Protein-disulfide isomerase (PDI) has been shown to act as a molecular chaperone during the refolding of denatured proteins in vitro. To investigate the role of this multifunctional protein within a cellular context, we have established a semi-permeabilized cell system that reconstitutes the synthesis, folding, modification, and assembly of procollagen as they would occur in the cell. We demonstrate here that P4-H associates transiently with the triple helical domain during the assembly of procollagen. The release of P4-H from the triple helical domain coincides with assembly into a thermally stable triple helix. However, if triple helix formation is prevented, P4-H remains associated, suggesting a role for this enzyme in preventing aggregation of this domain. We also show that PDI associates independently with the C-propeptide of monomeric procollagen chains prior to trimer formation, indicating a role for this protein in coordinating the assembly of heterotrimeric molecules. This demonstrates that PDI has multiple functions in the folding of the same protein, that is, as a catalyst for disulfide bond formation, as a subunit of P4-H during proline hydroxylation, and independently as a molecular chaperone during chain assembly.

Protein-disulfide isomerase is now firmly established as a multifunctional protein that both catalyzes the formation of disulfide bonds and acts as a subunit of prolyl 4-hydroxylase and microsomal triglyceride transfer protein (1). The function of PDI2 as a component of these enzymes appears to be to maintain the catalytic subunits in a soluble form rather than directly participating in catalysis (2, 3). In this respect, its function is independent of disulfide isomerase activity (4). More recently, PDI has been proposed to act as a molecular chaperone by binding to unfolded proteins, thereby preventing aggregation (5–8). This proposal is based on the observation that PDI assists in the refolding of certain denatured proteins in vitro, but this activity appears to be substrate specific, with no activity or even negative (antichaperone) activity being observed with some protein substrates (9–11). PDI also has been shown to interact with newly synthesized proteins (12, 13) and with cysteine mutants of human lysozyme (14), but whether this interaction reflects chaperone activity or the binding of PDI to its substrate during disulfide bond formation still needs to be determined.

PDI is clearly a key cellular folding enzyme that is important for the maturation of several secreted and membrane-associated proteins. This is particularly true for the folding and maturation of procollagen, where PDI is involved in a number of key stages. As the polypeptide chain is translocated across the membrane of the endoplasmic reticulum, intrachain disulfide bonds are formed within the N-propeptide and C-propeptide, and hydroxylation of proline and lysine residues occurs within the triple helical domain (15). Chains then associate via the C-propeptides to form homo- or heterotrimeric molecules. This allows the triple helical domain to form a nucleation point at its C-terminal end, ensuring correct alignment of the chains. The triple helix then folds in a C- to N-direction, with the N-propeptides finally associating and in some cases forming interchain disulfide bonds (16). PDI participates during proline hydroxylation as a subunit of prolyl 4-hydroxylase and also catalyzes the formation of both intra- (17) and interchain disulfide bonds (18).

Most of our understanding of how procollagen folds and assembles within the cell has come from studies of cells grown in culture, particularly either skin or tendon fibroblasts. Although this approach has provided us with a clear outline of the intracellular folding and assembly of procollagen, it does not lend itself to a more detailed analysis of the molecular recognition events occurring during assembly. To facilitate these studies, a semi-permeabilized cell system has been developed that reconstitutes the initial stages in the assembly and modification of procollagen as they would occur in an intact cell (19). Using this system, the translocation, disulfide bond formation, and assembly of procollagen into a correctly aligned triple helical molecule has been reconstructed, in a system that mimics the processes as they would occur within an intact cell (20). Here, we have extended these studies to investigate the role of PDI in the folding and assembly of procollagen. We have demonstrated that this protein not only participates in disulfide bond formation and proline hydroxylation but also acts as a molecular chaperone interacting specifically and independently with procollagen chains that remain monomeric, thereby preventing premature assembly or aggregation.

