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Mapping the binding surface of interleukin-8 complexed with an N-terminal fragment of the Type 1 human interleukin-8 receptor

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Abstract

Interleukin-8 and its receptors are key mediators of immune and inflammatory responses. Heteronuclear NMR spectroscopy has been utilized to map the binding surface on interleukin-8 (IL-8) for an N-terminal fragment of the human Type-1 IL-8 receptor. A peptide corresponding to residues 1–40 of the IL-8 type 1 receptor (IL8-r1) was titrated into a sample of uniformly ^{15}N-labeled IL-8. IL8-r1 binds to IL-8 with a dissociation constant of 170 ± 50 μM assuming the peptide binds with a stoichiometry of one peptide per IL-8 monomer, exchanges rapidly (> 900 s^{-1}) between free and bound states, and selectively perturbs the chemical environment of several IL-8 residues. The binding surface on IL-8 suggested by our results is comprised of residues in strand β3 of the β-sheet (Glu^{48} to Cys^{50}), the turn preceding β3 (Ser^{44}), the C-terminal α-helix (Val^{61}) and the irregular N-terminal loop region (Thr^{12}, Lys^{15}, Phe^{17}, His^{18}, Lys^{20} and Phe^{21}). The IL-8 dimer appears to present two symmetrical binding surfaces for the IL8-r1 peptide, suggesting two receptor peptides may bind per dimer.

Key words: Interleukin-8; Interleukin-8 receptor; Protein NMR; Cytokine-receptor interaction

1. Introduction

Interleukin-8 (IL-8) [1] is a dimeric chemokine (M_r ~ 16 kDa) released by several cell types, including monocytes, fibroblasts, endothelial cells, and keratinocytes, in response to an inflammatory stimulus [2]. In vivo and in vitro studies indicate that IL-8 functions in a wide range of proinflammatory activities, which include neutrophil activation [3], and the selective capacity to attract neutrophils [4] and T cells [1]. IL-8 is a member of the α-chemokine family of chemotactic cytokines which also includes platelet factor 4 (PF4), β-thromboglobulin, γ-interferon-induced protein, neutrophil activating protein 2 (NAP-2) and human growth related protein (GRO) (also known as melanoma growth stimulating activity; MGSA). Structurally, members of the family share 25–55% sequence identity and the sequence specific placement of four conserved cysteine residues. The three dimensional structures of two members of the family, IL-8 [5,6] and PF4 [7] have been determined and share a common polypeptide fold, consisting of two antiparallel α-helices lying on top of a six-stranded antiparallel β-sheet.

Members of the IL-8 family modulate the activities of their target cells by binding to cell surface receptors. The amino acid sequences of two classes of IL-8 receptors with nearly equal affinity for IL-8 have been determined (Type 1 and Type 2) [8–12]. The two classes are similar, sharing 74% sequence identity, but deviate substantially at their N-termini and in their ability to bind the cytokine GRO/MGSA and NAP-2 [12,13]. Both receptors are members of the rhodopsin family, receptors with seven membrane-spanning helices that couple to guanine nucleotide binding proteins [14]. Recent functional studies indicate that peptides generated from the N-termini of both receptor types inhibit IL-8 binding, and only peptides derived from the type 2 receptor inhibit GRO/MGSA binding to the intact IL-8 type 2 receptor [15]. Complementary mutagenesis studies of IL-8 have identified the N-terminus (Glu^4–Leu^5–Arg^6 and Ile^{10}) as important in receptor binding and cell activation [16,17]. However, this is not the only structurally pertinent region of IL-8 since GRO/MGSA contains the Glu-Leu-Arg sequence but inhibits IL-8 binding to Type 2 receptor only. Furthermore, IL-8 analogs shortened at the C-terminus show progressively reduced potency as residues are deleted [18].

