A computer-designed hydropathically complementary peptide to human interleukin 1β (IL1β) precursor sequence 204–215 recognized the 204–215 peptide as well as the entire IL1β 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 IL1β-(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 IL1β-(204-215)-peptide and IL1β 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 IL1β-(204-215)-peptide in such a way as to alter their hydrophatic 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 IL1β 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 proteins and peptides, such as angiotensin II [5], insulin [6], melanocyte-stimulating hormone [7], fibronectin [8], s-peptide [9,10], substance P [11], [Arg6]vasopressin [12], luteinizing hormone-releasing hormone [13], growth hormone-releasing hormone [14], interleukin 2 [15], c-raf protein [16], enkephalins [17] and cysitatin [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 hydropathic complementarity derived from the hydropathic anti-correlation of complementary codons [22]. On the basis of this observation, a computer program has recently been developed which allows deduction of a hydropathically 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 (δIL) hydropathically complementary to residues 204–215 of interleukin (IL1β) precursor. The IL1β 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 δIL peptide affinity columns, and assessing the ability of immobilized δIL peptide to recognize IL1β-(204-215)-peptide as well as full-length IL1β from crude synthetic peptide mixtures or bacterial lysates.

MATERIALS

9-Fluorenlymethoxy carbonyl (Fmoc)-derivatized amino acids and 4-hydroxymethylphenoxyacetate 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. (Weiterstadt, Germany). Reversed-phase h.p.l.c-purified human IL1β and Escherichia coli extracts containing recombinant human IL1β were a gift from Dr. M. L. Melli, Centro Ricerche Sclavo (Siena, Italy).
METHODS

Computerized design of hydropathically complementary peptides

Selection of hydropathically complementary sequences to target IL1β-(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 hydrophobicity 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 hydropathy unit range is used. Then, the moving average hydropathy \( a \) [26] of the target sequence is calculated according to the formula:

\[
a_k = \sum_{i=1}^{n} h_{i}
\]

where \( h_i \) is the hydropathy value of each residue \( A_i \) of the target sequence, \( k \) is \((n-s)\), 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 \( b \) of all the complementary sequences is determined according to the expression:

\[
b_k = \sum_{i=k}^{n} g_{i}
\]

where \( g_i \) 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, \( \Theta \), which is inversely proportional to the degree of complementarity on a moving average base, and defined as:

\[
\Theta = \frac{1}{\sum (a_i + b_i)^2/(n-2s)}
\]

is selected as the best recognition sequence. The complementary sequence characterized by the lowest hydropathic score, \( \Theta \), 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 \( C_{18} \) column (25 cm x 1 cm internal diameter), equilibrated at a flow rate of 3 ml/min with water/acetonitrile/trifluoroacetic acid (800:200:1, by vol.)

Affinity column preparation

Immobilization of APIL (GGGDGGGDGRDDG) (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 NaHCO\textsubscript{3}/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 x 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-NH\textsubscript{2}. In the case of δIL (GGHILLFFPIIIAASL) and scrambled δIL (SAIL; GGGLILIPSAAIFL), owing to their poor solubility in aqueous buffers, peptides (5 mg) were dissolved in 10 ml of dimethyl sulfoxide/water (9:1, v/v) containing 500 μl 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 δIL (5 mg) was also immobilized on treosyl-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 δIL and soluble IL1β-(204–215)-peptide were determined by competitive elution experiments [27,28]. The extent of IL1β-(204–215)-peptide elution volume retardation on the δIL-Eupergit column was measured in the presence of increasing amounts of IL1β-(208–215)-peptide dissolved in the elution buffer. Data were plotted according to:

\[
\frac{1}{(V-V_d)} = \frac{K_{M,dep}}{M_T} + \frac{[D]K_{M,dep}}{K_{M,dep}}
\]

where \( V \) is the elution volume of IL1β-(204–215)-peptide, \( V_d \) the column void volume, \( K_{M,dep} \), the dissociation constant for the interaction between immobilized δIL and soluble IL1β-(204–215)-peptide, \([D]\) the concentration of IL1β-(208–215)-peptide in the buffer, \( K_{M,dep} \) the dissociation constant for the interaction between immobilized δIL and competing soluble IL1β-(208–215)-peptide, and \( M_T \) the total amount of immobilized δIL. In the absence of competing peptide in the buffer, eqn. (1) becomes:

\[
\frac{1}{(V-V_d)} = \frac{K_{M,dep}}{M_T}
\]

for \( P \ll M_T \).

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

Samples of crude bacterial lysates and affinity-purified recombinant IL1β were analysed by SDS/PAGE on a glass column (2.5 mm x 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.