MATERIALS AND METHODS

Construction of Recombinant Plasmids—Recombinant p11IIIΔ1 and p021Δ1 have been described previously (21). Recombinant p011Δ1 was generated from COL1A1-CMV (22) by excision of an internal 2.5-kb Apal fragment and religation of the parental plasmid. An additional nucleotide was inserted by Pfu mutagenesis using the QuikChange mutagenesis kit (Stratagene Ltd., Cambridge, UK) at the sites which would be added in the future. This work was supported by The Royal Society. 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 1754 solely to indicate this fact.
Apol cleavage site to preserve the correct reading frame. Recombinant plasmid constructs were generated by polymerase chain reaction overlap extension using the principles outlined by Horton (23). Polymerase chain reactions (100 μl) comprised template DNA (500 ng), oligonucleotide primers (100 pmol each), in 10 mM KCl, 20 mM Tris-HCl, pH 8.8, 10 mM MgSO₄, 0.1% (w/v) Triton X-100, 300 μM each dNTP. Ten rounds of amplification were performed in the presence of 1 unit Vent DNA polymerase (New England Biolabs, Beverly, MA). Recombinant C-propeptide-minus was generated using a 5'- oligonucleotide primer (5'-GATGATGCAAGCGCGCA 3') complementary to the T3 promoter sequence upstream of the initiation codon in pCP1(III)Δ1, and a 3'- amplification primer (5'-TCCGGAGGTACCCGCTACTGGCAGTTGTTTG 3') complementary to a region 100 bp upstream of an XhoI site in pCP1(III)Δ1 and a 3' amplification primer (5'-TCCGGAGGTACCCGCTACTGGCAGTTGTTTG 3') complementary to a sequence 100 bp downstream of the stop codon in pCP1(III)Δ1. A KpnI site was incorporated to facilitate subsequent sub-cloning. Recombinant plasmids were linearized and transcribed using T3 RNA polymerase (Promega, Southampton, UK). Recombinant C-propeptide-minus was generated using a 5'- oligonucleotide primer (5'-AATGGAGCTCCTGGACCCATG 3') and subcloned into pBS-SK (Stratagene Ltd, Cambridge, UK). Recombinant C-propeptide-minus was generated using a 5'- oligonucleotide primer (5'-AATGGAGCTCCTGGACCCATG 3') and subcloned into pBS-SK (Stratagene Ltd, Cambridge, UK).

Transcription in Vitro—Transcription reactions were carried out as described by Guereich et al. (26). Recombinant plasmids were linearized and transcribed using T3 RNA polymerase (Promega, Southampton, UK). Transcription reactions (100 μl) were incubated at 37 °C for 4 h. Following purification over RNaseY columns (Qiagen, Dorking, UK), the RNA was resuspended in 100 μl of RNase-free water containing 1 mM DTT and 40 units of RNasin (Promega).

Translation in Vitro—RNA was translated using a rabbit reticulocyte lysate (FlexiLysate, Promega) for 60 min. at 30 °C. The translation reaction (25 μl) contained 17.5 μl of reticulocyte lysate, 0.5 μl of 1 mM amino acids (minus methionine), 0.5 μl of 100 mM KCl, 0.25 μl of ascorbic acid (5 mg/ml), 0.05 μl of T-[3H]methylene, (NEN Life Science Products), 1 μl of transcribed RNA and 1 μl (approximately 2 x 10⁶) of semipermeabilized cells (SP cells) prepared as described previously (19). Translations were initiated either in the presence or absence of 1 mM of Leu, Lys (Sigma) to inhibit the activity of endoplasmic reticulum (ER) hydroxylation enzymes.

Posttranslational Incubations—After 60 min of translation, cycloheximide was added to 5 μM, and samples were incubated for a further time periods up to 60 min at 30 °C in the presence or absence of 5 mM Fe(II) sulfate to allow hydroxylation to occur posttranslationally (20). SP cells were isolated by centrifugation at 13,000 x g for 5 min. Pellets were resuspended in RWM buffer prior to subsequent analysis. Samples were prepared for electrophoresis and treated with proteases or the chemical cross-linkers BMH or DSP (Pierce and Warriner Ltd., Chesh-ire, UK).

