The Propeptide of the Transforming Growth Factor-β Superfamily Member, Macrophage Inhibitory Cytokine-1 (MIC-1), Is a Multifunctional Domain That Can Facilitate Protein Folding and Secretion*

Received for publication, November 2, 2000, and in revised form, February 14, 2001
Published, JBC Papers in Press, February 26, 2001, DOI 10.1074/jbc.M010000200

W. Douglas Fairlie, Hong-Ping Zhang, Wan M. Wu, Susan L. Pankhurst, Asne R. Bauskin, Patricia K. Russell, Peter K. Brown, and Samuel N. Breit‡
From the Centre for Immunology, Saint Vincent’s Hospital and University of New South Wales, Victoria Street, Sydney, New South Wales 2010, Australia

Macrophage inhibitory cytokine-1 (MIC-1)1 is a divergent member of the transforming growth factor-β (TGF-β) superfamily. While it is synthesized in a pre-pro form, it is unique among superfamily members because it does not require its propeptide for correct folding or secretion of the mature peptide. To investigate factors that enable these propeptide independent events to occur, we constructed MIC-1/TGF-β1 chimeras, both with and without a propeptide. All chimeras without a propeptide secreted less efficiently compared with the corresponding constructs with propeptide. Folding and secretion were most affected after replacement of the predicted major α-helix in the mature protein, residues 56–68. Exchanging the human propeptide in this chimera with either the murine MIC-1 or TGF-β1 propeptide resulted in secretion of the unprocessed, monomeric chimera, suggesting a specific interaction between the human MIC-1 propeptide and mature peptide. Propeptide deletion mutants enabled identification of a region between residues 56 and 78, which is important for the interaction between the propeptide and the mature peptide. Cotransfection experiments demonstrated that the propeptide must be in cis with the mature peptide for this phenomenon to occur. These results suggest a model for TGF-β superfamily protein folding.

* This work was funded in part by grants from St. Vincent’s Hospital and by Meriton Apartments Proprietary Ltd. through a research and development syndicate arranged by Macquarie Bank Ltd., and by a New South Wales health research and development infrastructure grant. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed. Tel.: 61-2-8382-2590; Fax: 61-2-8382-2820; E-mail: s.breit@cfi.unsw.edu.au.
1 The abbreviations used are: MIC-1, macrophage inhibitory cytokine-1; TGF-β, transforming growth factor-β; CHO, Chinese hamster ovary; PAGE, polyacrylamide gel electrophoresis.

To induce cartilage formation and the early stages of endochondral bone formation (4), and to inhibit proliferation of primitive hemopoietic progenitors (6). The very high level of MIC-1 mRNA in the human placenta, relative to other tissues (2, 4, 6–8), also suggests a role in embryo implantation and placental function. Further, markedly elevated serum levels of MIC-1 occur during pregnancy, suggesting a more generalized function in this process (8). Finally, a number of recent reports have demonstrated that the MIC-1 promoter region is a target for the p53 tumor suppressor gene product (9–11) and that recombinant MIC-1 can suppress tumor cell growth in a number of cell line lines including human breast cancer cells (9). This last effect was a result of induction by MIC-1 of both G1 cell cycle arrest and apoptosis in breast tumor cells.

The MIC-1 protein is synthesized as a 308-amino acid polypeptide that encompasses a 29-amino acid signal peptide, a 167-amino acid propeptide, and a 112-amino acid mature region. The mature protein is secreted as a disulfide-linked homodimer of 112 amino acids which is released from the propeptide after intracellular cleavage at a typical RXR furin-like cleavage site (1). Importantly, unlike all other TGF-β superfamily members studied to date, the MIC-1 mature peptide can be correctly folded and secreted without a propeptide (12, 13). This unique property of MIC-1 makes it a particularly suitable molecule for the study of the factors that influence TGF-β superfamily protein folding. In the case of TGF-β1, activin A, Müllerian inhibitory substance, and bone morphogenetic protein-2, the propeptide region is known to be essential for the correct folding and secretion of the mature peptide (14–16). For example, no TGF-β1 or activin A mature peptides were detected in the supernatants from cells transfected with constructs with an in-frame deletion of their propeptides, and analysis of the cell lysates indicated that the activin A had formed large intracellular disulfide-linked aggregates (16).

