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Interaction of the Pre-Domain of Interleukin-1 with the Mature Domain

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The two interleukin 1 precursors (preIL-1α and β) with a molecular mass of 33 kDa are proteolytically processed to the mature carboxyl-terminal 17-kDa proteins. In this study we newly developed a monoclonal antibody against the precursor domain of IL-1β (ED7), of which the binding to preIL-1β was hindered by a polyclonal antibody against the mature domain of IL-1β. Immunoprecipitation of limited proteolysed preIL-1β by ED7 suggested that the epitope for ED7 may not be localized at the junction between preIL-1β and mature IL-1β. We therefore examined the possibility that the pre-domain of IL-1β might interact with the mature domain by using chemical cross-linking. V8 protease yielded a mature 17.5-kDa protein from untreated preIL-1β, but not chemically cross-linked preIL-1β. However, cleavage of the cross-link with 2-mercaptoethanol liberated a mature 17.5-kDa protein from preIL-1β, suggesting that the pre-domains of IL-1β might interact with the mature domain. Similar phenomena were observed with preIL-1α. Such an intermolecular interaction may inhibit or modulate the biological activity of mature IL-1s.

Key words: chemical cross-linking, intermolecular interaction, monoclonal antibody, pre-domain, preIL-1.

There are two types of interleukin-1 (IL-1), termed IL-1α and β. The two interleukin 1 precursors (preIL-1α and β) with a molecular mass of 33 kDa are proteolytically processed to the mature, active, carboxyl-terminal 17-kDa proteins by a calcium-activated protease, calpain [EC 3.4.22.17] (*1, 2*), and a novel cysteine protease, IL-1β converting enzyme (*3, 4*), respectively. Although both IL-1s can bind to their receptors with similar affinity and exert a wide variety of biological activities in common, preIL-1β is inactive, whereas preIL-1α is as active as the mature IL-1α (*5*). Since preIL-1α is known to be very easily degraded by cell-associated proteases (*5*), the possibility remains that some of the biological activity of preIL-1α is due to the mature IL-1α. However, it is also possible that preIL-1α is structurally different from preIL-1β, and, as a result, able to mediate biological activity.

It has been demonstrated that the amino-terminus of preIL-1α and β, namely, the precursor domain, causes a conformational change of the carboxyl-terminus, namely the mature domain (*6*). Proteinase K-sensitive regions of the mature domain of both IL-1s become proteinase K-insensitive with proteolytic removal of the precursor domain. At the same time, two cysteine residues in the mature domain of preIL-1β become inaccessible chemical modification. Trypsin cleaves preIL-1β into fragments of smaller size than mature IL-1β, but it hardly digests mature IL-1β (*7*). Sandwich enzyme-linked immunosorbent assays (ELISA) designed to detect mature 17-kDa IL-1β underestimate preIL-1β (*8*). Thus, these results indicate that the mature domain of preIL-1s differs in conformation from that of 17-kDa mature IL-1s.

In this study, we first describe a unique feature of a newly developed monoclonal antibody against the precursor domain of IL-1β (ED7), *i.e.*, that ED7 hardly bound to preIL-1β immobilized with a polyclonal antibody against the mature domain of IL-1β, although the polyclonal antibody can bind to preIL-1β immobilized with ED7. This may be explained by assuming that the epitope for ED7 is located close to some of the epitopes for the polyclonal antibody. We then demonstrate, by using chemical cross-linking, that the pre-domain of both IL-1s interacts with the corresponding mature domain.

MATERIALS AND METHODS

Construction of an Expression Vector for a Fusion Protein between Glutathione S Transferase (GST) and the Pre-Domain of PreIL-1β—Plasmid pBR322 harboring preIL-1β cDNA (pILβ, kindly provided by Dr. Don Carter) was digested with *Sac*I/*Hpa*II, followed by filling-in of the *Hpa*II site and insertion into the *Sac*I/*Sma*I sites of pUG131. The *Eco*RV/*Eco*RI fragment of the resultant plasmid was then inserted into the *Sma*I/*Eco*RI sites of the *Escherichia coli* expression vector, pGEX-3X (Pharmacia Biotech, Uppsala, Sweden). The resultant plasmid, pGILβH-53, encodes a fusion protein between GST and the pre-domain of preIL-1β (Glu^{6} to Arg^{127}), with the insertion