Proteolytic Digestion—Isolated SP cells were solubilized in C/T digest buffer (50 mM Tris-HCl, pH 7.4, containing 0.15 μ NaCl, 10 mM EDTA, 1% (w/v) Triton X-100) and centrifuged at 13,000 x g for 5 min to remove cell debris. The supernatant was recovered and then digested with a combination of chymotrypsin (250 μg/ml) and trypsin (100 μg/ml) (Sigma) for 1 min at 30 °C. The reactions were stopped by the addition of soybean trypsin inhibitor (Sigma) to a final concentration of 500 μg/ml and acidified by the addition of HCl to a final concentration of 100 mM. Samples were incubated with pepsin (100 μg/ml) for 2 h at 30 °C. The reactions were stopped by neutralization with Tris base (100 mM) and prepared for electrophoresis as described below.

Chemical Cross-linking—After translation, SP cell pellets were resuspended in a final volume of 50 μl of KH2O and chemical cross-linkers added from a 50 mM stock (prepared fresh in DMSO) to a final concentration of 1 mM for both DSP and BMH cross-linking experiments. Cross-linking of samples was performed for 10 min at 25 °C followed by a further incubation after addition of 100 mM glycine or 5 mM DTT to quench the DSP or BMH reactions, respectively.

Immunoprecipitation—Cross-linked samples were denatured by boiling for 5 min in SDS/Nonidet P-40 denaturation buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl containing 1% (w/v) SDS and 1% (v/v) Nonidet P-40). Insoluble material was removed by centrifugation at 13,000 x g for 10 min, and the supernatant was adjusted to a final volume of 1 ml of immunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, containing 0.15 μ NaCl, 10 mM EDTA, 1% (w/v) Triton X-100). Immunoprecipitations were preincubated at 4 °C for 40 min in IP buffer containing 50 μl of protein A-Sepharose (10% (w/v) in PBS) (Zymed Laboratories Inc., San Francisco, CA). The samples were centrifuged for 1 min at 10,000 x g to remove protein-A binding components. Supernatants were recovered and made up to a volume of 1 ml with IP buffer. Immunoprecipitation of cross-linked products was carried out at 4 °C in the presence of antibodies and 50 μl of protein A-Sepharose (10% (w/v) in PBS). The polyclonal antisera to bovine PDI and rat P4-H α subunit were used as described previously (2, 27). Immune complexes were retrieved by brief centrifugation (13,000 x g for 30 s) and washed twice in IP buffer, once in IP buffer containing 500 mM NaCl, and finally in IP buffer alone.

SDS-Polyacrylamide Gel Electrophoresis—Samples prepared for electrophoresis by the addition of SDS-PAGE loading buffer (0.0625 x Tris/HCl, pH 6.8, SDS (2% w/v), glycerol (10% w/v), bromophenol blue) in the presence or absence of 50 mM DTT and boiled for 5 min. After electrophoresis, gels were dried, processed for autoradiography, and exposed to Kodak X-Omat AR film.

RESULTS

Assembly of Procollagen Mini-chains in SP Cells—The main aim of this study was to investigate the role of resident proteins within the endoplasmic reticulum in the folding and assembly of procollagen. To facilitate these studies we constructed a variety of different procollagen “mini-chains” that contain deletions within the triple helical domain. These deletions preserve the Gly-X-Y triplet consensus and are not predicted to alter the folding of the chains. We also prepared a C-propeptide-minus (CP-minus) construct that contains all of the pro1(III)Δ1 chain apart from the C-propeptide, the last amino acid being at the C-proteinase cleavage site (Fig. 1). Previous experiments have shown that procollagen mini-chains translated in the presence of SP cells are efficiently translabeled, modified, and, in the case of pro1(III)Δ1, assembled into a correctly aligned triple helix (20, 25).