In the present paper the effects of receptor binding on the conformation of IL-8 were investigated by heteronuclear NMR spectroscopy. A fragment of the receptor, corresponding to residues 1–40 of the IL-8 type 1 recep-

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Abbreviations: GRO, growth related protein; IL-8, interleukin-8; IL8-r1, human type 1 IL-8 receptor 1–40 peptide; HSQC, heteronuclear single quantum coherence; MGSA, melanoma growth stimulating activity; NAP-2, neutrophil activating protein 2; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; PF4, platelet factor 4.

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tor (IL8-r1) was titrated into a sample of uniformly ^{15}N-labeled IL-8. The effects of IL8-r1 binding on the conformation of IL-8 were then monitored by acquisition of heteronuclear NMR spectra of the IL-8:IL8-r1 complex. We find that the receptor peptide binds to IL-8 with a dissociation constant of either 170 ± 50 μM or 340 ± 90 μM if the peptide binds to IL-8 with a stoichiometry of one peptide per IL-8 monomer or one peptide per IL-8 dimer, respectively; exchanges rapidly (> 900 s^{-1}) between free and bound states; and selectively perturbs the magnetic environment of several IL-8 residues.

2. Materials and methods

Recombinant IL-8 uniformly enriched with ^{15}N was prepared as described previously [19,20]. A peptide comprising the N-terminal 40 residues of human Type 1 IL-8 receptor (IL8-r1) was synthesized using solid-phase methods and purified by reverse phase high performance liquid chromatography. The amino acid sequence of this peptide substitutes Ser for Cys at position 30 to prevent disulfide formation and is as follows:

MSNITDPQMW[10] DFDDLNFTGM[20] PPADEDYSPS[30] MLETETLNKY[40]

NMR spectra were recorded on a Bruker AMX-500 spectrometer at 26.6°C and NMR data were processed using the in-house nmrPipe software (F. Delaglio, unpublished). Two-dimensional (2D) ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra [21] were acquired with 128 and 512 complex points in ω_1 and ω_2, respectively. The 3D ^{1}H-^{15}N NOESY-HSQC spectrum [22,23] was acquired with 108, 38 and 512 complex points in ω_1, ω_2 and ω_3, respectively. Quadrature detection in the indirect dimensions of all experiments was accomplished with the TPPI-States method [24].

The IL-8 NMR sample was 0.75 mM in monomer subunits, 50 mM potassium phosphate, pH 6.7, dissolved in 500 μl of 90% $^{1}H_2O$/10% $^{2}H_2O$. The ^{1}H-^{15}N HSQC spectrum of unligated IL-8 was sequence specifically assigned using previously reported chemical shifts [19,20]. Lyophilized aliquots containing 0.5 mgs of IL8-r1 peptide were dissolved into the IL-8 sample and a ^{1}H-^{15}N HSQC spectrum recorded. A total of four peptide additions were made resulting in final peptide concentrations of 0.22, 0.43, 0.65 and 0.86 mM. The final addition results in a IL8-r1 peptide to IL-8 monomer ratio of 1.25:1.

3. Results

A fragment of the IL-8 receptor, corresponding to residues 1–40 of the IL-8 type 1 receptor (IL8-r1) was titrated into a sample of uniformly ^{15}N-labeled IL-8. NMR spectra of the IL8-r1 peptide alone showed no evidence of structure. Titration of the IL8-r1 peptide perturbed the amide ^{1}H and ^{15}N chemical shifts of a select set of IL-8 residues. Some of these IL8-r1-dependent chemical shift changes are illustrated in Fig. 1, which shows an overlay of the ^{1}H-^{15}N HSQC spectra of free IL-8 (solid lines) and the IL-8:IL8-r1 complex (dashed lines) at a IL8-r1 peptide to IL-8 monomer ratio of 1.25:1. The ^{1}H-^{15}N cross-peaks arising from Thr[12] and Ser[44] of IL-8 are substantially affected by the addition of peptide. In contrast, the ^{1}H-^{15}N cross-peaks from Lys[54] and Trp[57] are unchanged, and cross-peaks from Arg[60], Lys[67] and Glu[70] are only minimally perturbed. The identity of all displaced cross peaks was confirmed through analysis of the 3D NOESY-^{1}H-^{15}N HSQC spectrum of the IL-8:IL8-r1 complex.