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Abbreviations: IL-1, interleukin-1; preIL-1, interleukin 1 precursor; ELISA, enzyme-linked immunosorbent assays; GST, glutathione S transferase; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, SDS polyacrylamide gel electrophoresis; i.p., intraperitoneally; BSOCOES, bis[2-(succinimidooxy carbonyl))ethyl]-sulfone; DSP, dithio bis(succinimidyl propionate).

of one amino acid, Ile, at the junction and with a C-terminal extra-pentapeptide, GlyIleHisArgAsp.

Expression and Purification of GST and the Fusion Protein—Bacterium JM109 harboring plasmid pGEX-3X or pGILβH-53 was grown, and the expression of both proteins was induced with 0.1 mM isopropyl-1-thio-β-D-galactoside at 25°C for 20 h. The cells were washed with 25 mM Tris-HCl (pH 8.0) containing 50 mM glucose and 10 mM EDTA, then lysed by repeated sonication in 20 mM phosphate-buffered saline (PBS, pH 7.4) containing 5 mM EDTA, 0.1 mM dithiothreitol, 1% NP40, and 2 mM phenylmethanesulfonyl fluoride (PMSF). The lysate was then centrifuged at 12,000 rpm at 4°C for 30 min. GST was recovered as the soluble fraction, whereas the fusion protein was recovered as the insoluble fraction. The soluble fraction containing GST was applied onto a glutathione Sepharose column and eluted with 50 mM Tris-HCl (pH 9.6) containing 5 mM glutathione. The insoluble fraction containing the fusion protein was subjected to 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) (*9*), followed by washing with distilled water and soaking in chilled 0.25 M KCl. The band with a molecular mass of 40 kDa corresponding to the fusion protein was cut out and electrophoretically eluted in a dialysis bag with gel electrophoresis running buffer at 100 V for 1 h. The purified proteins were dialyzed against PBS and stored at −80°C until use.

Generation of Monoclonal Antibodies against the Pre-Domain of PreIL-1β—A BALB/c mouse was injected intraperitoneally (i.p.) with 200 μl of an emulsion of 50 μg of the fusion protein and Freund's complete adjuvant (GIBCO). The same animal was injected i.p. with the same volume of an emulsion of the fusion protein and Freund's incomplete adjuvant at 2-week intervals. Two weeks after the third immunization, the mouse was finally boosted with a solution of the fusion protein in PBS, and 3 days later its spleen was used for cell fusion with mouse myeloma cells, NS-1, using PEG4000, as described previously (*10*). When the hybridomas had grown, the supernatant in each well was tested for antibody binding to a solid phase precoated with the fusion protein or GST.

Purification of PreIL-1β on an Immunoaffinity Column—One milligram of rabbit anti-human mature IL-1β antibody, kindly provided by Otsuka Pharmaceutical (Tokushima), was added to 0.5 ml of protein A Sepharose (Pharmacia), followed by incubation overnight at 4°C. The bound antibody was then chemically cross-linked with 5 mg/ml of dimethylsuberimidate 2HCl in 0.1 M borate buffer (pH 8.0) for 30 min at room temperature, according to the method previously described (*11*). After washing with PBS, the cross-linked beads were incubated in 0.1 M acetate buffer (pH 2.9) containing 0.15 M NaCl for 2 h at 4°C, followed by washing with PBS. HTB9 5637 cells, constitutive producers of preIL-1α and β (*1*), were lysed with an extraction buffer [20 mM Tris-HCl (pH 8.0) containing 9 mM 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, 1 mM PMSF, 50 μg/ml of leupeptin, and 5 mM ethylene glycol bis-β3-aminoethyl ether *N,N,N′,N′*-tetraacetic acid]. The cell lysate was centrifuged at 10,000 rpm for 5 min, and the supernatant was repeatedly adsorbed onto the cross-linked beads at the flow rate of 50 ml/h for 3 h. After washing with 25 ml of 20 mM Tris-HCl (pH 7.6) containing 0.5 M NaCl, 1 mM EDTA, and 0.5% NP40, and then 25 ml of 10 mM Tris-HCl (pH 7.6) containing 10 mM NaCl, the bound material was eluted with 5 ml of 0.1 M acetic acid (pH 2.4), followed by immediate neutralization and vacuum concentration. On the same immunoaffinity column, we successfully purified radiolabeled preIL-1β, as evidenced on SDS-PAGE and autoradiography (data not shown).