To assay chain assembly, the procollagen constructs were transcribed in vitro, and the RNA transcript that was synthesized was translated in the presence of SP cells. Translation products were separated by SDS-PAGE under either reducing or nonreducing conditions (Fig. 2). Separation of procollagen chains under reducing conditions demonstrated that single translation products were produced for pro1(III)Δ1 and pro2(II)Δ1 (Fig. 2, lanes 2 and 3). However, the translation products from the pro1(III)Δ1 and the CP-minus RNA transcripts were separated into two polypeptides (Fig. 2, lanes 1...

![Fig. 1. Schematic diagram representing the domains present in the procollagen chains described in the text. The numbers refer to the number of amino acids in each domain. The numbers in parentheses indicate the sizes of the full-length triple helical regions.](http://www.jbc.org/)

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and 4). We have shown previously for proc1(III)Δ1 that these products represent unmodified and modified forms of the protein (20). The modifications that occur to the procollagen chain include proline and lysine hydroxylation and glycosylation of hydroxylysine residues, which can cause a retardation of electrophoretic mobility (28). The unmodified form is unlikely to have been properly translocated across the ER membrane because it was susceptible to digestion with externally added proteases (results not shown). Because the CP-minus construct contains the same triple helical domain as proc1(III)Δ1, the two different translation products are likely to be modified and unmodified forms of the protein. The lack of any apparent mobility change with the proc1(III)Δ1 and proc1(Δ1) chains could reflect the lower content of potential hydroxyproline and hydroxylysine residues within the truncated helical domains in these chains.

When the same samples were separated under nonreducing conditions, higher molecular weight molecules were detected with proc1(III)Δ1 (Fig. 2, lane 5) and to a certain extent with proc1(Δ1) (Fig. 2, lane 6) translation products. We have demonstrated previously that for proc1(III)Δ1 this represents a correctly folded triple helical molecule with interchain disulfide bonds stabilizing the structure in both the N- and C-propeptides (20). When a similar analysis was carried out on the proc2(Δ1) and CP-minus constructs, no interchain disulfide bonded trimers were seen (Fig. 2, lanes 7 and 8, respectively). However, a faster migrating polypeptide was observed (Fig. 2, compare lane 3 with lane 7 and lane 4 with lane 8). This is due to the formation of intrachain disulfide bonds, which result in a more compact structure with a faster mobility than the fully reduced polypeptide. These results are in agreement with previously published results for proc2(Δ1) (25) and are as expected for the CP-minus construct because the C-propeptide mediates the initial interaction of the procollagen chains. These and previous published results demonstrate that the proc1(III)Δ1 chain efficiently forms trimers, the proc1(Δ1) chain forms some homotrimers at low efficiency, and the proc2(Δ1) and CP-minus chains do not form trimers.

**Interference of Prolyl 4-Hydroxylase with Unfolded Procollagen Chains**—Previous work on the substrate specificity of P4-H has demonstrated that the conformation of the procollagen triple helix determines enzyme activity (15). The enzyme will readily hydroxylate unhydroxylated procollagen chains as long as the chains have not formed a triple helix (29). It has also been shown that malformed chains may be isolated as stable complexes with P4-H (30). This suggests that in addition to its enzymatic role, P4-H may interact specifically with unfolded procollagen chains in a chaperone-like manner.