To date, most of the studies that have examined the folding of TGF-β superfamily proteins have concentrated on the role of the propeptide. Sha et al. (17) used deletion and insertion mutagenesis to identify regions of the propeptide important for secretion of biologically active mature TGF-β1 and determined that amino acids between residues 50 and 80 interact with the mature peptide in the latent complex form of TGF-β1. Elimination of all glycosylation sites on the TGF-β1 and -β2 propeptide prevents secretion of any mature protein (18, 19), and mutation of the TGF-β1 propeptide cysteine residues results in the secretion of differently assembled and processed forms (20). In addition, it has been shown that the Müllerian inhibitory substance propeptide helps to maintain the conformation of the
Müllerian inhibitory substance mature peptide after secretion by preventing aggregation (14). Only limited studies have focused on the mature peptide region, where the role of the cysteine residues has been examined for TGF-β1 and activin A, and all were found to be essential for the secretion of a fully bioactive mature protein (18, 21, 22).

No detailed studies on MIC-1 folding have yet been published, although recently we reported that the propeptide has a role in the intracellular quality control of MIC-1 secretion by targeting monomeric precursor forms in the endoplasmic reticulum to the proteasome for degradation (12). In the current paper we have addressed a number of aspects of folding and secretion of MIC-1. In particular we have determined sequence regions of the mature peptide which enable it to fold and be secreted in the absence of the propeptide. We have also identified a region of the MIC-1 propeptide which can assist in protein folding/secretion.

EXPERIMENTAL PROCEDURES

Cell Culture—Chinese hamster ovary cells (CHO-K1) were maintained as recommended by the American Type Culture Collection in Dulbecco's modified Eagle's medium/F-12 medium supplemented with 5% fetal bovine serum. For collection of conditioned medium for immunoprecipitation and Western blotting experiments, transfected cells were cultured in the absence of fetal bovine serum (see below).

Antisera—Anti-FLAG M2 antibody coupled to agarose used in immunoprecipitations was purchased from Sigma. Polyclonal antisera to MIC-1 (PAb 233) was raised by immunization of sheep with purified recombinant human MIC-1 mature protein (13).

Preparation of cDNA Constructs—The preparation of the base FLAG-tagged, full-length "long" construct (i.e. MIC-1 leader + propeptide + MIC-1 mature peptide; see Fig. 1, constructs A1–A3) and "short" mature peptide only construct (FSH leader sequence + MIC-1 mature peptide; Fig. 1, constructs F1–F3) has been described previously (1). The FLAG epitope was engineered onto the amino terminus of the mature peptide of all constructs (i.e. inserted immediately after the furin-like cleavage sequence of propeptide region; see Fig. 1) to facilitate immunoprecipitation of the secreted proteins. The relevant constructs were cloned into the pOcus-2 vector (Novagen) for the construction of the chimeras and other mutants, and the pIRE2-EGFP vector (CLONTECH) was used for transfection into CHO cells.

Both the long and short constructs described previously were amplified by polymerase chain reaction and either Pfu DNA polymerase (Promega) or Vent DNA polymerase (New England Biolabs) with the oligonucleotide primers 1 and 2 (for all primer sequences, see Table I), which added an XhoI and SacII + BglII sites onto their 5'- and 3'-ends, respectively, for insertion into the pOcus-2 vector (at the XhoI and BglII sites) or pIRE2-EGFP (at the XhoI and SacII sites). The various mutant/chimeric constructs were made using a whole plasmid polymerase chain reaction technique described previously (23). The primers for the site 1, 2, and 3 chimeras have also been described previously (23). For the propeptide deletion mutants (Fig. 1, constructs B–E), a single common reverse primer was used (primer 3) which corresponded to the 3'-end of the MIC-1 signal-peptide sequence, and the forward primers (primer 4, deletion 1–28; primer 5, deletion 1–78; primer 6, deletion 1–41; primer 7, deletion 1–55) were positioned so that the 5'-end started at the first base of the codon for the amino acid following the region of the propeptide to be deleted. The glycosylation mutant in which pro-Asn41 (asparagine residue 41 of the propeptide) was changed to serine codons and was kindly provided by Ignacio Anegon (20). The constructs with the simian TGF-β1 propeptide fused to the MIC-1 mature (Fig. 1, construct H), the sim-TGF-β1 propeptide was amplified with primers 10 and 11, which added an XhoI/SacII fragment from the long, short, or both constructs in the pIRE2-EGFP vector was replaced with the same fragment from the mutated construct in the pOcus-2 vector. For the propeptide deletion constructs, the XhoI/EcoRI fragment of the pIRE2-EGFP long construct was replaced with same fragment derived from the mutants in the pOcus-2 vector.