ELISA—Antibody binding to a solid phase precoated with the fusion protein, GST, or goat anti-mouse IgG (H+L) antibodies (CALTAG Lab., CA) was performed as previously described (*10*). Sandwich ELISA was also performed after immobilization of preIL-1β on an antibody-coated solid phase.

Immunoprecipitation of a ^{35}S-Labeled HTB9 5637 Cell Lysate—HTB9 5637 cells were metabolically labeled with L-[^{35}S]methionine as previously described, followed by cell lysis (*1, 7*). Immunoprecipitation was performed using protein A Sepharose conjugated with rabbit anti-mouse IgG_1 or IgM antibodies, as previously described (*1, 7*).

PreIL-1α and β Generated with an In Vitro Transcription-Translation System—The preIL-1α expression plasmid was constructed as follows. The *Pst*I-*Hin*cII fragment (0.9 kb) of pHL4 (*12*), which contains the coding region of preIL-1α, was inserted into pUG131 treated with *Pst*I and *Eco*RV. The resultant plasmid [pUHL(PH)] was digested with *Pst*I and *Sma*I, then inserted into pAM19 (Amersham) treated with *Pst*I and *Sma*I. The resultant plasmid [pAM(PH)] was linearized with *Sma*I, followed by *in vitro* transcription (Amersham), then *in vitro* translation (Promega). On the other hand, the preIL-1β expression plasmid was constructed as follows. The *Sac*I-*Pst*I fragment of pILβ, which contains a portion of the coding region of preIL-1β (Met^{11} to Ser^{269}), was inserted into pSP65 treated with *Sac*I and *Pst*I. The resultant plasmid (pSPILβ) was linearized with *Pst*I, followed by *in vitro* transcription-translation.

Chemical Cross-Linking of PreIL-1α or β—Chemical cross-linking was performed as described previously (*13*). PreIL-1α or β generated with an *in vitro* transcription-translation system (0.2 to 0.3 μl) was diluted in 20 μl of 75 mM sodium phosphate buffer (pH 7.5). The sample was cross-linked with 2 μl of 20 mM bis[2-(succinimidooxy carbonyl)ethyl]-sulfone (BSOCOES) or 40 mM dithio bis(succinimidyl propionate) (DSP) at room temperature for 10 min, followed by the addition of 4 μl of 1M glycine in 0.1 M sodium phosphate buffer (pH 7.5) to quench the reaction. A preliminary experiment showed that the amount of glycine used was large enough to totally abolish the reactivity of chemical cross-linkers, as evidenced by the absence of cross-linked hemoglobin on SDS-PAGE.

RESULTS AND DISCUSSION

Characteristics of Newly Developed Monoclonal Antibodies against the Pre-Domain of PreIL-1β—To generate monoclonal antibodies against the pre-domain of preIL-1β, we first purified a fusion protein between GST and the pre-domain of preIL-1β, in which the C-terminal end of GST was connected with the N-terminal end of the pre-domain, with the insertion of one amino acid, Ile, at the junction, as described under "MATERIALS AND METHODS." The fusion protein could be solubilized in 6 M guanidine hydrochloride or SDS-containing buffer, but could not be eluted from a glutathione Sepharose column under the

