Multiple Amino Acid Substitutions Suggest a Structural Basis for the Separation of Biological Activity and Receptor Binding in a Mutant Interleukin-1β Protein†

Philip E. Auron,*,‡ Gary J. Quigley,§ Lanny J. Rosenwasser,∥ and Lee Gehrke*,⊥

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02114, The Center for Blood Research, Boston, Massachusetts 02115, L. E. Martin Laboratories, Massachusetts General Hospital, Charlestown, Massachusetts 02129, Department of Chemistry, Hunter College, New York, New York 10021, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206, Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT: Receptor binding and biological activity properties of human interleukin-1β can be dissociated by mutating a single amino acid, arginine 127, to glycine (IL-1$\beta_{R\rightarrow G}$) [Gehrke et al. (1990) *J. Biol. Chem. 265,* 5922–5925]. The mechanism underlying the reduced biological activity has been examined by replacing arginine 127 with several other amino acids, followed by determination of biological activity using a T-helper cell proliferation assay. Mutant IL-1β proteins containing lysine, glutamic acid, tryptophan, or alanine in place of arginine 127 maintain biological activity. These data strongly suggest that IL-1β biological activity is not directly dependent upon the specific properties of charge, hydrophobicity/hydrophilicity, or side-chain group presented by the residue at position 127. Molecular modeling analyses indicate that the structural integrity of the antiparallel β-strand 1/12 pair is disturbed in the glycine 127 mutant protein. Collapse of β-strand 1 into a hydrated space between strands 1, 2, and 4 could structurally alter a cleft in IL-1β that contains a cluster of highly conserved amino acids, including a key aspartic acid residue [Ju et al. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88,* 2658–2662]. Mutagenesis data and the differential activities of the IL-1$\beta_{R\rightarrow G}$ and IL-1 receptor antagonist proteins in stimulating early and late gene expression [Conca et al. (1991) *J. Biol. Chem. 266,* 16265–16268] suggest that multiple receptor–ligand contacts, exclusive of those required for receptor binding, are required for the stimulation of full IL-1 biological activity.

The interleukin-1 proteins, IL-1α and IL-1β, are cytokines that are important for their roles in immunity, inflammation, and hematopoiesis (Dinarello, 1991). Both forms of the protein bind to the same cellular receptors (Dower et al., 1986; Kilian et al., 1986; Sims & Dower, 1990) and, with few exceptions (Boraschi et al., 1990; Calkins et al., 1990), elicit the same biological responses. The interleukin-1 proteins are potent hormones that mediate a variety of effects in many different cell types, suggesting that regulation of IL-1 expression and activity must be tightly controlled. A third form of IL-1, the naturally occurring receptor antagonist protein (IL-1ra) (Carter et al., 1990; Hannum et al., 1990; Seckinger et al., 1987) may play an important role in modulating the effects of IL-1. Interleukin-1 biological activity is initiated by interaction with either the type I or type II cellular IL-1 receptors (Dower et al., 1985; Sims & Dower, 1990; Sims et al., 1988; Spriggs et al., 1990), each of which possesses three immunoglobulin-fold extracellular ligand-binding domains. The IL-1ra also binds to IL-1 receptors, but this protein has not been reported to elicit biological responses (Dripps et al., 1991). The three-dimensional structures of both IL-1α and IL-1β have been reported (Clore et al., 1991; Driscoll et al., 1990; Finzel et al., 1989; Graves et al., 1990; Priestle et al., 1988, 1990), and both receptor types have been molecularly cloned and expressed (McMahan et al., 1991; Sims et al., 1988; Spriggs et al., 1990). The details of specific IL-1 receptor–ligand interactions and the signaling events which follow are, however, not yet resolved.

In the absence of a high-resolution structure for the IL-1 receptor–ligand complex, one approach for characterization of IL-1 structure–function relationships is to identify specific amino acids which might be important for either receptor binding or biological activity, to mutate these amino acids, and then to characterize the biological activity of the mutated protein. Using this approach, we recently characterized and reported a point mutant human IL-1β protein which binds cellular receptors with the same affinity as that of the native protein but exhibits minimal biological activity in a T-helper cell proliferation assay (Gehrke et al., 1990). By changing an arginine residue (R_{127})[1] in β-strand 1 of the native protein to a glycine, we demonstrated that the receptor binding and biological activity domains of the protein are at least partially distinct. Subsequently, Ju et al. (1991) and Young et al. (1990) reported similar effects resulting from mutating other amino acids located in the hydrogen-bonded antiparallel β-strands 1 and 12 of IL-1β. Ju et al. (1991) also showed that the IL-1ra protein can be converted to a partial agonist by mutating a residue in a similar structural location. These

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* Address correspondence to either of these authors.

‡ Department of Pathology, Harvard Medical School, The Center for Blood Research, and Massachusetts General Hospital.

§ Department of Chemistry, Hunter College.

∥ Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine.

⊥ Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, and Department of Anatomy and Cellular Biology, Harvard Medical School.

[1] The numbering convention corresponds to the human proIL-1β precursor sequence (Auron et al., 1984). The mature IL-1β protein comprises amino acids 117–269 of the precursor.

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data support our proposal (Gehrke et al., 1990) that structural features of the strand 1 and strand 12 environment are related to IL-1 biological activity.