To create the constructs with the simian TGF-β1 propeptide fused to the MIC-1 mature (Fig. 1, construct H), the sim-TGF-β1 propeptide was amplified with primers 10 and 11, which added XhoI and EcoRI restriction enzyme sites to the 5’- and 3’-ends, respectively. The template for this construct was sim-TGF-β1 with the codons for Cys-223 and Cys-225 mutated to serine codons and was kindly provided by Ignacio Anegon (20). The XhoI/EcoRI fragments from the long pIRE2-EGFP construct as well as the site 2 chimeric cloned into the pIRE2-EGFP vector were then replaced with the digested, amplified sim-TGF-β1 propeptide. The murine MIC-1 propeptide was added to the wild type or site 2 chimeric human MIC-1 following replacement of the XhoI and EcoRI fragment with the corresponding fragment of the long murine construct (Fig. 1, construct G).

Cell Transfections—Transient transfections were performed in CHO-K1 cells grown in six-well plates to 60–80% confluence. Plasmid

![Fig. 1. Arrangement of constructs.](http://www.jbc.org/)
The lysate was collected after centrifugation at 4 °C, in inhibitor mixture (Roche Molecular Biochemicals), and then incubated in the medium or cell lysates was performed by adding 10 mM of DTT formed under reducing conditions, and Western blotting.

The (F) or (R) after each sequence indicates whether the primer used is a forward or reverse primer, respectively. Underlined bases indicate the primer sequence added in the construction of chimera or the base pair change used in the construction of the glycosylation mutant.

| Primer no. | Primer sequence |
|------------|----------------|
| 1          | AATTCTCGAGGATATCATGCGCCACCGAAGACTCAG (F) |
| 2          | ACATGATACGTCGGCGTCATGACGTCGACGTCTAC (F) |
| 3          | GGCAGGCGCTCGTGGCGGCGCACGCTG (R) |
| 4          | CAGAGCTGGAGAGGATCCGACGGAGCTTTGC (R) |
| 5          | GCCGCCCTTCGAGGGCTC (F) |
| 6          | CAGACTGTTAATTCTCGAGGATATCATGCGCCACCGAAGACTCAG (F) |
| 7          | GCAGCTGGAGAGGATCCGACGGAGCTTTGC (R) |
| 8          | GCCGCCCTTCGAGGGCTC (F) |
| 9          | GCAGCTGGAGAGGATCCGACGGAGCTTTGC (F) |
| 10         | CAGAGCTGGAGAGGATCCGACGGAGCTTTGC (F) |
| 11         | AATTCTCGAGGATATCATGCGCCACCGAAGACTCAG (F) |

DNA (1 μg), purified using a Qiaprep Spin purification kit (Qiagen), was mixed with 9 μl of LipofectAMINE (Life Technologies, Inc.) for 15–30 min at room temperature and then added to cells in a total volume of 1 ml of serum-free Dulbecco’s modified Eagle’s medium/F-12 medium. After overnight incubation at 37 °C, the cells were washed with Dulbecco’s modified Eagle’s medium/12% medium containing 5% fetal bovine serum and incubated for 6 h at 37 °C in the same medium. Cells were washed with serum-free medium then maintained in 1 ml of the same medium for a further 48 h before collection for immunoprecipitation and Western blot analysis. For the experiment to determine whether propeptide could act both in cis and in trans, mutant and wild type constructs were transfected, as above, into a CHO-K1 cell line previously stably transfected with the human MIC-1 propeptide alone (12). Transfection efficiency was monitored using a fluorescent microscope that could detect the enhanced green fluorescent protein, a product of the pLRES2-EGFP vector, and was routinely in the order of 60–80%. Comparison between supernatants was only made from wells in which the cells were transfected to approximately the same degree.

Immunoprecipitation of the FLAG-tagged proteins in the conditioned medium was collected, and the cells were washed with ice-cold phosphate-buffered saline and then lysed. To perform lysis, cells were scraped off the wells in the presence of 0.5 ml of 50 mM Hepes, pH 7.0, containing 1% Triton X-100, 1 mM EDTA, and a protease inhibitor mixture (Roche Molecular Biochemicals), and then incubated on ice for 30 min. The lystate was collected after centrifugation at 4 °C, then resuspended in 0.5 ml of phosphate-buffered saline, or in the case of the lyses with the lysis buffer itself, and eluted by heating at 95 °C in SDS-PAGE (nonreducing or reducing) sample buffer.