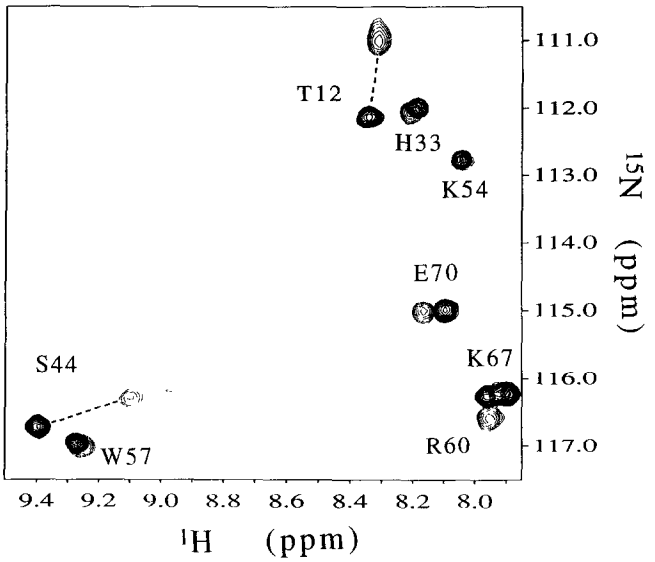

Fig. 1. Effects of IL8-r1 peptide binding on the ^{1}H-^{15}N HSQC spectrum of IL-8. A selected region of the overlaid ^{1}H-^{15}N HSQC spectra of free IL-8 and the IL-8:IL8-r1 complex is shown. Cross-peaks for free IL-8 and IL-8:IL8-r1 are drawn with solid and dashed lines, respectively. The ratio of IL8-r1 peptide to IL-8 monomer is 1.25:1.

Fig. 2 shows a plot of the chemical shift differences for selected residues between unligated IL-8 and the IL-8:IL8-r1 complex as function of peptide concentration. The chemical shifts are perturbed as peptide is added and approach limiting chemical shift deviations from unligated IL-8 at saturating IL8-r1 concentrations, indicating that the perturbations are a result of IL8-r1 binding and are unlikely to stem from changes in the solution conditions of the sample (i.e. pH, ionic strength, etc.). As a further check of this assumption the pH of the final IL-8:IL8-r1 complex (0.75 mM IL-8 monomer and 0.86 mM IL8-r1 peptide) was measured and a ^{1}H-^{15}N HSQC spectrum of a pH matched sample of unligated IL-8 was recorded. The unligated spectrum did not exhibit the chemical shift changes of the complexed sample (data not shown).

The appearance of a single set of NMR resonances indicates that the exchange process is fast on the NMR time scale. The IL-8 resonances in the presence of the IL8-r1 peptide are the population weighted average of the chemical shifts of free and bound IL-8 species. The dependence of these chemical shifts on peptide concentration therefore provides a convenient indirect measure of the concentration of the IL8-r1:IL-8 complex. Eq. 1 describes this dependency and was used to fit binding isotherms to the data by least-squares minimization (Fig. 2):

$$\Delta obs = \Delta max\ ([C]/[IL\text{-}8]_t) \quad (1)$$

where [C] is the concentration of the IL8-r1:IL-8 complex, $[IL\text{-}8]_t$ the total concentration of IL-8 (either dimer or monomer), Δobs the observed chemical shift change, and Δmax the chemical shift difference between the IL-8 and the IL8-r1 complex. The seven curves shown in Fig. 2 were fitted simultaneously by optimizing a single value for the dissociation constant and the values of Δmax for each curve. All the experimental data could be fitted within experimental error and there are no systematic deviations between the observed and calculated curves. Thus all seven binding isotherms are the same. The value of the dissociation constants obtained is 170 ± 50 μM for a model in which two IL8-r1 peptides bind per IL-8 dimer (i.e. one IL8-r1 peptide per IL-8 monomer), and 340 ± 90 μM for a model in which one IL8-r1 peptide binds per IL-8 dimer.