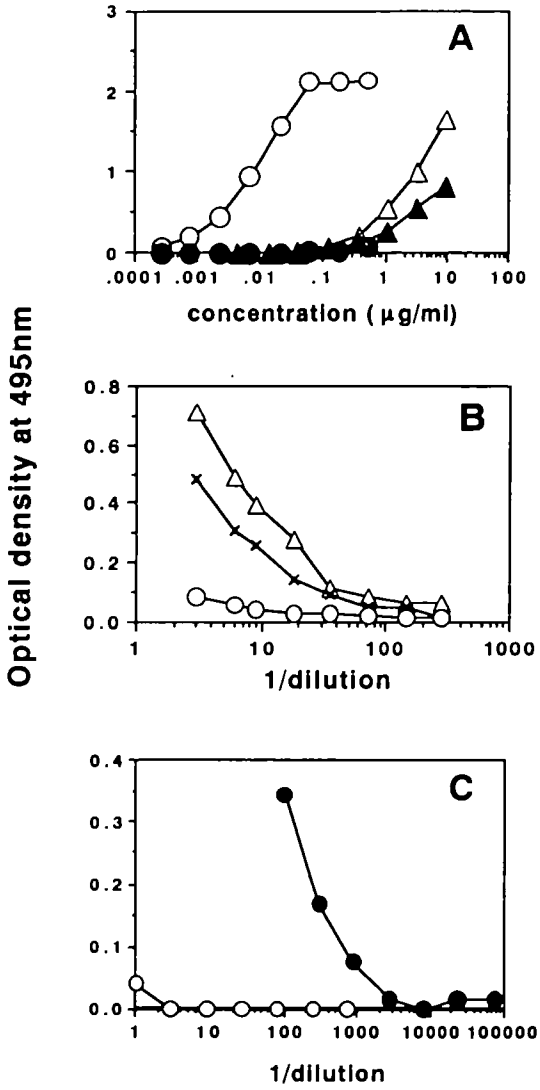

Fig. 1. **ELISA with two monoclonal antibodies.** (A) Each well of immunoassay microplates (Immulon 600; Greiner und Sohne, Germany) was coated with the fusion protein (open symbols) or GST (closed symbols) at 300 ng/ml for 2 h at room temperature, followed by blocking with 10% skim milk for 2 h at room temperature. Two monoclonal antibodies, namely, ED7 (circles) and DD7 (triangles), were added to wells at the various concentrations indicated, followed by the addition of biotinylated goat anti-mouse IgG+M antibodies and then streptoavidin horseradish peroxidase. The yellow color that developed was measured with a microplate reader at 495 nm. (B) Each well of immunoassay microplates was coated with appropriately diluted rabbit anti-mature IL-1β antibodies, followed by the addition of serially diluted preIL-1β. The inverse of the dilution is shown on the abscissa. Two monoclonal antibodies, namely, ED7 (circles) and DD7 (triangles), and biotinylated rabbit anti-mature IL-1β antibodies (crosses) were added to wells at 0.1, 5, and 10 μg/ml, respectively, followed by the addition of biotinylated goat anti-mouse IgG+M antibodies and then streptoavidin horseradish peroxidase for ED7 and DD7, or streptoavidin horseradish peroxidase for the rabbit antibody. (C) Each well of immunoassay microplates was coated with appropriately diluted rabbit anti-mature IL-1β antibodies (open circles) or ED7 (closed circles), followed by the addition of serially diluted preIL-1β. The inverse of the dilution is shown on the abscissa. Then, to the former wells, ED7 was added, whereas to the latter wells, biotinylated rabbit antibodies were added, followed by the same procedure as described in (B).

conditions tested. We therefore purified the fusion protein by SDS-PAGE. In contrast, GST could be purified on a glutathione Sepharose column. Its purity was confirmed by SDS-PAGE and silver staining (data not shown). The apparent molecular masses of the fusion protein and GST were 40 and 26 kDa, respectively, which are compatible with the fact that the pre-domain expressed in this study contains 128 amino acids.

We selected two monoclonal antibodies (ED7 and DD7) for this study. ED7 is IgG_1, whereas DD7 is IgM. As shown in Fig. 1A, ED7 almost exclusively bound to the fusion protein at the concentration tested, whereas DD7 bound to the fusion protein as well as GST. We then examined whether these antibodies bound to immunoaffinity-purified preIL-1β, which was immobilized to wells precoated with a rabbit anti-mature IL-1β antibody. DD7 and the rabbit anti-mature IL-1β antibody bound to preIL-1β much better than ED7, as shown in Fig. 1B. On a reversed sandwich ELISA in which ED7 was used to capture preIL-1β, the rabbit anti-mature IL-1β antibody could bind to preIL-1β immobilized with ED7, as shown in Fig. 1C. These findings may be explained by assuming that the epitope for ED7 is located close to some of the epitopes for the polyclonal antibody, although final proof of this assumption must await further studies involving a panel of monoclonal antibodies against mature IL-1β. Since ED7 bound almost exclusively to the fusion protein, we reasoned that ED7 may recognize a region in the pre-domain of preIL-1β. On the contrary, since DD7 bound to the fusion protein as well as GST, we reasoned that DD7 may recognize the junction between the N terminal portion of preIL-1β and the C-terminal portion of GST.