Western blot analysis was performed essentially as described previously (1). Membranes were probed with either sheep anti-MIC-1 polyclonal antiserum 233 (diluted 1:7,000) or anti-FLAG monoclonal antibody followed by either biotinylated sheep (1:1,000) (Sigma) or anti-mouse antiserum (1:1,000) (Amersham Pharmacia Biotech), respectively. Blots were then visualized on film after treatment with enhanced chemiluminescence reagents (PerkinElmer Life Sciences).

Rationale of MIC-1 Construct Design—The three-dimensional structures of the three mammalian TGF-β isoforms as well as bone morphogenetic proteins-2 and -7 and glial cell line-derived neurotrophic factor have all been solved (24–29). Comparison of these structures indicates that they are very similar in the overall fold of the subunits for each family member despite low amino acid sequence homology between family groups (26% between human TGF-β1 and human bone morphogenetic protein-7 and 12% between human TGF-β1 and human glial cell line-derived neurotrophic factor). This similarity suggested that it may be possible to construct chimeric molecules in which sequence regions of one family member are exchanged with the corresponding region of another family member. A similar approach has been used for the identification of TGF-β1 and TGF-β2 receptor binding regions where chimeric molecules involving both isoforms proved to be useful (30, 31). We therefore made a series of constructs in which three distinct sequence regions of the mature protein of MIC-1 were replaced with the corresponding regions of TGF-β1. The three regions of MIC-1 selected were: residues 24–37 (site 1) which corresponds to part of the extended loop region also referred to as “finger 1” in TGF-β1; residues 56–68 (site 2), corresponding to the major a-helix (also called the “heel” region) in TGF-β1; and residues 90–98 (site 3), which encompasses a type II β-turn and corresponds to the tip of “finger 2” of TGF-β1 (for the structural location each region see Fig. 2a; for sequences, see Fig. 2b). Additional constructs were also made in which the propeptide region was either deleted or replaced with the murine MIC-1 or sim-TGF-β1 propeptide.

The Propeptide of MIC-1 Enhances Secretion of MIC-1/TGF-β Chimeras—To determine whether each of the sequence substitutions described above would be tolerated and result in the secretion of a correctly processed dimeric molecule, the three MIC-1/TGF-β1 chimeric constructs with associated MIC-1 propeptide (see Fig. 1, constructs A1–A3) were transfected into CHO-K1 cells. Supernatants were collected and immunoprecipitated with anti-FLAG-coupled agarose and analyzed by Western blotting. Bands of the correct apparent molecular mass (30 kDa) for dimeric, FLAG-tagged MIC-1 were visualized on film after treatment with anti-MIC-1 polyclonal antiserum 233 (diluted 1:7,000) or anti-FLAG monoclonal antibody, respectively (1). Membranes were probed with either sheep anti-MIC-1 polyclonal antiserum 233 (diluted 1:7,000) or anti-FLAG monoclonal antibody.
similar bands were observed in the immunoprecipitated supernatant from cells transfected with the vector only (Fig. 3d, lane 3). Various additional higher molecular mass bands observed in lane 1 at ~33, 40, and 50 kDa correspond to aberrantly or differentially processed forms of MIC-1 which have been characterized previously (12).