Plots of the ^{15}N and ^{1}H chemical shift differences between the ^{1}H-^{15}N HSQC spectra of unligated IL-8 and the IL-8:IL8-r1 complex are displayed in Fig. 3A and B, respectively. The backbone ^{15}N chemical shifts of residues Thr[12], His[18], Lys[20], Glu[48], Leu[49], Cys[50] and Val[61] as well as the sidechain N^{ε} resonances of Q8, exhibit the largest displacements (Fig. 3A). The peptide dependent changes in the amide ^{1}H chemical shifts of IL-8 are less dramatic, with the backbone proton resonances of Lys[15], Phe[17], Phe[21], Ser[44] and Val[61] exhibiting the largest perturbations (Fig. 3B). The largest ^{15}N and ^{1}H chemical shift differences between unligated and complexed IL-8 are 1.77 (Leu[49]) and 0.3 (Ser[44]) ppm, respectively. Hence the chemical exchange rate between the free and bound states of the peptide must exceed 900 s^{-1} (i.e. $> 2\pi\Delta\delta$ where $\Delta\delta$ is the chemical shift difference in Hz between the free and bound states for a system where exchange is fast on the chemical shift scale).

4. Discussion

It is possible to postulate a binding area C on IL-8 for the IL8-r1 peptide from the observed peptide-dependent chemical shift changes in the resonances of IL-8. If we assume that the most pronounced perturbations of the magnetic environment of IL-8 residues occur at sites close to the bound peptide, resonances from those interacting residues should exhibit the largest chemical shift changes upon peptide binding. Fig. 4 shows a schematic representation of the solution structure of the IL-8 dimer with the Cα atoms of residues which exhibit the largest ^{15}N (> |0.47| ppm) and ^{1}H (> |0.1| ppm) chemical shift changes marked with black and white spheres, respectively. These residues form a cluster on each monomer unit with residues from strand β3 of the β-sheet (Glu[48] to Cys[50]), the turn preceding β3 (Ser[44]), the C-terminal α-helix (Val[61]), and the irregular N-terminal loop region (Thr[12], Lys[15], Phe[17], His[18], Lys[20] and Phe[21]). Thus, the IL-8 dimer contains two binding areas for the peptide, rather than one continuous surface, suggesting that two peptides bind per dimer, each one sliding into the cleft between strand β3 and the 12–21 loop. Since the IL8-r1 peptide is acidic (9 of 40 residues are aspartic or glutamic acids) basic residues of IL-8 may be involved in the interaction. Inspection of the region highlighted in Fig.