In this paper, we describe experiments and structural interpretation aimed at elucidating the basis for the loss of biological activity in the IL-1$\beta_{R\rightarrow G}$ protein (Gehrke et al., 1990); moreover, we speculate on features of the IL-1β molecule which are important for triggering biological functions. We have characterized the biological activity of IL-1β mutant proteins substituted with lysine (K), glutamic acid (E), tryptophan (W), and alanine (A) at the same site (position 127) as the previously reported glycine (G) substitution. While the biological activity of the IL-1$\beta_{R\rightarrow G}$ protein was greatly diminished as described previously, the K, E, W, and A substitutions did not have a significant effect upon the biological activity of the mutant proteins. The fact that the 127 position can tolerate all manner of replacements possessing a side-chain Cβ atom, including alanine, which is incapable of forming side-chain hydrogen bonds or salt bridges, leads us to conclude that the packing of amino acid residues plays a central role in maintaining the IL-1β function. Arginine 127 structurally supports other residues which define a cleft in the IL-1 molecule. Part of this cleft was originally noted by Clore et al. (1991) and has at its center the aspartic acid residue (D_{261}) which was mutated by Ju et al. (1991) to generate a protein which binds receptor but has diminished bioactivity. This "D_{261} cleft" contains a cluster of amino acids which are conserved in IL-1α and IL-1β proteins from five different species and which may be important for initiating the IL-1 biological response. Molecular modeling suggests that, by introducing the glycine, lacking a β-carbon, the structure of the protein may be perturbed through collapse of β-strand 1 into the hydrated space bounded by strands 1, 2, and 4. This loss of structural support for the D_{261} cleft is likely to be related to the loss of late signal transduction observed using the IL-1$\beta_{R\rightarrow G}$ mutant protein (Conca et al., 1991).

MATERIALS AND METHODS

Amino Acid Substitutions and Protein Expression. The synthesis of mature human IL-1β and IL-1β mutant proteins by in vitro transcription and translation has been described in previous publications (Conca et al., 1991; Gehrke et al., 1990; Jobling et al., 1988). Starting with the IL-1β cDNA (Auron et al., 1984), specific amino acid substitutions at position 127 (position 11 from the mature amino terminus) were introduced by subcloning annealed synthetic DNA oligonucleotides containing nucleotide changes in the Arg_{127} codon. The nucleotide sequence of DNA from recombinant clones was verified by chain termination DNA sequence analysis (Sanger et al., 1977). IL-1β messenger RNA was transcribed from a bacteriophage T7 promoter and translated in a messenger RNA-dependent rabbit reticulocyte protein synthesizing lysate (Promega Biotec) (Conca et al., 1991; Gehrke et al., 1990; Jobling et al., 1988). IL-1β protein concentrations were assayed by determining [^{35}S]methionine specific activity or by ELISA (Cistron Biotechnology).

IL-1β Biological Activity. The D10.G4.1 T-helper cell proliferation assay (Kaye et al., 1983) was used to determine IL-1 biological activity as described previously (Gehrke et al., 1990; Jobling et al., 1988).

Molecular Modeling. Protein structure modeling and inspection of human IL-1β were accomplished using the FRODO computer package (Jones, 1985) running on a Digital Equipment Corp. VAX minicomputer interfaced to an Evans and Sutherland color graphics display system. The coordinate data used for generating the human IL-1β structures used in this study were obtained from the Brookhaven Protein Data Bank as identification code 1I1B deposited by B. C. Finzel, L. L. Clancy, D. R. Holland, S. W. Muchmore, K. D. Watenpaugh, and H. M. Einspahr (January 15, 1990). These coordinates were derived from refinement of 2.0-Å X-ray diffraction data (Finzel et al., 1989).

RESULTS

The residues which replaced R_{127} in these experiments were selected to test the relative importance of amino acid charge, hydrophobicity/hydrophilicity, and structure in maintaining IL-1β biological activity. The native arginine was changed to a lysine in order to maintain the positive charge in the light of a slightly different side-chain structure. In a second construct, glutamic acid replaced arginine so that the impact of a negative, rather than a positive, charge at position 127 could be tested. In the IL-1 receptor antagonist molecule (IL-1ra) (Arend et al., 1990; Carter et al., 1990; Eisenberg et al., 1990; Hannum et al., 1990), a tryptophan is found in the position aligning with R_{127} in IL-1β (Figure 1); therefore, we replaced R_{127} with tryptophan, bearing its bulky hydrophobic side chain. In a fourth construct, alanine was substituted for Arg_{127}. Alanine, like glycine, is a small amino acid which has no potential for side-chain hydrogen bonding; however, the methyl group side chain of alanine (like the side chains of all other amino acids) offers greater steric hindrance to rotation about the N–Cα and Cα–C′ bonds than the glycine hydrogen, resulting in less backbone flexibility for alanine than glycine.

