Structure and function of interleukin-1, based on crystallographic and modeling studies

B. Veerapandian
Center for Advanced Research in Biotechnology, University of Maryland, Rockville, Maryland 20850 USA

INTRODUCTION

Interleukin-1 (IL1) is known to be an important mediator of the immune system, produced primarily by mononuclear phagocytes in response to injury and infection. IL1α and IL1β are two forms of the same class of interleukin-1, eliciting various biological activities, depending on the type of cell with which they interact. A closely related protein, IL1RA, acts as an antagonist by binding to IL1's receptor. We describe crystallographic and modeling studies of the structures and functional parts of these interleukins.

STRUCTURAL DETAILS OF IL1β AND IL1α

These two forms of IL1 are initially synthesized as 31-kD precursors and are cleaved by proteases to release mature biologically active 17-kD proteins. With an amino acid homology between them of ~25%, they are structurally similar and appear to carry out the same function by binding to a common receptor. For a detailed review of IL1, see references 1–3. To identify the structural requirements for receptor activation, we used protein crystallographic techniques to obtain the three-dimensional structure of IL1β. The detailed structure and a possible receptor binding epitope has been reported (4). Crystallographic and nuclear magnetic resonance (NMR) structures of these (5–8) and a few other site-directed mutants of IL1β (9) have been solved and the coordinates submitted to the Protein Data Bank.

THREE-DIMENSIONAL STRUCTURE OF IL1β

The molecule resembles a conical barrel with a shallow open face on one end and a closed face on the other. The molecule contains 12 antiparallel β-strands, where six of these (β1, β4, β5, β8, β9, and β12) constitute an antiparallel β barrel. The overall structure of the molecule consists of three similar fragments (F1, F2, F3), each containing two pairs of β strands. Three pairs of β strands (one pair from each of the fragments) form the six stranded barrel; the other three pairs cover one end of the barrel, referred to as the "closed end." The amino and carboxy termini are close to each other at the "open end" of the barrel. The molecule has internal pseudo threefold symmetry, with each subunit (F1, F2, F3) having a ββββLβ motif. There are five β-hairpins in this molecule, two of them in the open end and three at the closed end. The polypeptide α-carbon backbone of IL1β, viewed perpendicular to the barrel axis, is shown in Fig. 1. 24 hydrophobic side chains line the inner surface of the barrel and both the ends of the barrel have concentrations of exposed polar residues.

THREE-DIMENSIONAL STRUCTURE OF IL1α

The crystallographic structure of IL1α has been determined (8); its general fold is very similar to that of IL1β, having the same central β-barrel along with the adjoining loops. The major difference in the two molecules is an NH_2-terminal extension of 14 residues beyond the NH_2-terminus of IL1-β. As explained in reference 8, there are some additional features in IL1α: a short β-strand near the NH_2-terminus (residues 6–10), another short strand (residues 97–99) and about two turns of 3_{10} helix (residues 101–105). The strands are β1 = 14–23, β2 = 24–33, β3 = 34–40, β4 = 48–58, β5 = 59–68, β6 = 69–80, β7 = 81–91, β8 = 105–113, β9 = 114–121, β10 = 122–132, β11 = 133–140, β12 = 146–153. The pseudo threefold symmetry exhibited by IL1β is also present in IL1α, having 81 common atoms within a root mean square distance of 1.54 Å.

IL1-RECEPTOR ANTAGONIST

The pleiotropic activity of the same IL1 molecule with respect to different cells has to be regulated by the system or otherwise would possibly lead to a uncontrolled and undesirable effects. Recent reports show that there exists a naturally occurring protein which could act as an interleukin-1 receptor antagonist (IL1RA),

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regulating IL1 activity (10–12). IL1RA binds to the same receptor with an affinity equal to that of IL1α or IL1β but does not induce IL1-like biological activity, confirming that it is a protein antagonist of IL1. It is a 17-Kd polypeptide whose primary structure shares 26% amino acid homology to IL1β and 19% homology to IL1α. Conserved amino acids in IL1RA have a 41% homology to IL1β and 30% to IL1α. With the available structural information on IL1, a tentative model of IL1RA has been deduced and a comparison of the structures of IL1β, IL1α and IL1RA reveals significant functional aspects of these proteins.

MODELING STRATEGY

The available amino acid sequences of IL1, purified from various sources, were aligned by using a mutation data matrix, also by considering the features essential for IL1's common fold as obtained from the three-

TABLE 1 The amino acid sequences of IL1-β, IL1-α and IL1RA were aligned by using MACAW and also by considering the available three-dimensional structural details.