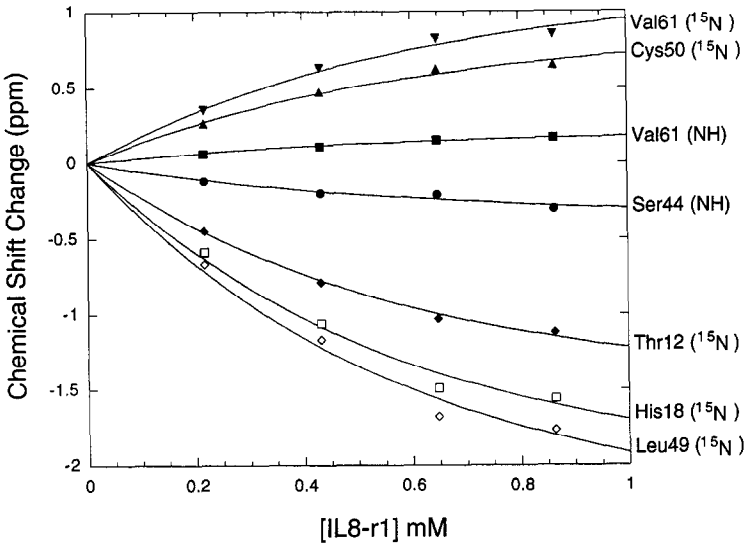

Fig. 2. Chemical shift differences between the ^{1}H-^{15}N HSQC spectra of unligated IL-8 and the IL-8:IL8-r1 complex as a function of IL8-r1 concentration. Each point results from subtracting the chemical shift of unligated IL-8 from the shifts of the IL-8:IL8-r1 complex. Residues Thr[12], His[18], Ser[44], Leu[49], Cys[50] and Val[61] are shown. 'HN' and '^{15}N' denote proton and ^{15}N chemical shifts, respectively. The solid lines represent non-linear least squares best fits to the data for simple equilibrium binding isotherms using the program FACSIMILE [26]. All seven curves were fitted simultaneously by optimizing the value of the maximum chemical shift change Δmax for each curve and a single value for the dissociation constant. The dissociation constant obtained in this manner is 170 ± 50 μM for a stoichiometry of two peptide molecules per dimer (i.e. one peptide per monomer) and 340 ± 90 μM for a stoichiometry of one peptide per dimer.

4 indicates that Lys[11], Lys[15], Lys[20], Lys[42] and Arg[47] are plausible candidates for this. In addition, Tyr[13], Phe[21] and Leu[49] may be involved in hydrophobic contacts with the receptor.

The peptide binding region deduced by NMR (Fig. 4) does not coincide with those residues identified as important for receptor interaction by previous mutagenesis studies of the IL-8 dimer [16,17]. These studies identified residues Glu[4] to Arg[6] and Ile[10] of IL-8 as important in receptor binding and activation [16,17]. Resonances from Lys[3], Glu[4] and Arg[5] are unresolved in the ^{1}H-^{15}N HSQC spectra of free and complexed IL-8, preventing accurate analysis of their peptide dependent chemical shift changes. These residues, however, are probably not substantially affected by peptide addition, since they are not significantly shifted upon complexation. Furthermore, the absence of any significant shift in the well resolved resonances of Ala[2] and Leu[5] indicates that the N-terminus of IL-8 which is unstructured in the free state [5,19], remains unstructured in the complexed form. We therefore believe that the N-terminal region of the IL-8 dimer may form contacts with regions other than the N-terminal segment of the IL8 receptor. Recent mutagenesis studies of the IL-8 receptor support this notion, indicating that both the N-terminus and the third extracellular loop of the receptor are essential determinants of IL-8 binding [25].

