Procollagen binds to both prolyl 4-hydroxylase/protein disulfide isomerase and HSP47 within the endoplasmic reticulum in the absence of ascorbate

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Abstract In cells, only properly folded procollagen trimers are secreted from the endoplasmic reticulum (ER), while improperly folded abnormal procollagens are retained within the ER. Ascorbic acid is a co-factor in procollagen hydroxylation, which in turn is required for trimer formation. We examined chaperone proteins which bound to procollagen in the absence of ascorbic acid, a model which mimics the human disease scurvy at the cellular level. We found that both prolyl 4-hydroxylase (P4-H)/protein disulfide isomerase (PDI) and HSP47 bound to procollagen in the absence of ascorbic acid. However, the binding of PDI to procollagen decreased when HSP47 was co-transfected, suggesting that HSP47 and PDI compete for binding to procollagen. These data indicate that P4-H/PDI and HSP47 have cooperative but distinct chaperone functions during procollagen biosynthesis.

Key words: Procollagen; Chaperone protein; HSP47; Protein disulfide isomerase; Prolyl 4-hydroxylase

1. Introduction

Procollagens are synthesized within the endoplasmic reticulum (ER), and only properly folded procollagen trimers are secreted from the cell, while improperly folded abnormal procollagens are retained within the ER [20]. Recently, molecular chaperones have been reported to play important roles in quality control [1]. HSP47 is an ER-resident stress protein which is thought to function as a molecular chaperone specific to procollagen biosynthesis [2,3]. Protein disulfide isomerase (PDI) is another ER-resident chaperone protein [4,5] which has dual functions in procollagen biosynthesis. As well as forming intra- and inter-chain disulfide bonds in the pro- or telopeptide regions of procollagen [6], PDI also functions as the β subunit of prolyl 4-hydroxylase (P4-H), an essential enzyme for procollagen biosynthesis [7,8]. Procollagen trimer formation is dependent upon correct hydroxylation of proline residues in the triple helical region. In addition, PDI has recently been reported to carry out a chaperone function during procollagen biosynthesis [9,10].

In this study, we focused on identification of chaperone proteins responsible for the ER retention of abnormal procollagen synthesized under pathophysiological conditions such as ascorbate depletion. Ascorbic acid is a co-factor of P4-H, and it is known that ascorbate deficiency causes the human disease scurvy by secreting improperly folded procollagens caused by hypohydroxylation of proline residues. In every cell line that we have examined, the expression of HSP47 always parallels that of collagen [2], so we could not separate the expression of these two proteins. Therefore, taking advantage of the fact that 293 cells do not produce any extracellular matrix proteins and express neither collagens nor HSP47 [11,12], we have transfected type III procollagen cDNA, with or without HSP47 cDNA, into 293 cells in order to analyze the function of HSP47 in quality control of procollagen synthesis.

2. Materials and methods

2.1. Antibodies

Rabbit antiserum against human/bovine type III collagen was purchased from LSL (Japan) and purified antibody against human type III collagen from Southern Biotechnology Associates, Inc. (USA). The rabbit antiserum against mouse HSP47 has been described elsewhere [13], and monoclonal antibody raised against HSP47 (SPA-470) was purchased from StressGen (Canada). Rabbit IgG against PDI was kindly provided by Dr. A. Yamamoto (Kansai Medical University, Japan). Mouse monoclonal antibodies against human P4-Hα and P4-Hβ were purchased from Fuji Chemical Co. (Japan). Rat monoclonal antibody against GRP94 (SPA-850) and rabbit polyclonal antibody against calnexin (SPA-860) were from StressGen, and rabbit polyclonal antibody against calreticulin was purchased from Affinity Bioreagents, Inc. (USA). Rat antiserum against mouse GRP78 was raised by Dr. M. Satoh (Aichi Human Service Center, Japan), and mouse monoclonal antibody against ERp72 was a kind gift from Dr. S. Saga (Aichi Medical University, Japan).