IL1-β	b1		b2	b3		b4
Il1-β no.	3----------12		17---21	25---29		40----------52
β-Rat	VPIRQLHCRLRD	EQQK	CLVLS	DP-C	ELKAL	HLNGQNISQQ	VVFSMSFVQGETS
β-Mouse	VPIRQLHYRLRD	EQQK	SLVLS	DP-Y	ELKAL	HLNGQNINQQ	VIFSMSFVQGEPS
β-Rabbit	AVRSLHCRLQD	AQQK	SLVLS	GT-Y	ELKAL	HLNAENLNQQ	VVFSMSFVQGEES
β-Sheep	AAVQSVKCKLQD	REQK	SLVLD	SP-C	VLKAL	HLLSQEMSRE	VVFCMSFVQGEER
β-Bovine	APVQSIKCKLQD	REQK	SLVLA	SP-C	VLKAL	HLLSQEMNRQ	VVFCMSFVQGEER
β-Human	APVRSLNCTLRD	SQQK	SLVMS	GP-Y	ELKAL	HLQGQDMEQQ	VVFSMSFVQGEES
RA-Human	RPSGRKSSKMQAFRIWD	VNQK	TFYLR	N--N	QLVAG	YLQGPNVNLE	EKIDVVPIEPH--
α-Human	SAPFSFLSNVKYNFMRIIKYEFILND	ALNQ	SIIRA	ND-Q	YLTAA	ALH--NLDEA	VKFDMGAYKSSKD
α-Bovine	SAHYSFQSNVKYNFMRVIHQECILMD	ALNQ	SIIRD	MSGP	YLTAT	TLN--NLDDA	VKFDMVAYVSEED
α-Rabbit	SVPYTFQRNMRYKYLRIIKQEFILMD	ALNQ	SLVRD	TSDQ	YLRAA	PLQ--NLGDA	VKFDMGVYMTSED
α-Mouse	SAPYTYQSDLRYKLMKLVRQKFVMND	SLNQ	TIYQD	VDKH	YLSTT	WLN--DLQQE	VKFDMYAYSSGGD
α-Rat	SAPHSFQNNLRYKLIRIVKQEFIMND	SLNQ	NIYVD	MDRI	HLKAA	SLN--DLQLE	VKFDMYAYSSGGD
IL1 pro no.	119---------128		133---137	141---145		156----------168

	b5		b6		b7		b8
Il1-β no.	55------62		66--------74		77-------85		100-----106
β-Rat	ND	KIPVALGL	KGK	NLYLSCVMK	DG	TPTLQLESV	D-PKQYPKKKMEKRF	VFNKIEV
β-Mouse	ND	KIPVALGL	KGK	NLYLSCVMK	DG	TPTLQLESV	D-PKQYPKKKMEKRF	VFNKIEV
β-Rabbit	ND	KIPVALGL	RGK	NLYLSCVMK	DD	KPTLQLESV	D-PNRYPKKKMEKRF	VFNKIEI
β-Sheep	DN	KIPVALGI	RDK	NLYLSCVKK	GD	TPTLQLEEV	D-PKVYPKRNMEKRF	VFYKTEI
β-Bovine	DN	KIPVALGI	KDK	NLYLSCVKK	GD	TPTLQLEEV	D-PKVYPKRNMEKRF	VFYKTEI
β-Human	ND	KIPVALGL	KEK	NLYLSCVLK	DD	KPTLQLESV	D-PKNYPKKKMEKRF	VFNKIEI
RA-Human	--	--ALFLGI	HGG	KMCLSCVKS	GD	ETRLQLEAV	NITDLSENRKQDKRF	AFIRSDS
α-Human	DA	KITVILRI	SKT	QLYVTAQDE	E-	QPVLLKEMP	P-EIPKTITGSETNL	LFFWETH
α-Bovine	-S	QLPVTLRI	SKT	QLFVSAQNE	E-	EPVLLKEMP	P---TPKIIKDETNL	LFFWEKH
α-Rabbit	-S	ILPVTLRI	SQT	PLFVSAQNE	E-	EPVLLKEMP	P--TPRIITDSESDI	LFFWETQ
α-Mouse	DS	KYPVTLKI	SDS	QLFVSAQGE	E-	QPVLLKELP	P--TPKLITGSETDL	IFFWKSI
α-Rat	DS	KYPVTLKV	SNT	QLFVSAQGE	E-	KPVLLKEIP	P--TPKLITGSETDL	IFFWEKI
IL1 pro no.	171------178		182-------190		193-------201		216-----222