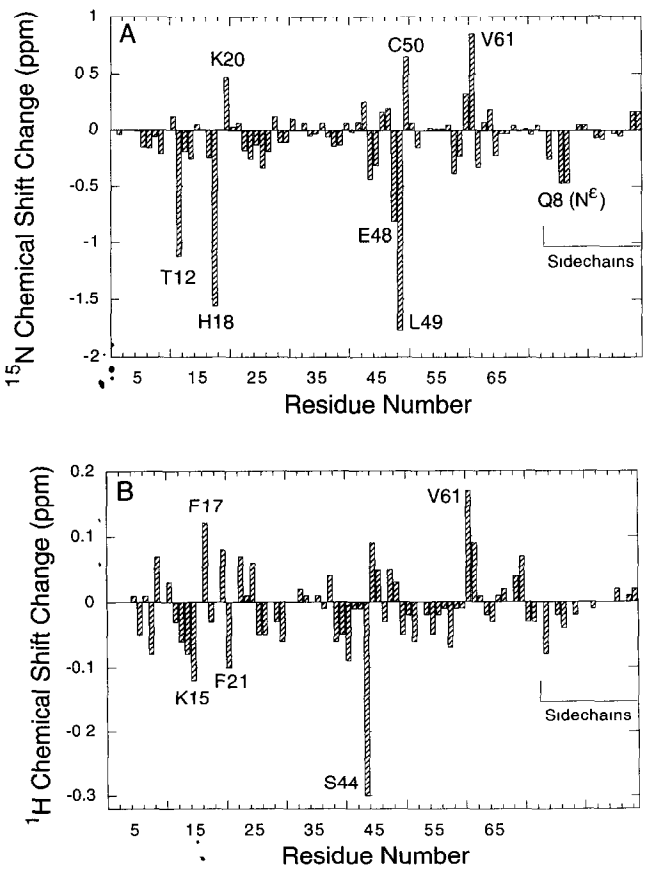

Fig. 3. (A) ^{15}N and (B) ^{1}H chemical shift differences between the ^{1}H-^{15}N HSQC spectra of unligated IL-8 and the IL-8:IL8-r1 complex versus sequence number. Excluded resonances are overlapped in the ^{1}H-^{15}N HSQC spectra. The ratio of IL8-r1 peptide to IL-8 monomer is 1.25:1 in the complex.

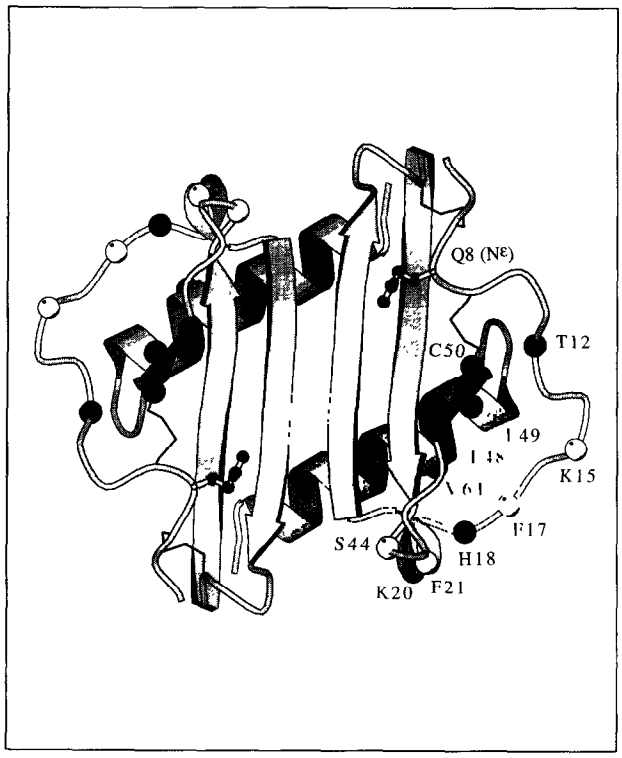

Fig. 4. Schematic ribbon diagram of the solution structure of the IL-8 dimer. The Cα atoms of residues that exhibit the largest ^{15}N (> |0.487| ppm) and ^{1}H (> |0.1| ppm) chemical shift changes are denoted with black and white spheres, respectively. The figure was generated using the program MOLSCRIPT [22]. The coordinates of IL-8 are taken from [5] (PDB accession code 1IL8).

Although certain features of the IL-8/IL8-r1 peptide complex seem to mimic the situation of the intact IL-8 receptor interaction [15], it has to be stressed that our results should be interpreted with caution since the peptide/protein model system lacks several key features of the intact IL-8:IL-8 receptor system. In particular, extracellular loops of the IL-8 receptor, N-glycosylation of residues at its N-terminus, a cysteine residue at position 30, and the unique local environment present at the surface of the membrane are absent in the peptide model. Nevertheless, our results may provide some guidance with regard to mutagenesis studies on particular regions of IL-8. Future NMR work on IL-8 interacting with peptide fragments derived from other extracellular segments of the IL-8 receptor should complement these studies.