2.2. Cell culture and drug treatment

Construction and cell culture of 293 cell lines expressing proα1(III) and/or HSP47 were as previously described [12]. For experiments in the presence of ascorbate, ascorbic acid phosphate was added to the culture medium at least 16 h before cell harvest [12]. α,α′-Dipyridyl was added to culture medium containing ascorbate 4 h before metabolic labeling, and was present during the labeling period, at a final concentration of 0.3 mM.

2.3. Western blotting, metabolic labeling and immunoprecipitation

Cell lysate (10 μg) was separated by SDS-PAGE, and immunoblotting using specific antibodies was performed as described previously [12]. Metabolic labeling, chemical cross-linking and immunoprecipitation procedures were carried out as described previously [12]. To label both ER-resident and secretory proteins efficiently, cells were meta-

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Abbreviations: ER, endoplasmic reticulum; PDI, protein disulfide isomerase; HSP, heat shock protein; GRP, glucose-regulated protein; BiP, heavy chain binding protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; P4-H, prolyl 4-hydroxylase; DTT, dithiothreitol; DSP, dithiobis(succinimidylpropionate)

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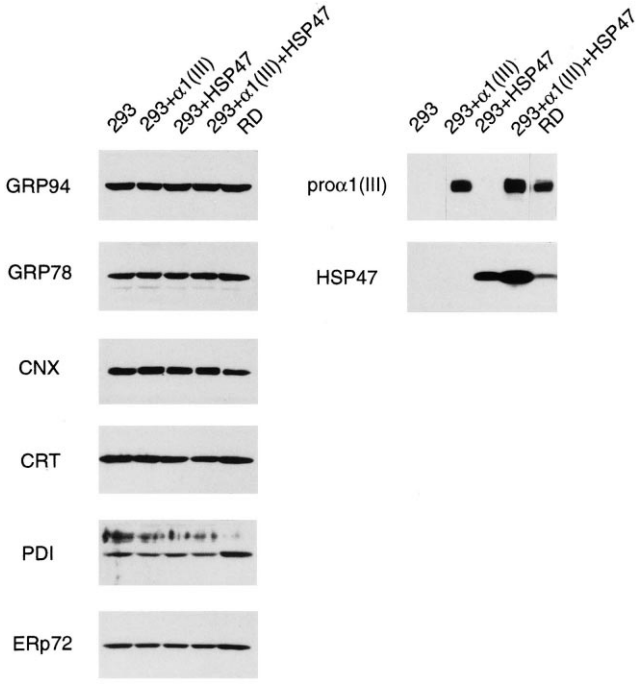

Fig. 1. Western blot analysis using antibodies against ER chaperone proteins and type III collagen. 293 cells, 293 cells stably transfected with proα1(III) chain (293+α1(III)), with mouse HSP47 (293+HSP47) or with both proα1(III) chain and mouse HSP47 (293+α1(III)+HSP47) and RD cells were cultured in the absence of ascorbate. Cells were lysed in a buffer containing 1% NP-40, and 10 μg of soluble fraction was resolved by SDS-PAGE. After blotting to a nitrocellulose membrane and probing with antibodies against the indicated proteins, immunoreactive bands were detected by enhanced chemiluminescence (Amersham).

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bolically labeled for 14 h with [^{35}S]methionine (EXPRE^{35}S^{35}S, DuPont-NEN) in the presence of 6 mg/l methionine, chased for 2 h in DMEM containing excess methionine, then labeled again for 1.5 h with [^{35}S]methionine in medium lacking methionine [14].