The approach we adopted to investigate this possibility was to allow the hydroxylation of newly synthesized procollagen chains to occur posttranslationally and assay the formation of a stable triple helix. We then determined whether P4-H or any other ER protein was associated with the folding chains. Translation reactions were carried out in the presence of a,a′-dipyridyl, an iron chelator that is a potent inhibitor of prolyl 4-hydroxylase, for 60 min. Ferrous iron was added in excess of the chelator, thereby activating P4-H and allowing P4-H oxidation and triple helical formation to occur. We have used such a protocol previously and demonstrated that proc1(III)Δ1 chains can fold to form correctly aligned triple helices within 60 min under these conditions (20). The results presented here (Fig. 3A) clearly show that after 60 min of translation in the presence of a,a′-dipyridyl, no modification to the proc1(III)Δ1 chain occurred, as judged by a lack of the appearance of a slower migrating polypeptide (Fig. 3A, compare lanes 1 and 2). How-
FIG. 4. Cross-linking of pro-collagen chains and immunoprecipitation with antiserum raised to PDI and P4-H α-subunit. RNA encoding the procollagen chains was translated in the presence of SP cells. Samples were translated in the presence of α,α′-dipyridyl and then incubated for various times as indicated with lanes 1–4 and 6–9 or without lanes 1 and 10; addition of 5 mM FeSO₄. Cells were isolated by centrifugation and resuspended in 50 μl of KHM buffer, and DSP was added to a final concentration of 1 mM. Cross-linking was quenched by the addition of 100 mM glycine, and the samples were denatured by boiling in SDS/Nonidet P-40 denaturation buffer. After removal of insoluble material and preclearing, samples were divided equally and immunoprecipitated with antiserum raised to PDI and P4-H α-subunit or β-subunit (PDI). DSP cross-links were cleaved by addition of SDS-PAGE buffer containing 50 mM DTT to immune complexes. Procollagen chains were resolved through 7.5% SDS-PAGE gels.

ever, when the incubation was continued for 60 min in the presence of excess iron, chains were modified, as judged by the appearance of a polypeptide with a slower mobility (Fig. 3A, lane 3). This posttranslational modification correlated with the formation of a protease-resistant triple helical fragment (Fig. 3B, lane 3). This experimental system allows us to accumulate unhydroxylated procollagen chains, which can be hydroxylated posttranslationally. For procollagen individually and then added the thiol-specific, non-cleavable, bi-functional cross-linking reagent BMH. BMH was used instead of DSP for these studies to identify proteins interacting at regions of the protein other than the triple helical domain. When separated under reducing conditions, the appearance of a radiolabeled higher molecular weight product would indicate cross-linking of procollagen to the immunoprecipitated protein. Clear cross-linked products were seen for the procollagen chain (Fig. 5, compare lanes 3 and 6). More diffuse cross-linked products were also seen for the procollagen chain (Fig. 5, compare lane 1 with lane 4 and lane 2 with lane 5). After immunoprecipitation, only the PDI antibody was able to precipitate cross-linked products (Fig. 5, lanes 8 and 9). The cross-linked products from the procollagen chain were resolved as distinct bands after immunoprecipitation (Fig. 5, lanes 8 and 9). The type III procollagen cross-linked products were not immunoprecipitated by the PDI antibody (Fig. 5, lane 7). We investigated this result further by expressing just the C-propeptide of type III procollagen (24) and cross-linking with BMH. Here again, no cross-links to PDI were observed (results not shown). These results suggest that PDI was able to interact with type I procollagen chains but not type III procollagen chains.

It is not clear from these results whether PDI interacts independently with monomeric procollagen chains or as a subunit of P4-H. To address this point, we used BMH to cross-link pro-collagen chains under conditions that result in P4-H binding, i.e., after translation in the presence of α,α′-dipyridyl. It was clear from the results (Fig. 6) that even under conditions that result in binding of P4-H to procollagen chain, no immunoprecipitation of BMH cross-links occurred (Fig. 6, lanes 2 and 4). Cross-linking to the procollagen chain was unaffected by the presence of α,α′-dipyridyl demonstrating that the interaction of PDI is independent of hydroxylation. We also cross-linked the C-propeptide construct with BMH under conditions where P4H remains bound to this chain, but no cross-link products were observed (results not shown). These results suggest that the BMH cross-links that we observed are a consequence of PDI interacting with procollagen chains independently and not in a complex with P4-H.