The results of the biological activity responses elicited by each of the proteins in the D10.G4.1 T-helper cell proliferation assay (Kaye et al., 1983) are shown in Figure 2. We have shown previously that the reticulocyte lysate in vitro translation system is a rapid method for expression of biologically active IL-1β proteins in yields of approximately 1–5 μg of IL-1β protein/mL of translation reaction mixture (Gehrke et al., 1990; Jobling et al., 1988). The advantages of this system are that mutant proteins can be generated and tested rapidly without extensive purification and without the use of denaturants to increase solubility of expressed proteins. The data (Figure 2) demonstrate that the biological activity profiles of the wild-type (WT), R → E, R → K, R → W, and R → A proteins are similar. We have subsequently learned that substituting serine at position 127 has no effect upon IL-1β biological activity and receptor binding affinity (K. Vosbeck, personal communication). The IL-1$\beta_{R\rightarrow G}$ protein elicits minimal activity at picomolar concentrations, where the wild-type and other position 127 muteins are maximally active, but the IL-1$\beta_{R\rightarrow G}$ mutein stimulates a full response at high (nanomolar) concentrations [Figure 2 and Gehrke et al. (1990)]. The amino acid substitution data strongly suggest that neither charge, hydrogen-bonding potential, nor specific amino acid side-chain groups at position 127 play a significant role in maintaining the biological activity of the protein. Furthermore, the fact that the IL-1$\beta_{R\rightarrow W}$ mutant is fully active suggests that the tryptophan positioned similarly in the IL-1ra molecule is not directly responsible for the absence of IL-1ra agonist activity.

In order to begin to interpret the unexpected generic activity of the substitution mutants, the IL-1 crystal structure was examined. Previous studies (Clore et al., 1990· Finzel et al., 1989; Priestle et al., 1988) had revealed that Arg_{127} is positioned near a hydrated pocket, located between strands