2.4. In vitro translation and translocation using semipermeabilized cells

In vitro translation and translocation was performed using in vitro transcribed bovine type X procollagen RNA, as described previously [15]. Plasmid encoding bovine type X procollagen (pGEM-BX) was kindly provided by Dr. N. Bulleid (University of Manchester, UK) [16]. The *Eco*RI fragment of pGEM-BX was inverted and SP6 RNA polymerase was used to transcribe RNA in vitro. 293 cells and 293+HSP47 cells (the latter being 293 cells stably transfected with mouse HSP47) were treated with 30 μg/ml digitonin to obtain semipermeabilized cells [15]. RNA was translated in rabbit reticulocyte

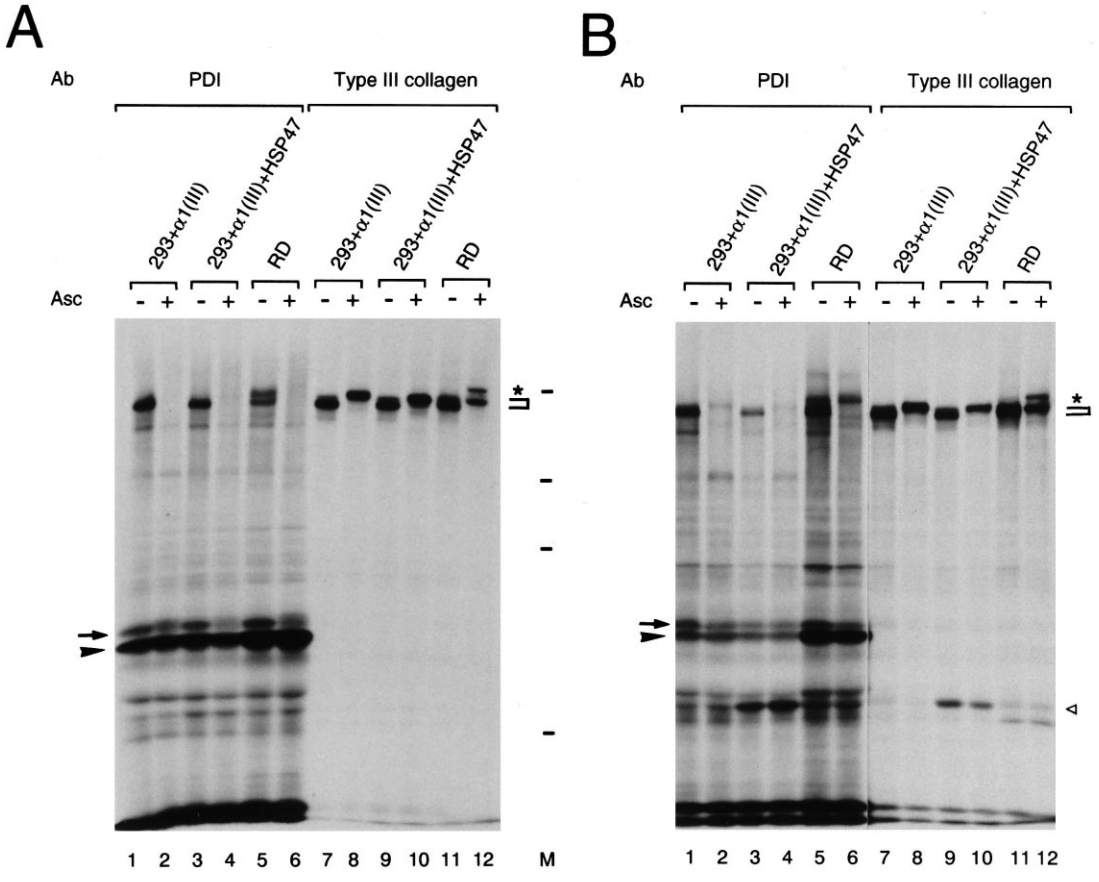

Fig. 2. Coprecipitation of type III procollagen with PDI. Cells were cultured in the presence (Asc+) or absence (Asc−) of ascorbate. Metabolic labeling was performed for 14 h with [^{35}S]methionine and chased in normal growth medium for 2 h, then labeled again for 1.5 h with [^{35}S]methionine. Cells were lysed in a buffer containing 1% NP-40 without pre-treatment (A), or after addition of the cross-linking reagent DSP (B). Immunoprecipitation was performed using specific antibodies against PDI (lanes 1–6) or against type III collagen (lanes 7–12). The positions of molecular mass markers (200, 117, 75 and 42.9 kDa) are shown in lane M. Arrowheads indicate PDI, and arrows indicate the α subunit of P4-H (see Fig. 4). Brackets denote the positions of proα1(III) chains. Asterisks indicate a collagenous protein which was expressed in RD cells and was recognized by polyclonal antibody raised against type III collagen (LSL Co.). This band was considered to represent type IV procollagen (see Section 3).