There are two explanations for the lack of PDI cross-links to procollagen chains. Thus, P4-H was bound most abundantly to procollagen chains that were unhydroxylated (Fig. 4, lanes 1, 5, 6, and 10). Once the chains were hydroxylated and folded to form a triple helix, most of the P4-H dissociated (Fig. 4, lanes 1–4 and 6–9). Such a transient association of P4-H with procollagen chains suggests an interaction with unhydroxylated chains followed by a dissociation of P4-H following hydroxylation and folding. The decrease in the interaction of P4-H with the procollagen chains was hydroxylation dependent and was not due to degradation because an equivalent amount of material was cross-linked and immunoprecipitated when the samples were incubated for 60 min posttranslationally in the absence of added iron (Fig. 4, lanes 5 and 10). These results clearly show that P4-H binds to the unhydroxylated chains and will, therefore, prevent the triple helical domain from interacting prior to the formation of a stable triple helix.
proa1(III)Δ1 chains. Either the cross-links only occur with monomeric chains or there are sequence differences between the various chains that preclude cysteine specific cross-links to this chain. To investigate this point, we constructed a proa1(III)Δ1 chain that we predicted would remain monomeric. We have recently identified the sequence of amino acids within the C-propeptide that are responsible for selective association of procollagen chains (25). Hence, we replaced the proa1(III) sequence that directs homotrimer assembly with the corresponding sequence from the proa2(I) C-propeptide. This resulting molecule, which we called proa1(III)Δ1-alt, has exactly the same sequence as proa1(III)Δ1 apart from a stretch of 23 amino acids within the C-propeptide. As predicted, this chain, unlike proa1(III)Δ1, was unable to associate to form an interchain disulfide-bonded trimer when translated in the presence of SP cells (Fig. 7A, compare lanes 3 and 4). The synthesized product did show an increase in electrophoretic mobility when separated under nonreducing conditions, indicating that the interchain disulfide bonds had formed. When the translation products were cross-linked with BMH and immunoprecipitated with antibody to PDI, clear PDI-procollagen products were formed (Fig. 7B, lane 3). Because the only difference between this chain and proa1(III)Δ1 is that this chain remains monomeric and has a slightly altered C-propeptide, we can deduce that PDI interacts at the C-propeptide either specifically with monomeric chains or via the specific altered sequence. This interaction may serve to chaperone the monomeric procollagen chains prior to their assembly into trimers.

DISCUSSION

The biosynthesis of multisubunit proteins entering the secretory pathway is regulated at the ER, where the individual subunits are synthesized and their assembly is coordinated. This regulation ensures that unassembled subunits are prevented from being transported out of the ER and are either degraded or maintained in an assembly competent state by interacting with ER resident proteins (31). The mechanism underlying this “quality control” appears to involve the binding of unassembled subunits to a variety of ER proteins until assembly occurs. The assembled complex is then released and can be transported from the ER. Such a mechanism has been likened to affinity chromatography, with the “matrix” being the resident proteins in the ER and the selective interactions occurring via oligosaccharide side chains (32), hydrophobic regions in the protein (33), of free thiol residues (34) or with specific molecular chaperones (35, 36). This study has demonstrated that protein-disulfide isomerase also plays a role in this regulation by binding to procollagen chains either as a subunit of prolyl 4-hydroxylase or independently.