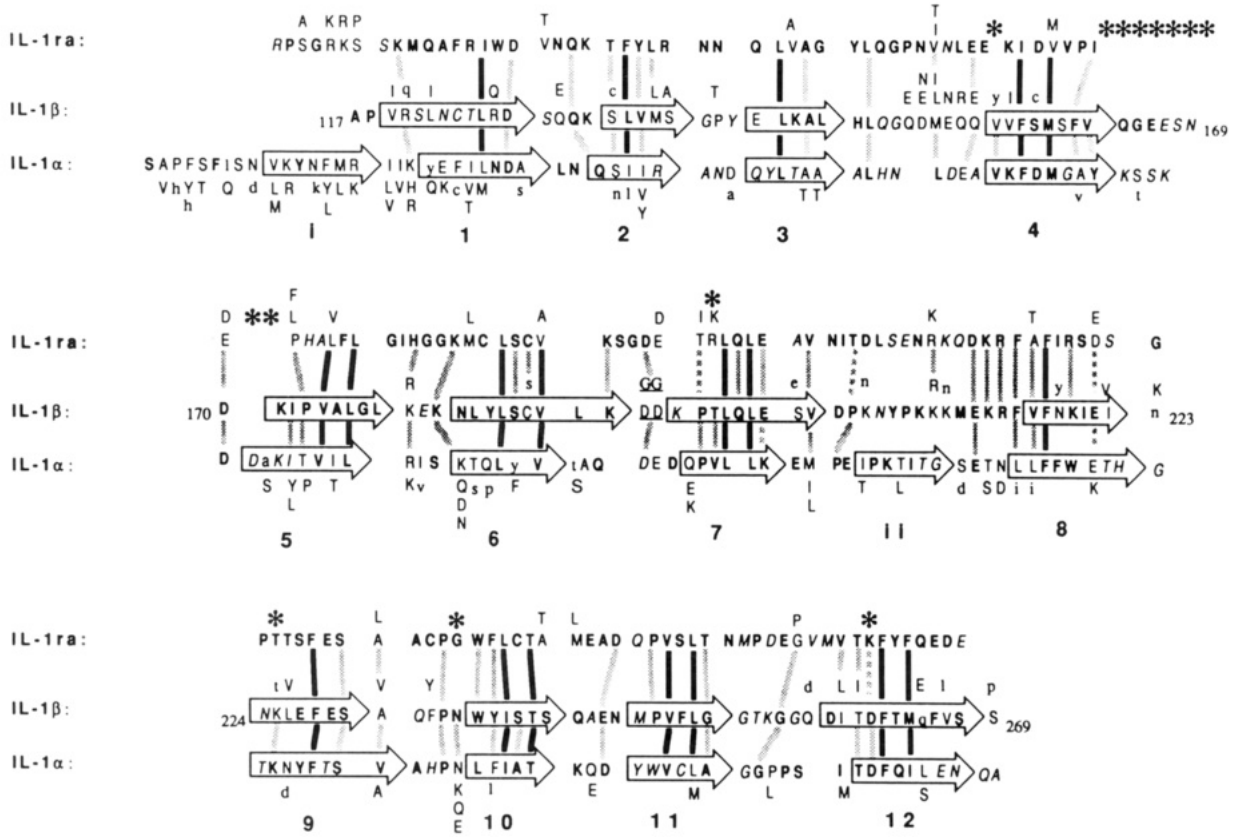

FIGURE 1: Primary sequence and secondary structure homology alignment for IL-1α, IL-1β, and IL-1ra. The ten IL-1α and IL-1β sequences used for the alignment were identical to those used by Yanofsky and Zurawski (1990), while the three IL-1ra sequences were those reported by Eisenberg et al. (1991). The sequences of other IL-1 superfamily members used for determining conserved core residues in the IL-1 fold included two fibroblast growth factors described by Zhu et al. (1991) and the five Kunitz-type protease inhibitor sequences analyzed by Onesti et al. (1991). The three continuous sequences used in the figure represent the human forms of IL-1α, IL-1β, and IL-1ra. Amino acid single-letter code representations are as follows: bold characters, absolutely conserved residues; italics, not conserved; plain type, semiconserved as indicated by the adjacent single-letter codes or by lower-case serif type which designates that only one molecule out of those analyzed deviated from the absolutely conserved consensus; underscored residues, a +1/−1 position uncertainty; large Arabic numerals (1–12), β-strands (arrows) common to IL-1α and IL-1β as derived by X-ray crystallography (Graves et al., 1990; Priestle et al., 1988); similar Roman numerals (i and ii), an additional hydrogen-bonded antiparallel pair found only in IL-1α (Graves et al., 1990). Gray vertical bars indicate homologous residues, black vertical bars indicate hydrophobic core residues common to all IL-1 fold proteins, and broken bars locate possible homologies. With the exception of the strand 6 alignments, gaps introduced to preserve the primary sequence alignment of related residues do not interfere with the conserved β-strand core sequences common to all IL-1 fold molecules. Asterisks (*) identify IL-1α and IL-1β residues absent from IL-1ra molecules.

1, 2, and 4, that contains three bound water molecules. This hydrated pocket is bordered by amino acids L_{126}, R_{127}, D_{128}, S_{133}, L_{134}, Q_{155}, and V_{156} (Figure 3). An analysis of the X-ray crystallographic coordinates (Finzel et al., 1989) shows that the Cγ of R_{127} is in contact with the carbonyl oxygen of K_{132} (Figure 4A,B). There appears to be little other than this R_{127}·K_{132} interaction preventing strand 1 and its hydrogen-bonded antiparallel strand 12 partner from displacing the water molecules and collapsing into the hydrated pocket. Thus, by complete elimination of the position 127 side chain (i.e., the R → G substitution), residue 127 could move past the K_{132} oxygen, allowing the strand 1/12 antiparallel pair to move into the hydrated pocket. A Cβ at position 127 would be predicted to prevent the displacement movement in the absence of a Cγ atom and maintain the structural features and biological activity found with the IL-1$\beta_{R\rightarrow A}$ mutant protein. That the IL-1$\beta_{R\rightarrow G}$ mutant protein is biologically active at high concentration (Figure 2) might be explained by the increased rotation permitted around the α-carbon of glycine, allowing multiple conformations with potential for generating biologically active forms.

The fact that the IL-1$\beta_{R\rightarrow G}$ mutant is biologically active at high concentrations (Figure 2), coupled with the observation that the arginine at position 127 is not completely conserved among IL-1α and IL-1β proteins (Figure 1), led us to consider the possibility that R_{127} is not the determinant residue responsible for triggering IL-1β biological activity. An alternate explanation is that the effects of the displacement of the strand 1/12 pair into the hydrated pocket (described above) might be transmitted to an adjacent region of the protein containing the key residues required for triggering activity. Ju et al. (1991) identified D_{261} as a perfectly conserved amino acid in IL-1 proteins which is important for IL-1 biological activity, and the proximity of D_{261} to the R_{127} to the hydrated pocket is shown in Figure 4, parts A and C. The region of the IL-1β molecular surface encompassing residue D_{261} was described as a "cleft" and proposed as a potential receptor binding site by Clore et al. (1991) on the basis of its shape and accessibility.

Our interpretation of this D_{261} cleft is shown in Figure 5B as a depressed saucerlike accessible surface approximately 15 Å in diameter. Figure 5A is presented for orientation with reference to the view published by Priestle et al. (1988). The D_{261} residue is located at the geometric center of the saucer (Figure 5B), whose circular rim is defined by the exposed side chains of amino acids (in clockwise order) K_{254}, N_{223}, N_{224}, K_{225}, S_{129}, Q_{130}, and Q_{242} and the peptide backbone of residues G_{256}, G_{255}, and K_{254}. The D_{261}, T_{260}, and E_{227} side chains are located within the saucer and, unlike the amino acids which form the rim, possess buried peptide backbones which are

