(204–215)-peptide decreases with increasing amounts of IL1 β -(208–215)-peptide in the buffer. Amino acid analysis of measured amounts of δ IL-Eupergit indicated the presence of 0.3 μ mol of

immobilized peptide per ml of resin, and in the total column volume (2.3 ml) $M_T = 0.7$ μ mol. Using this value as the functional capacity of the column, from the intercept and slope of the plot $1/(V-V_0)$ against IL1 β -(208–215)-peptide concentration, the values of $K_{M/P}$ (10 μ M) and $K_{M/D}$ (80 μ M) were graphically calculated. In agreement with data reported previously [9,10,12,16], the IL1 β -(208–215)-peptide (which is shorter) recognizes immobilized δ IL with weaker affinity ($K_{M/D} = 80$ μ M).

Hydrophatically complementary peptide recognition: selectivity

In order to evaluate the selectivity of hydrophatically complementary peptide recognition, the ability of immobilized δ IL to recognize the target peptide and protein from crude mixtures containing different components in different amounts was tested. A complex synthetic peptide mixture containing 20 different peptides each at a concentration of 1 mg/ml was prepared (Fig. 5b), and to 1 ml of the mixture 20 μ g of h.p.l.c.-purified IL1 β -(204–215)-peptide was added (Fig. 5c). In this mixture, IL1 β -(205–215)-peptide was eluted close to other components and appeared as a shoulder of a main peak. The mixture was made up of peptides ranging from 8 to 20 amino acids in length, with net charges at pH 7.0 from -3 to $+6$, and encompassing a wide range of hydrophobicity.

To the δ IL-Eupergit column, equilibrated with 50 mM-Tris, pH 6.8, at a flow rate of 1 ml/min, a 2 ml portion of the mixture was applied, with continuous monitoring of the eluate. After elution of the main peak of non-retarded material at the void volume of the column, the buffer was changed to 0.1 M-acetic acid (Fig. 5a). The peaks corresponding to the flow-through material (peak 1) and to the acid-eluted material (peak 2) were collected and analysed by reversed-phase h.p.l.c. under the same conditions as used for analysis of the crude mixture (Figs. 5d and 5e). Whereas peak 2 contained mainly IL1 β -(204–215)-peptide, peak 1, corresponding to the unbound material, contained the majority of unrelated peptides originally present in the crude mixture. Identity of affinity-purified IL1 β -(204–215)-peptide with

synthetic h.p.l.c.-purified IL1 β -(204–215)-peptide was also confirmed by comparing their tryptic maps (results not shown).

The same mixture was also applied to the δ IL–Sephacrose column under the same experimental conditions as used for the δ IL–Eupergit column. In this case, a slightly different reversed-phase h.p.l.c. elution gradient was adopted better to identify IL1 β -(204–215)-peptide in the crude mixture. The IL1 β -(204–215)-peptide was successfully separated from the peptide mixture (Fig. 6). Equal selectivity in the recognition was demonstrated with *E. coli* extracts containing recombinant IL1 β (Fig. 7). A 1 ml portion of *E. coli* lysate was applied to the δ IL–Eupergit column equilibrated with 50 mM-Tris, pH 6.8. After elution of non-retarded material, the eluent was changed to 1.0 M-Tris, pH 6.8. Bound material, denoted as peak 2, was analysed by reversed-phase h.p.l.c. (Fig. 7b) and by h.p.e.c. (Fig. 7c). Recovery of starting material was close to 70%, and 50 μ g of recombinant IL1 β was purified from 1 ml of crude extract in 35 min on the 2.3 ml total volume affinity column. The same column was used for more than 30 runs without apparent loss of capacity.