As indicated previously, MIC-1 is unique among the TGF-β superfamily members studied to date in that it can be secreted from a construct in which the propeptide is deleted. To identify sequence regions of the mature MIC-1 protein which enable it to fold correctly without association or interaction with its propeptide, the three chimeras were expressed using constructs in which the propeptide had been deleted (Fig. 1, constructs F1–F3). An FSH leader sequence was fused to these proteins to enable secretion. This adds three extra amino acids to each subunit resulting in slower migration of the expressed proteins on SDS-PAGE compared with those expressed from the full-length constructs. Bands corresponding to the molecular mass of dimeric MIC-1 were observed for both the wild type MIC-1 and the site 1 and site 3 replacements (Fig. 3, a, b, and d, lanes 2) following immunoprecipitation and immunoblotting of the supernatants from CHO cells transfected with these constructs. This indicates that these proteins were correctly assembled and secreted despite the deletion of the propeptide. However, the level of secretion of the site 1 and site 3 chimeras was consistently less than the corresponding constructs with a propeptide. In the case of the site 2 replacement, no measurable protein was secreted from the expressed construct without propeptide (Fig. 3c, lane 2), demonstrating that this protein cannot be folded and/or secreted without the presence of the propeptide. Analysis of the cell lysates for all truncated constructs demonstrated that the mutant proteins were synthesized at approximately the same level (Fig. 3, lower panel), confirming that the markedly lower level of protein observed in the supernatant for the site 2 chimaera in the absence of its propeptide is caused by its inefficient folding/secretion. These combined results therefore suggest that the MIC-1 propeptide, although not essential, can assist in folding/secretion. Furthermore, it appears that the site 2 (α-helix) sequence is one region of MIC-1 which enables it to be secreted in the absence of the propeptide.

The MIC-1 Propeptide Carbohydrate Moiety Is Not Essential for Secretion of the Site 2 MIC-1/TGF-β Chimaera—The carbohydrate moieties on the propeptide of the TGF-β1 and -β2 propeptides are important for the secretion of biologically active TGF-β mature peptides. Glycosylation of the MIC-1 propeptide occurs at a single N-linked glycosylation site at residue pro-Asn41 (residue asparagine 41 of the propeptide) (12). Therefore, it was of interest to determine whether the carbohydrate moiety played a role in MIC-1 folding and secretion and in particular to determine whether it was essential for secretion of the site 2 MIC-1/TGF-β chimera. To achieve this, pro-Asn41 of the propeptide was mutated to a serine residue on constructs with both the wild type and site 2 chimeric mature peptides (Fig. 1, construct 1).

Both the wild type and site 2 chimera mature peptides were secreted when expressed from constructs with either glycosylated or unglycosylated propeptide (Fig. 4). Although a lower level of secretion was observed for the site 2 chimera mature peptide from the unglycosylated propeptide (Fig. 4, lane 4) compared with the glycosylated construct (Fig. 4, lane 3), a corresponding lower level of synthesis was also observed in the cell lysate for the same construct (Fig. 4, lysate). It can therefore be concluded that the propeptide carbohydrate does not play a role in facilitating secretion of the site 2 chimera.

Expression of the Correctly Processed and Assembled Site 2 Chimera Only Occurs with the Human MIC-1 Propeptide—To the best of our knowledge, only one previous attempt has been made to interchange the propeptides belonging to different TGF-β superfamily members (16). This earlier study involved exchanging the activin A propeptide for the TGF-β propeptide. The results demonstrated that the mature protein was secreted, albeit with low efficiency, when the non-native propeptide was introduced. As MIC-1 is secreted without a propeptide, it was of interest to establish whether the protein would undergo correct assembly, processing, and secretion after replacement of the MIC-1 propeptide with that of the closely related murine MIC-1 propeptide (65% identical) or the more distantly related and quite dissimilar TGF-β1 propeptide. More importantly, we also wanted to establish whether these other propeptides could facilitate folding and secretion of the site 2 chimeric mature peptide. It should be noted that in these experiments we utilized the simian TGF-β1 propeptide, which has the cys-
Proteins immunoprecipitated from the supernatant of CHO-K1 cells transiently transfected with the wild type MIC-1 mature peptide sequence fused to either the murine MIC-1 and TGF-β1 propeptide or (lanes 1 and 3) the MIC-1/TGF-β1 site 2 chimera (lanes 3 and 4) and then transiently transfected into CHO cells and the supernatants analyzed as described previously. The Western blot of the immunoprecipitated cell lysate for the site 2 chimera constructs with or without mutated glycosylation site is shown below. Lane V corresponds to the lysate for cells transfected with vector only. The band shown in the lysates corresponds to the correct size for the unprocessed precursor proteins.

Deletion of portions of the propeptide attached to the wild type mature protein appeared to have little effect on the secretion of the wild type MIC-1 (data not shown). In the case of the MIC-1/TGF-β1 site 2 chimera, deletion of residues 1–28, 1–41 (which includes the glycosylation site), and 1–55 resulted in only a marginal progressive decrease in the level of secretion observed (Fig. 6, upper panel, lanes 1–3). The deletion of residues 1–78, however, essentially prevented secretion of any chimeric protein (Fig. 6, lane 4). The cell lysates for each of these constructs indicated approximately equal levels of synthesis of each precursor protein (Fig. 6, lower panel, lanes 1–4).