	b9		b10		b11		b12	
Il1-β no.	109----114		120----125		130-----135		142----------152	
β-Rat	KT	KVEFES	AQFPN	WYISTS	QAEH	RPVFLG	NSNG--R	DIVDFTMEPVSS	152
β-Mouse	KS	KVEFES	AEFPN	WYISTS	QAEH	KPVFLG	NNSG--Q	DIIDFTMESVSS	152
β-Rabbit	KD	KLEFES	AQFPN	WYISTS	QTEY	MPVFLG	NNSG--Q	DLIDFSMEFVSS	152
β-Sheep	KN	TVEFES	VLYPN	WYISTS	QIEE	KPVFLG	RFRG-GQ	DITDFRMETLSP	153
β-Bovine	KN	TVEFES	VLYPN	WYISTS	QIEE	RPVFLG	HFRA-GQ	DITDFRMETLSP	153
β-Human	NN	KLEFES	AQFPN	WYISTS	QAEN	MPVFLG	GTKG-GQ	DITDFTMQFVSS	153
RA-Human	GP	TTSFES	AACPG	WFLCTA	MEAD	QPVSLT	NMPDEGV	MVTKFYFQEDE	152
α-Human	GT	KNYFTS	VAHPN	LFIATK	Q--D	YWVCLA	GGP---P	SITDFQILENQA	159
α-Bovine	GS	MDYFKS	VAHPK	LFIATK	Q--E	KLVHMA	SGP---P	SITDFQILEK--	155
α-Rabbit	GN	KNYFKS	AANPQ	LFIATK	P--E	HLVHMA	RGL---P	SMTDFQIS----	150
α-Mouse	NS	KNYFTS	AAYPE	LFIATK	E--Q	SRVHLA	RGL---P	SMTDFQIS----	155
α-Rat	NS	KNYFTS	AAFPE	LLIATK	E--Q	SQVHLA	RGL---P	SMIDFQIS----	156
IL1 pro no.	225----230		236----241		246-----251		258----------268		269

The β-strands (β1 to β12) of IL1β are also marked.

FIGURE 1 The α-carbon backbone atoms of IL-1 β, viewed perpendicular to the axis of the barrel.

dimensional structures of IL1-β and IL1-α (Table 1). While doing so, it was kept in mind that the matching patterns of residue conservation should be higher in the β-barrel core, and the fold not only maintains the structural integrity of any protein but also enables the placement of the functionally important residues in correct juxtaposition. Because IL1 and IL1RA bind to the same receptor, their binding surface should be similar but their opposite functional characteristics suggests that the functional aspect of IL1 should be absent in IL1RA. Because their functions are quite different, the alignment was carried out manually wherever the residues concerned with the proposed epitope appeared. Initial alignment was done by comparing the three-dimensional structures of human IL1-β and IL1-α and the alignment was extended to the rest of the sequences. In the case IL1-RA the sequence similarity seems to be closer to IL1β than IL1α.

Model building was performed by using graphics programs FRODO on Evans and Sutherland PS390 graphics system and QUANTA (Polygen Co.) on a Silicon Graphics 4D/70 IRIS workstation with the procedure as follows. Based on the main chain of IL1-β, a model of IL1RA was obtained using the sequence alignment (Table 1). The side chains of the IL1β were replaced for the appropriate residue of IL1RA to generate the required primary sequence, leaving the backbone unaltered. Conformational angles of the new side chains were retained as a start and a visual scanning of torsion angles about each bond of a residue was done to look for any van der Waals contacts between atoms on either side of the bond. The optimal alignment of the primary sequences, however, required deletions at 23, between 51 and 56, and an insertion of Ile between