DISCUSSION

Hydropathically complementary peptides designed *de novo* show sufficient selectivity to be used successfully as peptide ligands for peptide/protein purification from crude extracts [12,16]. A detailed understanding of the structural features required to produce the recognition is provided by spectroscopic characterization of complementary peptide complexes. Studies carried out mainly by n.m.r. on angiotensin II [31] and adrenocorticotropin [32] failed to provide any information, probably because the binding affinity of the system was too low to produce spectroscopic transitions [31], or because the medium conditions were not such as to optimize the recognition [32]. On the other hand, preliminary spectroscopic studies on the c-raf [16] and Big endothelin [33] fragments indicated that conformational transitions are associated with complementary peptide recognition. In these two systems, the binding affinities were fully characterized as well as the optimal medium composition required for binding.

The major goals of the present study were to demonstrate the possibility of computer generating, on the basis of complementary hydropathy, a peptide able to recognize with high affinity a selected fragment of the IL1 β molecule, to evaluate the specificity and selectivity of the interaction, and to investigate its use as an affinity ligand for purification of the target protein. Interaction selectivity has been unequivocally proved by the ability of immobilized δ IL to recognize IL1 β -(204–215)-peptide and full-length IL1 β from complex mixtures containing different unrelated molecules (Figs. 5, 6 and 7). Immobilized δ IL retains its binding properties independently of the type of solid support used for the preparation of the affinity column (Figs. 3 and 6).

Interaction specificity between complementary peptides was clearly demonstrated in this study by the displacing effect of competing IL1 β -(208–215)-peptide in the buffer (Figs. 2c and 4), and by the inability of IL1 β -(204–215)-peptide and mature IL1 β to bind to columns derivatized with unrelated peptides (Figs. 2 and 3).

Although spectroscopic analysis of the complementary peptide complex was not possible because of the poor solubility of δ IL in aqueous buffers, the failure of APIL, the charge-complementary peptide to IL1 β -(204–215)-peptide, to recognize IL1 β -(204–215)-peptide excluded simple ionic-exchange phenomena as the driving force for the interaction. Similarly, the presence of hydrophobic interactions as the dominant mechanism for binding has been ruled out by the ability of δ IL to recognize IL1 β -(204–215)-

peptide as well as mature IL1 β from complex mixtures containing different molecules covering a wide range of hydrophobicities (Figs. 5, 6 and 7).

Furthermore, the failure of immobilized scrambled δ IL to recognize IL1 β -(204–215)-peptide has stressed the role of hydrophobic profile complementarity in producing the interaction, and at the same time provided further evidence of specificity. Finally, immobilized δ IL proved to be a sequence-directed ligand useful for affinity purification of recombinant IL1 β directly from a crude *E. coli* extract (Fig. 7). Recovery of starting material was high and the total purification time was limited to 35 min. Contaminants in the extract, often able to poison irreversibly reversed-phase h.p.l.c. columns in a few runs, were efficiently removed. The ligand seems to be stable to bacterial extracts, since the same column was used for more than 30 runs without apparent loss of capacity. Even if the binding affinities between complementary peptides are low compared with polyclonal antibody recognition, they are sufficiently high to allow the preparation of affinity columns with moderate ligand density. In certain instances, when the target molecule for purification is particularly unstable, low affinity could be an advantage, since very mild elution conditions can be used to elute the bound molecule. Another attractive feature of using hydropathically complementary peptides as sequence-directed ligands resides in their synthetic nature, which overcomes all the biological contamination problems often associated with the use of immunoaffinity sorbents. Further, the small ligand size allows easy separation of leaked or fragmented ligand from the purified macromolecule simply by dialysis or gel filtration.