Table 1. Specificity of IL1β-(204–215)-peptide binding to immobilized δIL

<table>
<thead>
<tr>
<th>Column</th>
<th>Ligand loading (μmol)</th>
<th>Retardation volume (ml)</th>
<th>Dissociation constant ( K_{M,dep} ) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(APIL)-Eupergit</td>
<td>2.5</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>(Gly-NH\textsubscript{2})-Eupergit</td>
<td>90.0</td>
<td>1.0</td>
<td>90</td>
</tr>
<tr>
<td>(SAIL)-Eupergit</td>
<td>0.6</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>(δIL)-Eupergit</td>
<td>0.7</td>
<td>350.0*</td>
<td>0.02</td>
</tr>
<tr>
<td>(δIL)-Sepharose</td>
<td>0.9</td>
<td>450.0*</td>
<td>0.02</td>
</tr>
<tr>
<td>(Gly-NH\textsubscript{2})-Sepharose</td>
<td>3.0</td>
<td>1.0</td>
<td>30</td>
</tr>
</tbody>
</table>

* Values determined by extrapolation at [D] = 0.
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 hydropathically complementary peptides to the IL1β(204–215)-peptide sequence

A peptide with hydropathic complementarity to the exposed and flexible IL1β(204–215)-peptide site (KNYPKKKMEKRF).

Fig. 1. Hydropathic profiles of IL1β(204–215)-peptide (A), δIL (■) and SδIL (●). 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 IL1β(204–215)-peptide to various affinity columns

Left: binding of h.p.l.c.-purified IL1β(204–215)-peptide to δIL 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 μg) of IL1β(204–215)-peptide dissolved in 200 μl 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-IL1β(204–215)-peptide. Right: binding of h.p.l.c.-purified IL1β(204–215)-peptide to Gly-NH₂-Eupergit (d), SδIL-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 IL1β to δIL–Eupergit (a), δIL–Sepharose (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 μg) of IL1β dissolved in 200 μl of eluting 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 hydropathically complementary peptide dissociation constants

The δIL–Eupergit column was equilibrated at a flow rate of 2 ml/min with 0.1 M-Tris, pH 6.8 containing IL1β(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 μg) of IL1β(204–215)-peptide dissolved in 200 μl of eluting buffer were injected on the column. Extent of IL1β(204–215)-peptide retardation on IL1β(204–215)-peptide competitor concentration is plotted in the inset according to eqn. (2).

[30] was designed using a computer program able to generate peptide sequences hydropathically complementary on a moving average basis to a target sequence using only the amino acid sequence of the target. The computer-generated complementary peptide (HLLFPiIIiAASL) (δIL) to the IL1β(204–215)-peptide...
sequence was designed using the software AMINOMAT (see the Methods section), and its hydropathic profile is reported in Fig. 1, and compared with the IL1β-(204-215) peptide profile. The complementary peptide (δIL) 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 IL1β and its fragment 204-215. In order to evaluate the sequence specificity of the interaction, and the role of complementarity of hydropathic profiles, the δIL sequence was scrambled in such a way as to abolish the hydropathic complementarity (Fig. 1). This peptide, denoted scrambled δIL (SδIL) (LILIHPSSAAFL), was also synthesized with a triglycine spacer at the N-terminus for subsequent immobilization on the solid phase. In a similar way, the IL1β-(204-215)-peptide sequence was scrambled (MFYKKENKKKK). A comparison was also made between the binding properties of the computer-generated hydropathically complementary peptide and a peptide obtained by complementing the charge of IL1β-(204-215)-peptide. This peptide was designed by replacing each negatively charged residue in the IL1β-(204-215)-peptide sequence with arginine, each positively charged residue with aspartate, and the remaining residues with glycine. Denoted APIL (DGGGDDGGRDDG), it was synthesized with a triglycine spacer at the N-terminus, and purified and immobilized on Eupergit C 30N to prepare an affinity column.