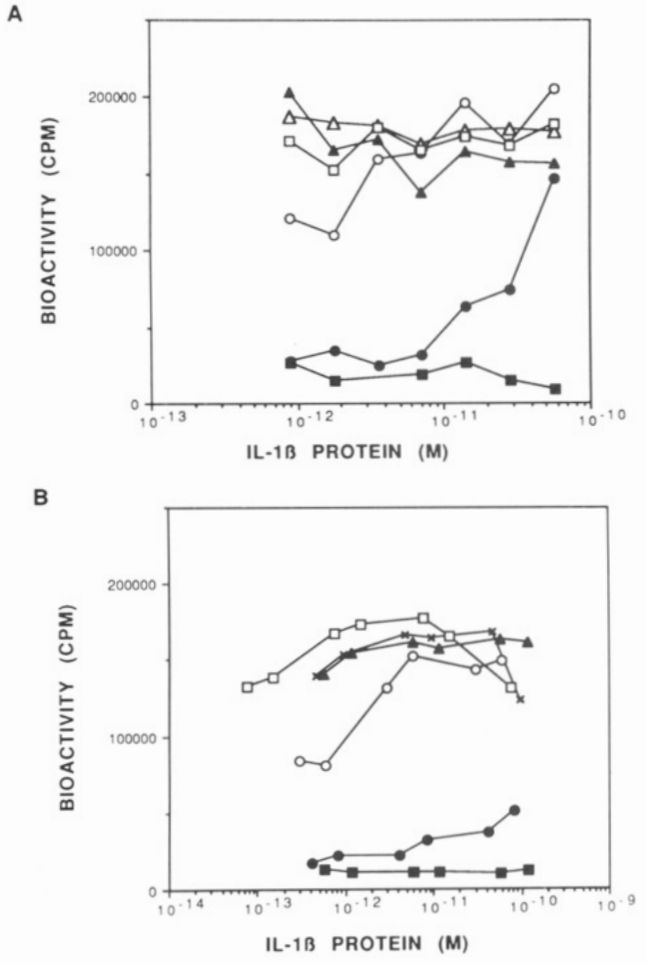

FIGURE 2: Thymocyte proliferation activity for various R_{127} substitution mutants of human IL-1β revealing that only R → G has diminished activity. Parts A and B represent two data sets where the results of testing R → E, R → G, and R → K are duplicated while the R → A and R → W data are shown only in parts A and B, respectively. Symbols indicate the activity of specific mutants: open circles, R → E; open squares, R → K; open triangles, R → A; closed circles, R → G; closed triangles, wild-type IL-1β; ×, R → W; closed squares, control reticulocyte lysate (no added messenger RNA). WT: wild-type (nonmutated) IL-1β.

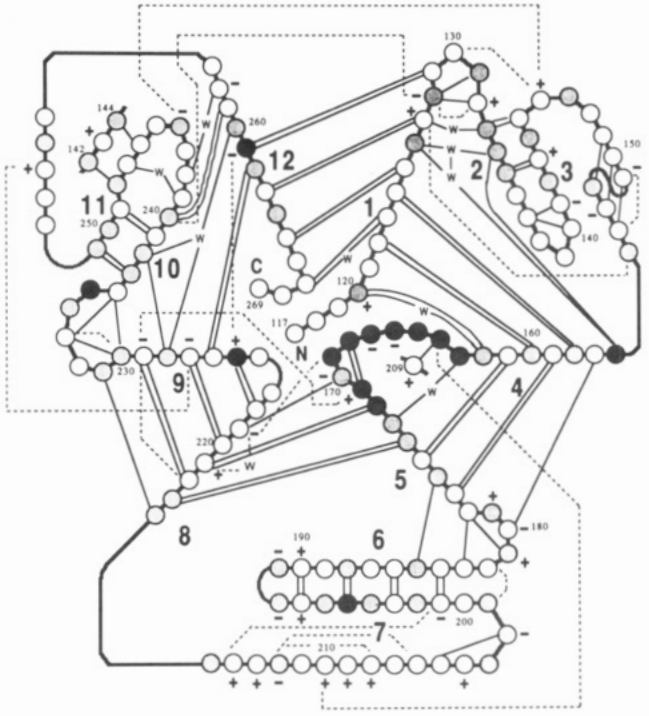

FIGURE 3: Schematic representation of the human IL-1β crystal structure showing the extensive cooperative interactions, 3-fold symmetry, and the location of bound water molecules between strands 1, 2, and 4. Heavy solid black lines and circles represent successive amino acids, and light solid lines represent main-chain, peptide backbone hydrogen bonds as described by Finzel et al. (1989). Dotted lines represent side-chain hydrogen bonds and salt bridges as reported (Finzel et al., 1989; Veerapandian et al., 1992) or identified by direct examination of the X-ray crystal structure as indicated under Materials and Methods. The amino acid identity is indexed by selective numbering. Identification of conserved residues was derived from the alignment analysis shown in Figure 1. Gray circles represent those residues which are conserved among IL-1α, IL-1β, and IL-1ra; solid circles indicate residues conserved in IL-1α and IL-1β, but not IL-1ra; open circles indicate residues not conserved.

packed along with F_{262}, T_{263}, and M_{264} beneath the saucer floor, thus stabilizing the structure. In this way, a continuous, structurally integrated network of residues constituting the cleft floor extends throughout the length of strand 12 from residue T_{260} through residue M_{264}. The Q_{265} residue of strand 12 continues this network into strand 1 (Figure 4A,C) by stacking upon the side chain of T_{125} as well as the peptide backbone of R_{127}. This cooperative arrangement, along with the antiparallel hydrogen bonding between the peptide backbones of β-strands 1 and 12, provides a likely mechanism for the transfer of structural effects among the members of this network. Therefore, the conformation of the D_{261} cleft appears to be dependent upon the integrity of the above described network which extends from the hydrated pocket to residue D_{261}. The closely packed residues T_{260}, D_{261}, F_{262}, and M_{264}, along with Q_{265} [which packs against R_{127} in strand 1 (Figure 5)], are well conserved in IL-1α and IL-1β. In particular, the absolutely conserved F_{262}, which is buried beneath D_{261}, appears to position D_{261} properly for surface exposure. On the basis of these data, we conclude that subtle conformational changes resulting from changing R_{127} to G_{127} may cause a concomitant change in the accessibility of D_{261}, with an accompanying decrease in biological activity. This argument may also provide an explanation for the behavior of the T_{125} → G mutant protein described by Young et al. (1990), which also binds receptor but has diminished biological activity.