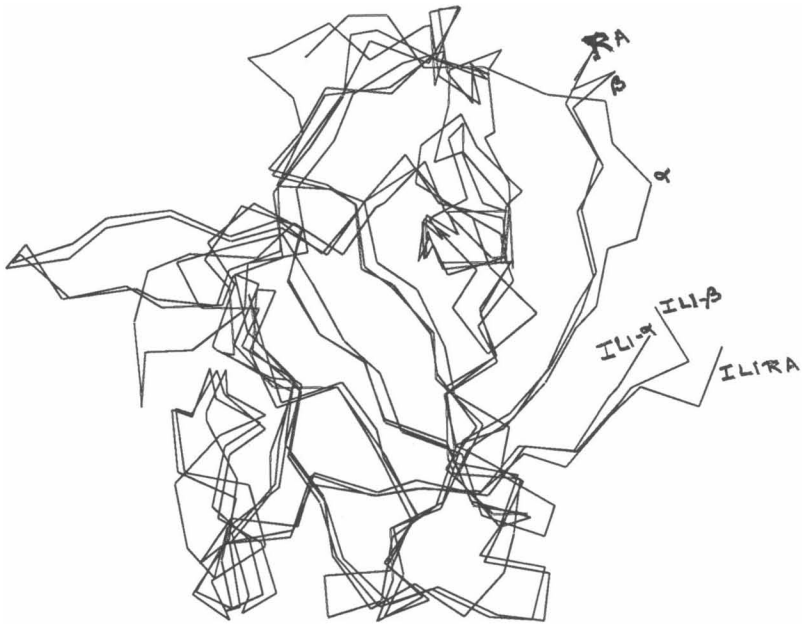

FIGURE 2 Superposition of the α-carbon backbones of IL1α, IL1β and IL1RA.

positions 86 and 87, Glu between 139 and 140 (IL1β numbering). Insertions and deletions with respect to IL1α have been done, wherever necessary. All the deletions and insertions are apparently on the loop regions which are present on the surface of the molecule and not within the barrel staves. The next step involved several cycles of sterochemical regularization and energy minimization by using the program QUANTA and obtained model was then subjected to molecular dynamics coupled with energy minimization and regularization procedure using the program XPLOR.

The crystallographic and deduced models possess the same IL1-fold (secondary structure) and maintain very similar backbone hydrogen bonding pattern and also have similar nonbonding energies. Superposition of the α-carbon backbones of IL1α, IL1β, and IL1RA are shown in Fig. 2, which reveals that their spatial structure are close to each other in all regions of the protein except in the regions of deletion and insertion. We proposed in our earlier paper that the receptor binding epitope of IL1 should be large, formed by various segments of the molecule. Similar regions such as the surface polar loops exist in the IL1RA structure and so it could be conceived that these loops are required for the binding. The observable major difference is that the binding loop, having the immunostimulatory sequence 50–56 (EESNDKI), is not present in IL1RA and there are subtle changes observed in the open end of the barrel. These small structural differences might play a major role in the functional aspects of IL1. The coordinates of the model have been deposited in the Protein Data Bank and are also available from the author.

REFERENCES

1. Dinarello, C. A. 1991. Interleukin-1 and Interleukin-1 antagonism. *Blood.* 77:1627–1652.
2. Durum, K. S., J. J. Oppenheim, and R. Neta. 1990. *In* Immunophysiology, Chapter 11, Immunophysiologic role of Interleukin-1. J. J. Oppenheim, and E. M. Shevach, editors. Oxford University Press, New York, 210–225.
3. Giovine, F. S., and G. W. Duff. 1990. Overview of the biology of IL-1. *Immunol. Today.* Jan:13–20.
4. Veerapandian, B., G. L. Gilliland, R. Ragg, A. L. Svensson, Y. Nasui, Y. Hirai, and T. L. Poulos. 1992. Functional implications of Interleukin-1β based on the three-dimensional structure. *Proteins.* 12:10–23.
5. Priestle, J. P., H.-P. Schar, and M. G. Grutter. 1989. Refinement of the structure of IL1-β, at 2.0 Å resolution. *Proc. Natl. Acad. Sci. USA* 86:9667–9671.
6. Finzel, B. C., L. L. Clancy, D. R. Holland, S. W. Muchmore, K. D. Watenpaugh, and H. M. Einspahr. 1989. Crystal structure solution of IL1-β at 2.0 Å resolution. *J. Mol. Biol.* 209:779–791.
7. Clore, G. M., P. T. Wingfield, and M. Gronenborn. 1991. Structure of IL1-β, derived from 3-D, 4-D NMR spectroscopy. *Biochemistry.* 30:2315–2323.
8. Graves, B. J., M. H. Hatada, W. A. Hendrickson, J. K. Miller, V. S. Madison, and Y. Satow. 1990. Structure of IL1-α at 2.7 Å resolution. *Biochemistry.* 29:2679–2684.
9. Veerapandian, B, et al. 1991. Crystallographic structures of site-directed mutants of IL1-β. (in preparation, coordinates submitted in PDB).
10. Hannum, C. H., C. J. Wilcox, W. P. Arend. et al. 1990. *Nature (Lond.).* 343:336–340.
11. Carter, D. B. et al. 1990. *Nature (Lond.).* 344:633–637.
12. Eisenberg, S. P., R. J. Evans, W. P. Arend, E. Verderber, M. T. Brewer, C. H. Hannum, and R. C. Thompson. 1990. *Nature (Lond.).* 343:341–346.