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References

[1] Larsen, C.G., Anderson, A.O., Apella, E., Oppenheim, J.J. and Matsushima, K. (1989) Science 243, 1464–1466.
[2] Matsushima, K. and Oppenheim, J.J. (1989) Cytokine 1, 2–13.
[3] Thelen, M., Peveri, P., Kernen, P., von Tscharner, V., Walz, A. and Baggiolini, M. (1988) FASEB J. 2, 2702–2706.
[4] Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E.A., Apella, E., Oppenheim, J.J. and Leonard, E. (1987) Proc. Natl. Acad. Sci. USA 84, 9233–9237.
[5] Clore, G.M., Appella, E., Yamada, M., Matsushima, K. and Gronenborn, A.M. (1990) Biochemistry 29, 1689–1696.
[6] Baldwin, E.T., Weber, I.T., St. Charles, R., Xuan, J.C., Apella, E., Yamada, M., Matsushima, K., Edwards, B.F., Clore, G.M., Gronenborn, A.M. and Wlodawer, A. (1991) Proc. Natl. Acad. Sci USA 88, 502–506.
[7] St. Charles, R., Walz, D.A. and Edwards, B.F.P. (1989) J. Biol. Chem. 264, 2092–2099.
[8] Beckman, M.P., Munger, W.E., Kozlosky, C., VandenBos, T., Price, V., Lyman, S., Gerard, N.P., Gerard, C. and Cerretti, D.P. (1991) Biochem. Biophys. Res. Commun. 179, 784–789.
[9] Holmes, W.E., Lee, J., Kuang, W.J., Rice, G.C. and Wood, W.I. (1991) Science 253, 1278–1280.
[10] Murphy, P.M. and Tiffany, H.L. (1991) Science 253, 1280–1283.
[11] Thomas, K.M., Taylor, L. and Navarro, J. (1991) J. Biol. Chem. 266, 14839–14841.
[12] Cerretti, D.P., Kozlosky, C.J., VandenBos, T., Nelson, N., Gearing, D. and Beckman, M.P. (1993) Mol. Immunol. 30, 359–367.
[13] Lee, J., Kuang, W.J., Rice, G.C. and Wood, W.I. (1992) J. Immunol. 148, 1261–1264.
[14] Dohlman, H.G., Caron, M.G. and Lefkowitz, R.J. (1987) Biochemistry 26, 2657–2664.
[15] Gayle III, R.B., Sleath, P.R., Birks, C.W., Weerawarna, K.S., Ceretti, D.P., Kozlosky, C.J., Nelson, N., VandenBos, T. and Beckmann, M.P. (1992) J. Biol. Chem. 268, 7283–7289.
[16] Hebert, C.A., Vitangcol, R.V. and Baker, J.B. (1991) J. Biol. Chem. 266, 18989–18994.
[17] Moser, B., Dewald, B., Barella, L., Schumacher, C., Baggiolini, M. and Clark-Lewis, I. (1993) J. Biol. Chem. 268, 7125–7128.
[18] Clark-Lewis, I., Shumacher, C., Baggiolini, M. and Moser, B. (1991) J. Biol. Chem. 266, 23128–23134.
[19] Grasberger, B.L., Gronenborn, A.M. and Clore, G.M. (1993) J. Mol. Biol. 230, 364–372.
[20] Clore, G.M., Apella, E., Yamada, M., Matsushima, K. and Gronenborn, A.M. (1989) J. Biol. Chem. 264, 18907–18911.
[21] Bodenhausen, G. and Ruben, D.L. (1980) Chem. Phys. Lett. 69, 185–188.
[22] Marion, D., Driscoll, P.C., Kay, L.E., Wingfield, P.T., Bax, A., Gronenborn, A.M. and Clore, G.M. (1989) Biochemistry 28, 6150–6156.
[23] Zuiderweg, E.R.P. and Fesik, S.W. (1989) Biochemistry 28, 2387–2391.
[24] Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989) J. Magn. Reson. 85, 393–399.
[25] Herbert, C.A., Chuntharapai, A., Smith, M., Colby, T., Kim, J. and Horuk, R. (1992) J. Biol. Chem. 268, 18549–18553.
[26] Chance, E.M., Curtis, A.R., Jones, I.P. and Kirby, C.R. (1977) A.E.R.E. Report No. 8775, United Kingdom Atomic Energy Authority, Harwell.
[27] Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.