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REFERENCES

1. Bost, K. L., Smith, E. M. & Blalock, J. E. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1372–1375
2. Blalock, J. E. & Bost, K. L. (1986) *Biochem. J.* **234**, 679–683
3. Smith, L. R., Bost, K. L. & Blalock, J. E. (1987) *J. Immunol.* **138**, 7–9
4. Bost, K. L. & Blalock, J. E. (1989) *Methods Enzymol.* **178**, 16–28
5. Elton, T. S., Dion, L. D., Bost, K. L., Oparil, S. & Blalock, J. E. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2518–2522
6. Knutson, V. P. (1988) *J. Biol. Chem.* **263**, 14146–14151
7. Al-Obeidi, F. A., Hruby, V. J., Sharma, S. D., Hadley, H. E. & De L. Castrucci, A. M. (1988) in *Peptides* (Rivier, J. E. & Marshall, G. R., eds.), pp. 530–532, ESCOM, The Netherlands
8. Pasqualini, R., Chamone, D. F. & Brentani, R. R. (1989) *J. Biol. Chem.* **264**, 14566–14570
9. Shai, Y., Flashner, M. & Chaiken, I. M. (1987) *Biochemistry* **26**, 669–675
10. Shai, Y., Brunch, T. K. & Chaiken, I. M. (1989) *Biochemistry* **28**, 8804–8811
11. Pasqual, D. W., Blalock, J. E. & Bost, K. L. (1989) *J. Immunol.* **143**, 3697–3702
12. Fassina, G., Zama, M., Burke, M. B. & Chaiken, I. M. (1989) *Biochemistry* **28**, 8811–8818
13. Muchalkey, J. J., Neill, J. D., Dion, L. D., Bost, K. L. & Blalock, J. E. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9714–9718
14. Grosvenor, C. E. & Balint, K. (1989) U.S. Patent 4833 166
15. Weigent, D. A., Hoepflich, P. D., Bost, K. L., Brunch, T. K., Reiher, W. E. & Blalock, J. E. (1986) *Biochem. Biophys. Res. Commun.* **139**, 367–374
16. Fassina, G., Roller, P. P., Olson, A. D., Thorgeirsson, S. S., & Omichinski, J. G. (1989) *J. Biol. Chem.* **264**, 11252–11257
17. Carr, D. J. J., Blalock, J. E. & Bost, K. L. (1989) *Immunol. Lett.* **20**, 181–186
18. Ghiso, J., Saball, E., Leoni, J., Rostagno, A. & Frangione, B. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1288–1291
19. Bost, K. L., Smith, E. M. & Blalock, J. E. (1985) *Biochem. Biophys. Res. Commun.* **128**, 1373–1380

20. Draper, K. G. (1989) *Biochem. Biophys. Res. Commun.* **163**, 466–470
21. Chaiken, I. M., Fassina, G. & Shai, Y. (1989) *Protein Recognition of Immobilized Ligands* (Hutchens, J. W., ed.), pp. 257–268, Alan Liss, New York
22. Blalock, J. E. & Smith, E. M. (1984) *Biochem. Biophys. Res. Commun.* **121**, 203–207
23. Fassina, G., Thorgeirsson, S. S. & Omichinski, J. G. (1989) *Methods in Protein Sequence Analysis* (Wittman-Liebold, B., ed.), pp. 431–4438, Springer Verlag, Berlin
24. Dinarello, C. A. (1984) *Rev. Infect. Dis.* **6**, 51–95
25. March, C. J., Mosley, B., Larsen, A., Cerretti, A. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P. & Cosman, D. (1985) *Nature (London)* **315**, 641–647
26. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132
27. Chaiken, I. M. (1987) *Analytical Affinity Chromatography*, CRC Press, Boca Raton, FL
28. Fassina, G. & Chaiken, I. M. (1987) *Adv. Chromatogr.* **27**, 247–297
29. Einarsson, S., Josefsson, B. & Lagerkvist, S. (1983) *J. Chromatogr.* **282**, 609–618
30. Priestle, J. P., Shar, H. P. & Grutter, M. G. (1988) *EMBO J.* **7**, 339–343
31. Eaton, H. L., Austin, R. E., Fesik, S. W. & Martin, S. F. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9767–9769
32. Najem, E. S., Corigliano-Murphy, A. & Ferretti, J. A. (1989) *FEBS Lett.* **250**, 405–410
33. Fassina, G. & Cassani, G. (1990) *Proceedings of the Protein Society Meeting, San Diego, CA*, abstract no. T28

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