To confirm that ED7 and DD7 recognize the pre-domain of preIL-1β, we next examined whether or not these antibodies immunoprecipitate metabolically labeled preIL-1β from a HTB9 5637 cell lysate, which contains preIL-1β.

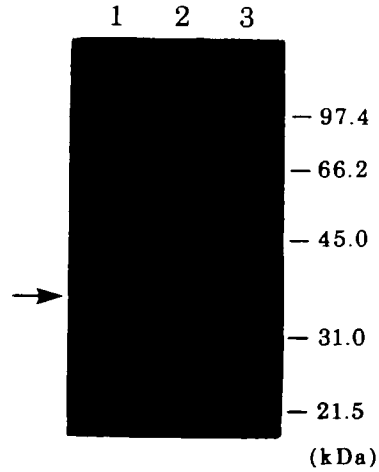

Fig. 2. **Immunoprecipitation of preIL-1β with a rabbit anti-mature IL-1β antibody, ED7, and DD7.** HTB9 5637 cells were metabolically labeled with [^{35}S]methionine, then cell lysates were immunoprecipitated with a rabbit anti-mature IL-1β antibody (lane 1), ED7 (lane 2), and DD7 (lane 3), according to the method described under "MATERIALS AND METHODS." Immunoprecipitates were analyzed by 10% SDS-PAGE, followed by autoradiography. The arrow indicates the position of preIL-1β.

ED7 and the rabbit anti-mature IL-1β antibody immunoprecipitated preIL-1β effectively, whereas DD7 did so ineffectively (Fig. 2). Since the hybridoma producing DD7 was not stable, we abandoned further characterization of DD7.

Immunoprecipitation of Limited Proteolysed PreIL-1β with ED7—To gain more information on the epitope for ED7, we performed immunoprecipitation of limited proteolysed preIL-1β with ED7. PreIL-1β was generated with an *in vitro* transcription-translation system as described under "MATERIALS AND METHODS." V8 protease was used for limited proteolysis, because we (*7*) and others (*14*) found that V8 protease degraded preIL-1β from the N-terminal end to generate a mature 17.5-kDa protein. ED7 immunoprecipitated a 33-kDa protein as well as a 29-kDa protein (indicated by arrows), but hardly immunoprecipitated a 17.5-kDa protein. In contrast, the rabbit anti-mature IL-1β antibody immunoprecipitated all three proteins (Fig. 3). A previous report described that the 17.5-kDa fragment obtained on V8 protease digestion has an extra pentapeptide at the N-terminal end of mature IL-1β (*14*), although we have not yet confirmed this ourselves. The epitope for ED7, therefore, may not be localized at the junction between preIL-1β and mature IL-1β. In spite of this mapping, the binding of ED7 to preIL-1β was sterically hindered by a polyclonal antibody against mature IL-1β. This suggests that the pre-domain of preIL-1β might interact with the mature domain.

Chemical Cross-Linking of PreIL-1α and β—To examine the possibility that the pre-domain of preIL-1β interacts with the mature domain, we tried to demonstrate by chemical cross-linking that the fusion protein between GST and the pre-domain of IL-1β interacts with mature IL-1β, but without success. We therefore examined the effect of chemical cross-linking of preIL-1β on digestion with V8 protease. As shown in Fig. 4, V8 protease at 1 μg/ml yielded a 17.5-kDa fragment from untreated preIL-1β (lane 3), whereas the protease, even at 10 μg/ml, did not yield the fragment from chemically cross-linked preIL-1β with BSOCOES (lane 8). However, the protease at 10 μg/ml degraded cross-linked preIL-1β to generate a fragment with a molecular mass of around 29 kDa (lane 8), suggesting that the N-terminal portion of preIL-1β may not be cross-linked. It should be noted here that the amount of glycine used to quench the reaction was large enough to totally abolish the reactivity of chemical cross-linkers, as evidenced by the absence of cross-linked hemoglobin on