Hydropathically complementary peptide recognition: specificity

Binding characteristics of IL1β-(204-215)-peptide and IL1β towards immobilized δIL were evaluated by zonal and competitive elution experiments. Affinity columns derivatized with δIL were equilibrated with 50 mM-Tris, pH 6.8, at a flow rate of 1 ml/min. Samples of IL1β-(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 mM-acetic acid, 1 mM-Tris, pH 6.8, or 0.1 mM-IL1β-(204-215)-peptide (Fig. 2). In agreement with data reported previously [12,16,18], binding between hydropathically 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 IL1β-(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 IL1β-(204-215)-peptide which contains a tyrosine residue. Further evidence of binding specificity is provided by the inability of IL1β-(204-215)-peptide to bind to affinity columns derivatized with Gly-NH$_2$ (Fig. 2d). The hydropathic profile dependence of the interaction was demonstrated by the lack of recognition properties of a scrambled δIL 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 hydropathic 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 IL1β-(204-215)-peptide charge-complementary peptide (APIL) to bind
IL1β-(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 IL1β-(204-215)-peptide. Further evidence of sequence specificity was clearly shown by the inability of the scrambled IL1β-(204-215)-peptide to bind to the δIL–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 δIL peptide is also able to recognize the complementary sequence in the full-length IL1β molecule (Fig. 3a), whereas IL1β 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. δIL 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 IL1β molecule (Fig. 3b). In Table 1 are reported the observed retention volumes of IL1β-(204-215)-peptide on all the columns tested, and, from the $M_r$ values determined by amino acid analysis of measured amounts of derivatized supports, the derived dissociation constants $K_{M_r}$ are calculated according to eqn. (1) or (2).

**Determination of dissociation constants for hydropathically complementary peptide recognition**

Dissociation constants for the interaction between Eupergit-immobilized δIL and soluble IL1β-(204-215)-peptide were determined by zonal elution of fixed amounts of IL1β-(204-215)-peptide (100 μg) in the presence of various amounts of competing IL1β-(208-215)-peptide dissolved in the elution buffer, 50 mM-Tris, pH 6.8 (Fig. 4). The extent of IL1β-(204-215)-peptide retardation on the δIL–Eupergit column was plotted against IL1β-(208-215)-peptide concentration according to eqn. (1) (Fig. 4, inset). As predicted by eqn. (1), the elution volume of IL1β-(204-215)-peptide decreases with increasing amounts of IL1β-(208-215)-peptide in the buffer. Amino acid analysis of measured amounts of δIL–Eupergit indicated the presence of 0.3 μmol of immobilized peptide per ml of resin, and in the total column volume (2.3 ml) $M_r = 0.7$ μmol. Using this value as the functional capacity of the column, from the intercept and slope of the plot $1/(V_1 - V_0)$ against IL1β-(208-215)-peptide concentration, the values of $K_{M_r}$ (10 μM) and $K_{M_D}$ (80 μM) were graphically calculated. In agreement with data reported previously [9,10,12,16], the IL1β-(208-215)-peptide (which is shorter) recognizes immobilized δIL with weaker affinity ($K_{M_D} = 80$ μM).

**Hydropathically complementary peptide recognition: selectivity**

In order to evaluate the selectivity of hydropathically complementary peptide recognition, the ability of immobilized δIL 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 μg of h.p.l.c.-purified IL1β-(204-215)-peptide was added (Fig. 5c). In this mixture, IL1β-(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 δIL–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 IL1β-(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 IL1β-(204-215)-peptide with
synthetic h.p.l.c.-purified IL1β-(204-215)-peptide was also confirmed by comparing their tryptic maps (results not shown).

The same mixture was also applied to the δIL-Sepharose column under the same experimental conditions as used for the δIL-Eupergit column. In this case, a slightly different reversed-phase h.p.l.c. elution gradient was adopted better to identify IL1β-(204-215)-peptide in the crude mixture. The IL1β-(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 IL1β (Fig. 7). A 1 ml portion of E. coli lysate was applied to the δIL-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 h.p.e.c. (Fig. 7c). Recovery of starting material was close to 70%, and 50 μg of recombinant IL1β 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 adenocorticotropin [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 IL1β 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 δIL to recognize IL1β-(204-215)-peptide and full-length IL1β from complex mixtures containing different unrelated molecules (Figs. 5, 6 and 7). Immobilized δIL 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 IL1β-(208-215)-peptide in the buffer (Figs. 2c and 4), and by the inability of IL1β-(204-215)-peptide and mature IL1β 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 δIL in aqueous buffers, the failure of APIL, the charge-complementary peptide to IL1β-(204-215)-peptide, to recognize IL1β-(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 δIL to recognize IL1β-(204-215)-peptide as well as mature IL1β from complex mixtures containing different molecules covering a wide range of hydrophobics (Figs. 5, 6 and 7).

Furthermore, the failure of immobilized scrambled δIL to recognize IL1β-(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 δIL proved to be a sequence-directed ligand useful for affinity purification of recombinant IL1β 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 immunoadfinity sorbents. Further, the small ligand size allows easy separation of leaked or fragmented ligand from the purified macromolecule simply by dialysis or gel filtration.

We are grateful to Dr. Maria Luisa Mellì, Centro Ricerche Scavo (Siena, Italy), for providing the recombinant E. coli IL1β extract, and to Simona Germani for her excellent technical assistance.

**REFERENCES**

A hydropathically complementary peptide to human interleukin 1β


Received 27 March 1991/5 August 1991; accepted 22 August 1991