These results therefore indicate that the region of the propeptide necessary for folding and secretion of MIC-1, in which the α-helix region has been replaced with the corresponding region of TGF-β1, lies between residues Pro59 and Pro79.

The Propeptide Must Be in cis with the Site 2 Chimeric Mature Peptide for Secretion to Occur.—To determine whether the MIC-1 propeptide could assist in secretion of the MIC-1/TGF-β1 site 2 chimera in trans as well as in cis, the site 2 chimeric construct (without associated propeptide) was transfected into a cell line previously stably transfected with the (FLAG-tagged) MIC-1 propeptide (12). The supernatants were then analyzed as previously. No secretion of the chimeric protein into the supernatant was observed (Fig. 7, upper panel, lane 3) although, as would be expected, secretion of the wild type MIC-1 mature peptide did occur (Fig. 7, upper panel, lane 2). The cell lysates were also analyzed to confirm synthesis of the propeptide, and a band of ~28 kDa, consistent with the correct size of the MIC-1 propeptide, was present in all samples (Fig. 7, lower panel, lanes 2 and 3). No similar band was seen in the lysate of untransfected CHO cells (Fig. 7, lower panel, lane 1). These results demonstrate that the propeptide must be in cis with the mature peptide region to enable secretion of the site 2 chimera.

DISCUSSION

We have shown recently that the MIC-1 propeptide has a novel role in proteasomal targeting (12). In addition, the MIC-1 mature peptide was unique among TGF-β1 superfamily members studied to date in that the propeptide region was neither
required for the correct folding and assembling of subunits, nor was it necessary for secretion of the mature protein. Indeed, MIC-1 secreted from constructs without the propeptide is both biologically active (32) and immunogenically identical to MIC-1 produced from full-length constructs (13). There is also no evidence that the MIC-1 propeptide is required for secretion of the mature protein. Indeed, it was necessary for secretion of the mature protein. This demonstrates that the MIC-1 propeptide can facilitate mature peptide folding despite not actually being required.

Although all of the regions exchanged in the chimeras resulted in reduced secretion of the mature peptide in the absence of the propeptide, the sequence region that was most dependent on the propeptide for secretion was the predicted major β-helix (residues 56–68). It is likely, therefore, that it is the physicochemical properties of this region which substantially contribute to the ability of MIC-1 to be secreted without its propeptide. The corollary of this is that in members of the TGF-β superfamily which cannot be secreted without their propeptides, it may be the properties of their β-helical region which are, at least in part, responsible for their requirement for the propeptide to achieve a correctly folded and secreted mature protein.

In the β-helical region in question, at least six (mainly hydrophobic) amino acids from this region of TGF-β1, -β2, -β3, and bone morphogenetic protein-7 are present at the dimer interface and make contact with amino acids on the opposite subunit. At least one of these residues participates in hydrophobic bonding with residues on the other subunit (24–27). Therefore, in the case of the site 2 chimera, it is possible that the propeptide region may play a role in ensuring that the appropriate intersubunit contacts can be made and/or that the subunits only align in the correct orientation. Alternatively, or concomitantly, the propeptide may also act to ensure that other inappropriate contacts do not occur. This is discussed further below. Consistent with these hypotheses is the previous finding of Gray and Mason (16) who used dimer-specific antisera to determine that intracellular dimer formation of activin A was dependent on the presence of the propeptide. Our data, which showed that replacement of the human propeptide with either the murine MIC-1 or TGF-β1 propeptide resulted in the secretion of just the unprocessed monomeric site 2 chimera, also supports this idea.
A potential clue as to why the propeptide is not required in MIC-1 folding/secretion may be found in hydrophilicity plots of the α-helix region of various TGF-β superfamily proteins (Fig. 8). Compared with other proteins that have been shown to require their propeptides for folding and secretion, the MIC-1 helix is significantly more hydrophilic across the entire region. This perhaps suggests that it may be possible to predict other family members that do not require propeptides for folding and secretion based upon the hydrophilicity in this region.