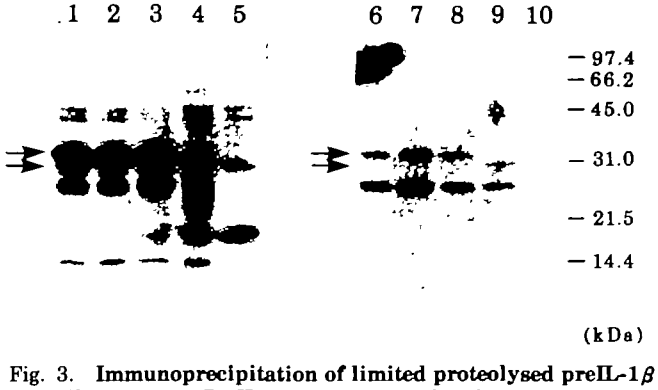

Fig. 3. **Immunoprecipitation of limited proteolysed preIL-1β with V8 protease.** PreIL-1β was generated with an *in vitro* transcription-translation system as described under "MATERIALS AND METHODS," followed by limited proteolysis with V8 protease at the concentration of 0 μg/ml (lanes 1 and 6), 0.01 μg/ml (lanes 2 and 7), 0.1 μg/ml (lanes 3 and 8), 1 μg/ml (lanes 4 and 9), and 10 μg/ml (lanes 5 and 10) for 30 min at 37°C. Limited proteolysed preIL-1β was then immunoprecipitated with a rabbit anti-mature IL-1β antibody (lanes 1 to 5) or ED7 (lanes 6 to 10). The arrows indicate the positions of 33- and 29-kDa proteins.

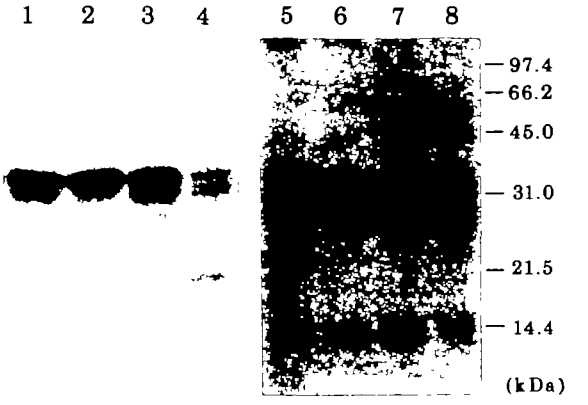

Fig. 4. **Effect of chemical cross-linking of preIL-1β on the susceptibility to V8 protease.** PreIL-1β generated with an *in vitro* transcription-translation system was chemically cross-linked with 2 mM BSOCOES (lanes 5 to 8). Untreated preIL-1β as well as chemically cross-linked preIL-1β was then digested with V8 protease at the concentration of 0 μg/ml (lanes 1 and 5), 0.1 μg/ml (lanes 2 and 6), 1 μg/ml (lanes 3 and 7), and 10 μg/ml (lanes 4 and 8), followed by analysis by SDS-PAGE and autoradiography.