DISCUSSION

Stimulation of IL-1 biological activity has proved to be more complex than a simple one-step binding/induction reaction. Mutation studies strongly suggest that the strand 1/12 pair of the IL-1β molecule is critical for triggering biological activity, as evidenced by the behavior of the Arg_{127} (Gehrke et al., 1990), T_{125} (Young et al., 1990), and D_{261} (Ju et al., 1991) mutant proteins. Despite the fact that all three of these mutations significantly decrease IL-1β biological activity without disrupting receptor binding, none completely obliterates the activity response to generate a pure receptor antagonist. We reported previously (Conca et al., 1991) that the IL-1ra, wild-type IL-1β, and IL-1$\beta_{R\rightarrow G}$ proteins can be distinguished on the basis of their effects on induction of transcription in fibroblasts. When fibroblasts are incubated with the IL-1ra, there is no stimulation of immediate early (*fos*/*jun*) or late (procollagenase and prostromelysin) gene expression (Conca et al., 1991). The IL-1ra, therefore, binds receptor but does not induce changes in gene expression (Dripps et al., 1991). The IL-1$\beta_{R\rightarrow G}$ mutein, however, binds receptor and in addition stimulates immediate early gene expression without inducing accumulation of late mRNAs (Conca et al., 1991). In these experiments, only the wild-type IL-1β protein

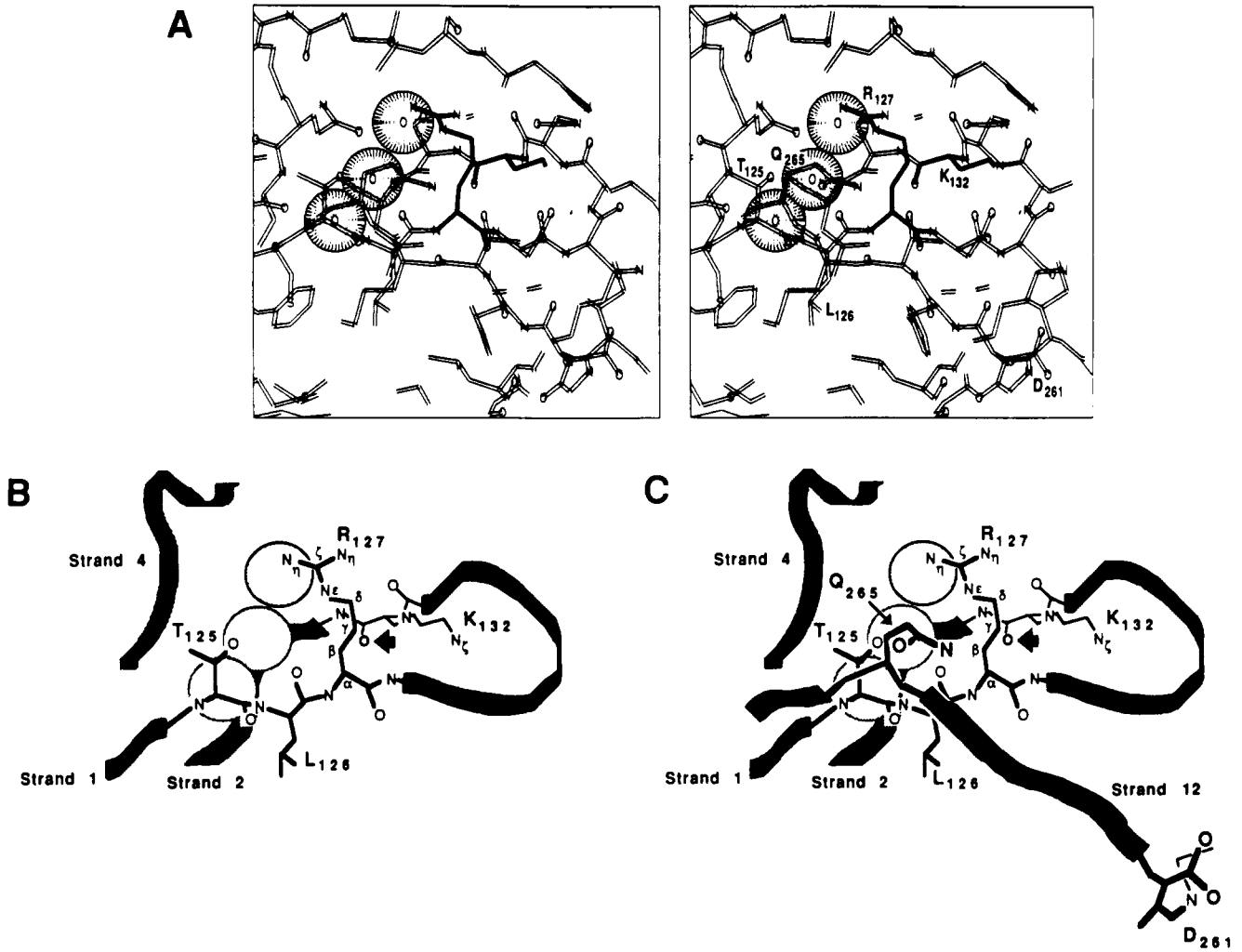

FIGURE 4: Hydrated pocket bordered by β-strands 1, 2, and 4. (A) Stereoview showing the structural relationships of R_{127} and adjacent amino acids to the three bound water molecules located in a hydrated pocket. These pictures were produced using the program PLOT1, written by G. J. Quigley. (B) Schematic representation of a portion of the data presented in part A showing the proximity of the Cβ and Cγ atoms to the carbonyl oxygen of K_{132}. (C) Schematic representation similar to that shown in part B except that a portion of the strand 12 antiparallel β-strand is added to show the location of Q_{265} and to illustrate the concept that structural perturbations in the R_{127} region could affect nearby amino acids such as D_{261}. The large bold arrow in parts B and C identifies the carbonyl oxygen of K_{132}. Ribbons locate the paths of relevant peptide backbone segments.

was competent to bind receptor and induce transcription of both early and late genes.