The apparent capacity of the MIC-1 propeptide to facilitate protein folding is of particular interest as it appears that the mature peptide region has evolved independently of the propeptide region such that the propeptide is no longer required for this particular function. An important practical consequence of this finding is that it may be possible to engineer other TGF-β superfamily proteins to be secreted without the necessity of their propeptides by making mutations within specific sequence regions. This is currently under investigation in our laboratory.

The fact that the site 2 chimera is only secreted from a construct with the propeptide made it a useful model protein for the study of which regions of the propeptide are important in the folding process. Deletion mutants were used to determine that amino acids 56–78 of the propeptide are essential for secretion of the mature chimeric peptide. Furthermore, the deletion of the correctly assembled and processed site 2 chimera, it is likely that an additional specific interaction between the mature peptide secretion (16). The level of protein observed in the supernatant, however, was very low (2.4% or 9% of that secreted from a wild type, full-length TGF-β1 or activin A construct, respectively), and hence the difference between the studies may simply reflect a difference in the sensitivities of the assays used for detection (enzyme-linked immunosorbent assay and bioactivity for TGF-β1 and activin A compared with immunoprecipitation followed by Western blotting for MIC-1).

In this paper we demonstrated that the MIC-1 propeptide is a multifunctional domain that, in addition to its previously reported role in proteasomal targeting, can also facilitate protein folding and secretion. We believe that the mechanism of propeptide-mediated folding in MIC-1 may provide some important insights that could be applicable to other superfamily members. One model that we propose is that the propeptide may act similarly to an intramolecular chaperone. In this context the propeptide acts by masking particular regions along the mature peptide which may form inappropriate interactions during the folding process. For TGF-β superfamily members the major α-helix in particular may be a specific region that requires masking. Because the TGF-β propeptide itself was unable to facilitate folding/secretion of the site 2 MIC-1/TGF-β1 chimera, it is likely that an additional specific interaction between the MIC-1 propeptide and the mature peptide region (outside of the α-helix) occurs. A schematic representation of how this may occur is presented in Fig. 9. Herein we indicate at least two contacts between the propeptide and mature peptide: an undefined specific interaction involving residues outside of the α-helix and amino acids in the propeptide; and a second, perhaps less specific (hydrophobic) interaction, which provides the masking and occurs between the mature peptide α-helix and the propeptide. In at least one of these interactions, residues between 56–78 of the propeptide are important. Additional contacts and masking of regions other than the α-helix not shown in the figure are also likely. Further studies will be required to confirm the validity of this model, and these are currently in progress in this laboratory.

Unlike another study of this superfamily, the MIC-1 propeptide only facilitated folding in cis. In the case of TGF-β1 and activin A, the independent cotransfection of the propeptide and mature peptide regions of both of these proteins rescued mature peptide secretion (16). The level of protein observed in the supernatant, however, was very low (2.4% or 9% of that secreted from a wild type, full-length TGF-β1 or activin A construct, respectively), and hence the difference between the studies may simply reflect a difference in the sensitivities of the assays used for detection (enzyme-linked immunosorbent assay and bioactivity for TGF-β1 and activin A compared with immunoprecipitation followed by Western blotting for MIC-1).

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In this paper we demonstrated that the MIC-1 propeptide is a multifunctional domain that, in addition to its previously reported role in proteasomal targeting, can also facilitate protein folding and secretion. We believe that the mechanism of propeptide-mediated folding in MIC-1 may provide some important insights that could be applicable to other superfamily members. One model that we propose is that the propeptide may act similarly to an intramolecular chaperone. In this context the propeptide acts by masking particular regions along the mature peptide which may form inappropriate interactions during the folding process. For TGF-β superfamily members the major α-helix in particular may be a specific region that requires masking. Because the TGF-β propeptide itself was unable to facilitate folding/secretion of the site 2 MIC-1/TGF-β1 chimera, it is likely that an additional specific interaction between the MIC-1 propeptide and the mature peptide region (outside of the α-helix) occurs. A schematic representation of how this may occur is presented in Fig. 9. Herein we indicate at least two contacts between the propeptide and mature peptide: an undefined specific interaction involving residues outside of the α-helix and amino acids in the propeptide; and a second, perhaps less specific (hydrophobic) interaction, which provides the masking and occurs between the mature peptide α-helix and the propeptide. In at least one of these interactions, residues between 56–78 of the propeptide are important. Additional contacts and masking of regions other than the α-helix not shown in the figure are also likely. Further studies will be required to confirm the validity of this model, and these are currently in progress in this laboratory.