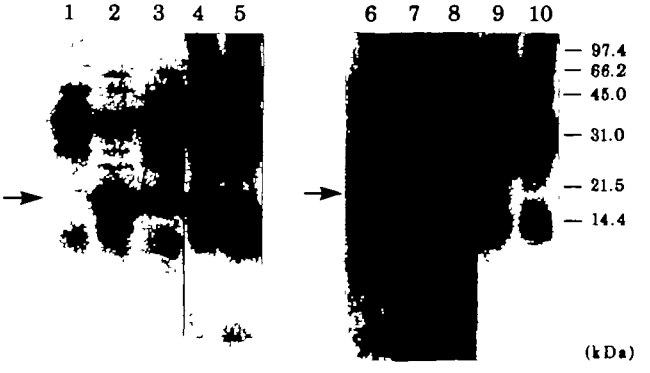

Fig. 5. **Cleavage of protease-digested chemically cross-linked preIL-1α and β with 2-mercaptoethanol.** PreIL-1α and β generated with an *in vitro* transcription-translation system were chemically cross-linked with 4 mM DSP. (1) Untreated preIL-1α as well as chemically cross-linked preIL-1α was then digested with ArgC protease at the concentration of 10 μg/ml for 30 min at 37°C. After inactivation of the ArgC protease by boiling for 5 min, 2-mercaptoethanol was added at the concentration of 5%, followed by boiling for 5 min. Lane 1, untreated preIL-1α; lane 2, preIL-1α treated with ArgC protease; lane 3, cross-linked preIL-1α treated with ArgC protease, and then cleaved; lane 4, cross-linked preIL-1α treated with ArgC protease; lane 5, cross-linked preIL-1α. (2) In the case of preIL-1β, V8 protease was used at the concentration of 10 μg/ml, and the protease was inactivated by boiling in the presence of 2% SDS for 5 min. Lane 6, untreated preIL-1β; lane 7, preIL-1β treated with V8 protease; lane 8, cross-linked preIL-1β treated with V8 protease, and then cleaved; lane 9, cross-linked preIL-1β treated with V8 protease; lane 10, cross-linked preIL-1β. Only samples in lanes 3 and 8 contain reducing agent. The arrows indicate the positions of a 17-kDa protein (lanes 2 and 3) and a 17.5-kDa protein (lanes 7 and 8).

SDS-PAGE (data not shown). Therefore it is unlikely that V8 protease was inactivated by the remaining chemical cross-linkers. In addition, cross-linked preIL-1β as well as the fragment with a molecular mass of 29 kDa migrated as diffuse bands, suggesting that cross-linking occurs at a somewhat internal portion of preIL-1β. A diffuse band(s) corresponding to a molecular mass of around 14-kDa was observed in all lanes for cross-linked preIL-1s, which may represent small fragments which could be detected in nontreated preIL-1s (*e.g.*, Fig. 5, lanes 1 and 6).

We obtained similar findings with preIL-1α using ArgC protease for the limited proteolysis. Our previous reports (*1, 7*) and unpublished results showed that trypsin as well as ArgC protease degraded preIL-1α from the N-terminal end to generate a mature 17-kDa protein. Although ArgC protease yielded a 17-kDa protein from untreated preIL-1α, it did not yield a 17-kDa protein from chemically cross-linked preIL-1α with BSOCOES (data not shown).

Although the result in Fig. 4 suggests that the pre-domain of IL-1β is cross-linked with the mature domain, the possibility remains that the chemical modification of preIL-1β with BSOCOES simply inhibits the digestion with V8 protease. We then employed the cleavable chemical cross-linker, DSP, to demonstrate directly that the pre-domain interacts with the mature domain. As shown in Fig. 5, chemically cross-linked preIL-1α and β with DSP were "resistant" to digestion with ArgC protease and V8 protease, respectively, in the same manner as with BSOCOES (lanes 4 and 9). These two proteases at 10 μg/ml did not yield 17 and 17.5-kDa proteins from preIL-1α and β, respectively. In this case, we also observed that V8 protease degraded cross-linked preIL-1β to generate a fragment with a molecular mass of around 29 kDa (lane 9), confirming the above finding with BSOCOES. Moreover, the cleavage of protease-digested chemically cross-linked preIL-1α and β with 2-mercaptoethanol yielded a small but significant amount of fragments with molecular masses of 17 kDa (for α) (lane 3) and 17.5 kDa (for β) (lane 8). Moreover, polyclonal anti-mature IL-1β antibody immunoprecipitated 17.5-kDa protein liberated from V8 protease-digested chemically cross-linked preIL-1β with 2-mercaptoethanol (Fig. 6). Although the molecular identification of these two proteins needs more thorough biochemical examination, the exact matching of the molecular masses (lanes 2 and 3; lanes 7 and 8) suggests that the 17 and 17.5-kDa proteins are the mature forms of IL-1s.