The ability of the IL-1$\beta_{R\rightarrow G}$ protein to stimulate immediate early but not late genes suggests that this mutein has a functional interaction with receptor to induce some, but not all, signal transduction events required for late gene expression. Stimulation of late transcription may therefore require receptor–ligand contacts that are absent when either the IL-1ra or the IL-1$\beta_{R\rightarrow G}$ protein interacts with receptor. The strong implication is, therefore, that multiple triggering contacts between IL-1β and the IL-1 receptor are likely to be required for stimulating full biological activity. Clore et al. (1991) also discuss multiple IL-1β–receptor contacts. Because of the proposed structural effects upon D_{261} caused by the R_{127} mutation (Figure 4) and the defective nature of the R_{127} mutein in stimulating late fibroblast gene expression (Conca et al., 1991), the D_{261} may represent a "late trigger" for IL-1 biological activity. We also speculate that the "early trigger" for IL-1 biological activity may reside in the β-bulge domain located between strands 4 and 5 (Figure 3). The structural significance of the bulge domain was hypothesized by Veerapandian et al. (1992), and it is interesting that this domain corresponds to a peptide previously demonstrated to have partial agonist activity (Antoni et al., 1986). Our speculation that the bulge domain may represent the early trigger is based in part upon the fact that the residues comprising the β-bulge are found in both IL-1α and IL-1β but are absent from the IL-1ra (Figure 1, asterisks at the end of strand 4).

Despite the fact that the properties of the IL-1$\beta_{R\rightarrow G}$ mutein define separable biological activity and receptor binding domains for IL-1β (Gehrke et al., 1990), it is likely that R_{127} is not a determinant residue for IL-1 biological activity. Several arguments or lines of experimentation are consistent with this statement. First, it is reasonable to predict that key amino acids responsible for IL-1 biological activity are likely to be very highly conserved. Although D_{261} fulfills this requirement, R_{127} is not conserved between IL-1α and IL-1β proteins (Figure 1), and it therefore seems less likely to be a determinant residue. Second, on the basis of the substitution data presented in Figure 1 and the modeling data shown in Figure 4, we propose that the charged character of the arginine side chain is not critical; rather, substituting glycine for arginine at position 127 more likely causes a conformational change in the protein. In support of the proposed conformational change, preliminary experiments involving analysis of antibody reactivity and susceptibility to proteolytic hydrolysis in vitro strongly suggest that the conformation of the R_{127} mutein is not equivalent to that of the wild-type IL-1β protein (data not shown). Third,