The procollagen trimer is folded and assembled through a series of distinct intermediates; the coordination of this assembly is crucial to produce a correctly folded, thermally stable triple helical molecule. One consequence of the sequence of events occurring during procollagen folding is that the individual chains have to be maintained in a soluble form prior to assembly occurring. The triple helical domain is inherently insoluble and must be prevented from self-association for several minutes, (37) because the chains associate at their C-propeptides and the triple helical domain folds in the C to N direction (16). We show here that P4-H plays a key role in ensuring that the triple helical domain remains soluble by binding to unhydroxylated chains. The binding of P4-H to its substrate in the absence of the co-factors iron and ascorbate has been shown previously using purified proteins (38). Our observation that this interaction also occurs during biosynthe-
observed an interaction of the C-propeptides of these chains with PDI. The proα1 chain of type I procollagen has previously been shown to be able to form homotrimers at a low efficiency (39). This was also the case here, with the majority of the synthesized chains remaining monomeric. The proα1 chain of type III efficiently form homotrimers and, significantly, did not interact with PDI at its C-propeptide. However, when this chain was altered to prevent association by changing its recognition site to that of the proα2-chain of type I, an interaction with PDI could be detected. We and others have previously shown that point mutations within the C-propeptide can cause misfolding of this domain (21), which leads to binding to ER proteins, such as immunoglobulin heavy chain-binding protein (BiP) (40). However, in these cases, the pro-chains were unable to form correct intrachain disulfide bonds and migrated as a diffuse smear when separated under nonreducing conditions. The pro-chain we have constructed here with an altered recognition site was able to form intrachain disulfide bonds. The polypeptide synthesized migrated as a sharp band when separated under nonreducing conditions, which co-migrated with the wild type protein. This indicates that the C-propeptide folded correctly but was unable to assemble due to its altered recognition site. These results clearly demonstrate that PDI plays a crucial role in binding to the C-propeptide, thereby coordinating heterotrimer assembly.

A growing body of evidence is accumulating that suggests a key role for the collagen binding protein HSP47 in the biosynthesis of procollagen (36). Co-immunoprecipitation experiments have shown that procollagen within the ER is associated with HSP47 (41) and can dissociate upon transport to the Golgi apparatus (42). It has been reported that HSP47 binds to the N-propeptide of type I procollagen (43), yet other workers have also reported binding to the triple helical domain (42). During our studies, we were unable to immunoprecipitate our procollagen constructs with anti-HSP47 antibodies after cross-linking. It could be that our shortened triple helical domains do not contain the binding site for this molecule or could simply reflect inefficient cross-linking. The folding and assembly of procollagen is a complex process and may require a number of different chaperone proteins to ensure efficient folding, assembly, and intracellular transport. There is also likely to be redundancy in the involvement of accessory proteins, as has been illustrated by the successful expression and assembly of procollagen in insect cells (44), which are unlikely to contain HSP47. Clearly, PDI, P4-H, and HSP47 may have overlapping functions as molecular chaperones during procollagen biosynthesis. The interactions described here provide the first direct evidence for a chaperone role for PDI (and P4-H) during assembly of procollagen chains by preventing their premature and hence nonproductive interaction. The interaction of procollagen intermediates with PDI may also, by virtue of its KDEL-retention sequence, provide a mechanism for retention of non-triple helical procollagen chains.

Acknowledgments—We thank Steve McLaughlin for critical reading of the manuscript and Darwin Prockop for the cDNA clones to the procollagen molecules.

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Fig. 7. Characterization of the assembly and cross-linking to PDI of proα1(III)Δ1:alt. A, RNAs encoding proα1(III)Δ1 and proα1(III)Δ1:alt chains were translated in the presence of SP cells. Isolated SP cells were washed and resuspended in 50 μl of KHM. 10-μl aliquots of the proα1(III)Δ1 and proα1(III)Δ1:alt translation products were removed for analysis under reducing (lanes 1 and 2) and nonreducing (lanes 3 and 4) conditions. B, RNAs encoding proα1(III)Δ1, proα2(III)Δ1, and proα1(III)Δ1:alt chains were translated in the presence of SP cells. Isolated SP cells were washed, incubated in 50 μl of KHM containing 1 mM BMH for 10 min, and then quenched with 5 mM DTT. Samples were treated with SDS/Nonidet P-40 denaturation buffer, and insoluble material was removed by centrifugation. After preclearing, samples were immunoprecipitated with antiserum raised against PDI. Immunoprecipitated material cross-linked to proα1(III)Δ1 (lane 1), proα2(III)Δ1 (lane 2), and proα1(III)Δ1:alt (lane 3) chains was resolved on a 7.5% reducing SDS-PAGE gel.
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J. Biol. Chem. 1998, 273:9637-9643.
doi: 10.1074/jbc.273.16.9637

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