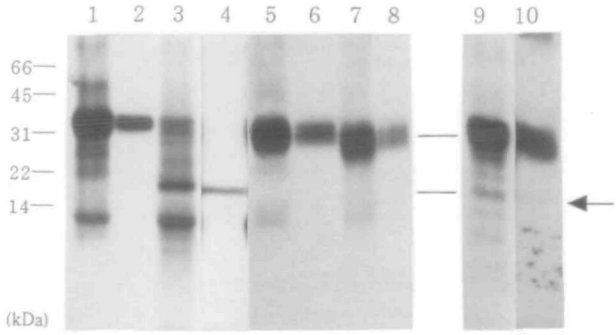

Fig. 6. **Immunoprecipitation of the cleaved products of V8 protease-digested chemically cross-linked preIL-1β.** PreIL-1β generated by with an *in vitro* transcription-translation system was chemically cross-linked with 4 mM DSP. V8 protease was used at the concentration of 10 μg/ml, and the protease was inactivated by 1 mM diisopropylfluorophosphate, followed by immunoprecipitation by polyclonal anti-IL-1β antibody. Lane 1, untreated preIL-1β; lane 2, untreated preIL-1β, immunoprecipitated; lane 3, preIL-1β treated with V8 protease; lane 4, preIL-1β treated with V8 protease, immunoprecipitated; lane 5, cross-linked preIL-1β; lane 6, cross-linked preIL-1β, immunoprecipitated; lane 7, cross-linked preIL-1β treated with V8 protease; lane 8, cross-linked preIL-1β treated with V8 protease, immunoprecipitated; lane 9, cross-linked preIL-1β treated with V8 protease and then cleaved; lane 10, cross-linked preIL-1β treated with V8 protease and then cleaved, immunoprecipitated. The arrows indicate the position of a 17.5-kDa protein.

There are several possible explanations for why only a small amount of the 17- or 17.5-kDa protein was liberated from protease-digested cross-linked preIL-1s. We and others suggested that the initial cleavage of the N-terminal portion of preIL-1α and β, even at the distal end, facilitates the subsequent proteolytic cleavage (*6, 7*), which may explain why only a small amount of a fragment with a molecular mass of 17.5 kDa was generated from chemically cross-linked preIL-1β with V8 protease. In contrast, ArgC protease seems to cleave preIL-1α at one site, yet only a small amount of a fragment with a molecular mass of 17 kDa was generated from chemically cross-linked preIL-1α, suggesting the alternative possibility that alteration of the tertiary structure due to chemical cross-linking may also reduce the susceptibility to digestion by such proteases. Whatever mechanism is responsible for this phenomenon, the experimental data in Figs. 5 and 6 indicate that mature IL-1s are cross-linked with pre-domains even after the cleavage with proteases, and thus are liberated with 2-mercaptoethanol.

These findings therefore support the view that the pre-domain of preIL-1β masks the functional domain of mature IL-1β. This has been suggested by several groups (*6, 14*), partly because ELISA with an antibody against the mature IL-1β underestimates preIL-1β (*8*). Moreover, it is quite possible that some of the biological activity of preIL-1α may be actually due to proteolysed mature IL-1α, although we cannot exclude the possibility that preIL-1α may be structurally different from preIL-1β in an as yet unknown way that makes it biologically active.

Since we failed to demonstrate by chemical cross-linking that the fusion protein between GST and the pre-domain of IL-1β interacts with mature IL-1β, the mature domain in preIL-1β may be structurally different from the cleaved mature peptide. This is consistent with a previous report that the proteinase K-sensitive region of the mature domain of both IL-1s becomes proteinase K-insensitive with proteolytic removal of the precursor domain (*6*). Thus, once cleaved, the pre-domain may not interact with mature IL-1β. Although the possibility remains that the pre-domain of IL-1β in the fusion protein may be structurally different from that in preIL-1β, our study demonstrated that the pre-domain in the fusion protein is to some extent antigenically similar to that in preIL-1β. A recent report described that, upon proteolytic processing, not only mature IL-1β but also the pre-domain is released extracellularly (*15*). Future work should determine whether the pre-domain released can serve as a modulator of the biological activity of IL-1β.

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