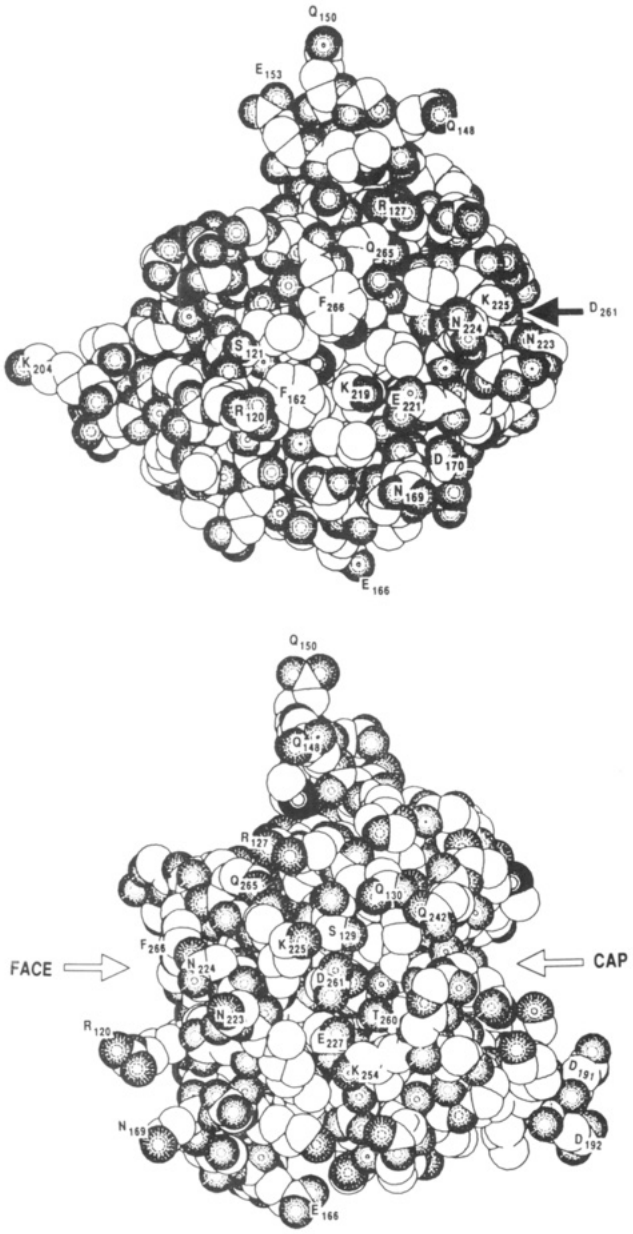

FIGURE 5: Space-filling views of the human IL-1β D_{261} cleft showing the relative locations of various amino acids, including R_{127} and Q_{265}. (A, top) View down the pseudo-3-fold axis of human IL-1β (i.e., looking into the face) showing solvent-accessible charged and hydrophobic residues (as indicated) which are displayed at the front of the β-barrel. This barrel structure was described previously (Finzel et al., 1989; Priestle et al., 1988). The arrow locates D_{261} and the cleft in which it is located and indicates the direction of view used to generate the figure shown in part B. (B, bottom) View of the side of human IL-1β as indicated in part A. The D_{261} cleft and adjacent amino acids are presented. These pictures were produced using the program PLOT1, written by G. J. Quigley. Filled symbols represent nitrogen, oxygen, and sulfur atoms. Open symbols are carbon atoms. Carbon atoms are drawn with a scaled diameter of 3.4 Å.

two-dimensional NMR experiments conducted using the functionally related IL-1β $Thr_{125} \rightarrow Gly_{125}$ mutein show that although there are no gross structural changes, small conformational changes are detected distal to the site of the mutation, suggesting that the alteration in bioactivity may not be localized directly to the Thr_{125} residue (Peter Young, personal communication). It is conceivable that the distal site involves the D_{261} region and that a conformational change involving strand 12 displacement of bound water is also transmitted, in a manner similar to that shown in Figure 4, from Thr_{125} to D_{261}. In sum, we propose that the integrity of

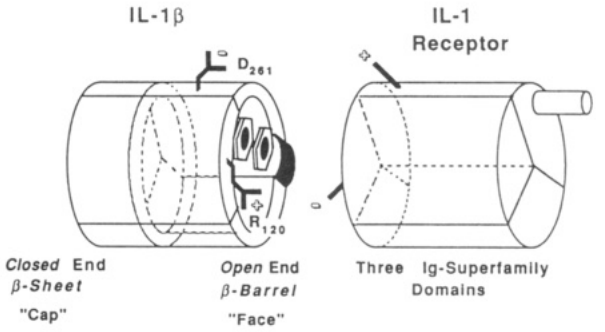

FIGURE 6: General conceptual model for an IL-1–receptor interaction, suggesting possible combined features of type I and type II receptors. The open end of the IL-1 β-barrel, which exhibits a 3-fold symmetry, is shown interacting with a putative 3-fold IL-1 extracellular receptor domain (soluble IL-1 receptor). The R_{120} and D_{261} residues that are postulated to play a role respectively in ligand binding and activity triggering are indicated as are the hydrophobic F_{162} and F_{266} residues (indicated by the hexagonal plates). These hydrophobic residues are located at the IL-1 surface in a location that would position them at the ligand–receptor interface. The putative charged residues shown on the IL-1 receptor may interact with the R_{120} and D_{261} residues of IL-1. The β-bulge region is shown as a shaded area on the face of the IL-1β molecule.

the R_{127} region is essential for triggering a biological response and that the major consequence of the $Arg_{127} \rightarrow Gly_{127}$ mutation is to cause a conformational change that is transmitted to other key amino acids, notably D_{261} (Figure 4).

The IL-1β structure can be viewed as consisting of two structural motifs, each of which is a β-sheet consisting of six antiparallel β-strands. One sheet forms a continuous β-barrel, while the other forms a "cap" which closes one end of the barrel (Figure 5). A 3-fold symmetry axis characteristic of all IL-1 fold molecules (Graves et al., 1990) passes down the center of the barrel; thus, the IL-1β structure resembles a "cup" in which one end is closed (cap end) and the other is open (face end). Veerapandian et al. (1992) proposed that a cluster of charged and polar groups presented at the open end of the β-barrel may be important for receptor binding, and data reported by Clore et al. (1991) and Labriola-Tomkins et al. (1991) strongly suggest that it is indeed the open, or face, end of the IL-1β molecule that interacts with receptor. The primary amino acid sequence of the extracellular domain of the IL-1 receptor (Sims et al., 1988) suggests potential 3-fold symmetry, and a conceptual model for IL-1–receptor association is presented in Figure 6. This model provides a structural paradigm to consider the differential receptor binding and biological activity attributes of IL-1β, IL-1ra, and the IL-1$\beta_{R \rightarrow G}$ mutein. A charged surface representing the rim of the β-barrel plus a hydrophobic core represented by the two essential solvent-exposed phenylalanine side chains [positions 162 and 266 (Labriola-Tompkins et al., 1991)] may provide the common binding epitope found in all three forms of IL-1 (IL-1α, IL-1β, and IL-1ra). The β-bulge, which is absent from the IL-1ra, may constitute a trigger for the early signal events, whereas the D_{261} region (found instead as a lysine in the IL-1ra) provides a likely trigger for the late signals. The behavior of the IL-1$\beta_{R \rightarrow G}$ mutein in stimulating early but not late signal transduction events (Conca et al., 1991) is consistent with the model in that the β-bulge region (putative early trigger) is intact while the D_{261} region (putative late trigger) may be compromised. Additional mutagenesis is required to test these predictions.

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