lysates (FlexiLysate, Promega) at 30°C. Proteinase K digestion (200 μg/ml) was carried out on ice for 30 min, and terminated by adding phenylmethylsulfonyl fluoride to a final concentration of 2 mg/ml and boiling for 2 min in Laemmli's sample buffer [17]. For pepsin digestion, semipermeabilized cells were pelleted by centrifugation at 13 000 × *g* for 5 min, resuspended in 20 μl of 0.5 M acetic acid containing 1% Triton X-100, and then digested with 100 μg/ml pepsin for 14 h at 4°C. Digestion was terminated by adding NaOH to neutralize the pH and boiling for 2 min in Laemmli's sample buffer [16].

3. Results

3.1. Expression of ER chaperone proteins

We first examined the expression levels of several ER chaperone proteins in the cell lines used in this study by Western blot analysis with specific antibodies (Fig. 1). These experiments were performed in the absence of ascorbate to detect the intracellular accumulation of type III procollagen, because procollagens are rapidly secreted from the cell in the presence of ascorbate [12]. The RD cell line (ATCC CCL 136) was established from a human rhabdomyosarcoma, and is known to secrete normal type III collagen. We observed some differences in the expression levels of ER chaperone proteins between RD cells and 293 cells, but no significant differences among 293 cells and cell lines derived from 293 cells. It should be noted that HSP47 was barely detectable in 293 cells. No change was detected in the expression level of any of these ER chaperone proteins when the cells were cultured in the presence of ascorbate (data not shown). This suggests that stable overexpression of HSP47 or type III procollagen did not alter the levels of other ER chaperone proteins expressed in 293 cell lines.

3.2. Coprecipitation of type III procollagen with PDI

When cells are cultured in the absence of ascorbic acid, hydroxylation of procollagens is inhibited, and the resulting hypohydroxylated procollagens are retained within the ER because they cannot form properly folded helical trimers. We examined the interaction of type III procollagen with ER chaperone proteins by immunoprecipitation following culture in the presence or absence of ascorbic acid. Among the ER chaperone proteins examined (PDI, GRP94, GRP78, calnexin and calreticulin), clear coprecipitation of type III procollagen was observed only when polyclonal antibody against PDI was used in the absence of ascorbic acid (Fig. 2A). Coprecipitation of proα1(III) chain was undetectable in ascorbate-treated cells (Fig. 2A). These results were observed in both RD cells and 293 cells transfected with type III procollagen. The cell lysates contained comparable amounts of radiolabeled proα1(III) chain under the experimental conditions used (Fig. 2A). The different electrophoretic mobilities of the proα1(III) chain in the presence or absence of ascorbate reflect the differences in hydroxylation of the procollagen chain.

Transient association of PDI with the proα1(III) chain was examined in the presence of ascorbate by treatment with a chemical cross-linking reagent (DSP) prior to cell lysis. Coprecipitation of proα1(III) chain with PDI was detected at low but reproducible levels in cell lysates treated with ascorbate (Fig. 2B). It is noteworthy that in 293+α1(III)+HSP47 cells, smaller amounts of proα1(III) chain were coprecipitated by anti-PDI antibody than in 293+α1(III) cells (Fig. 2B).

The slowly migrating band which was precipitated by antibody against type III collagen in RD cells (indicated by an asterisk in Fig. 2) was shown to be type IV procollagen (data not shown).

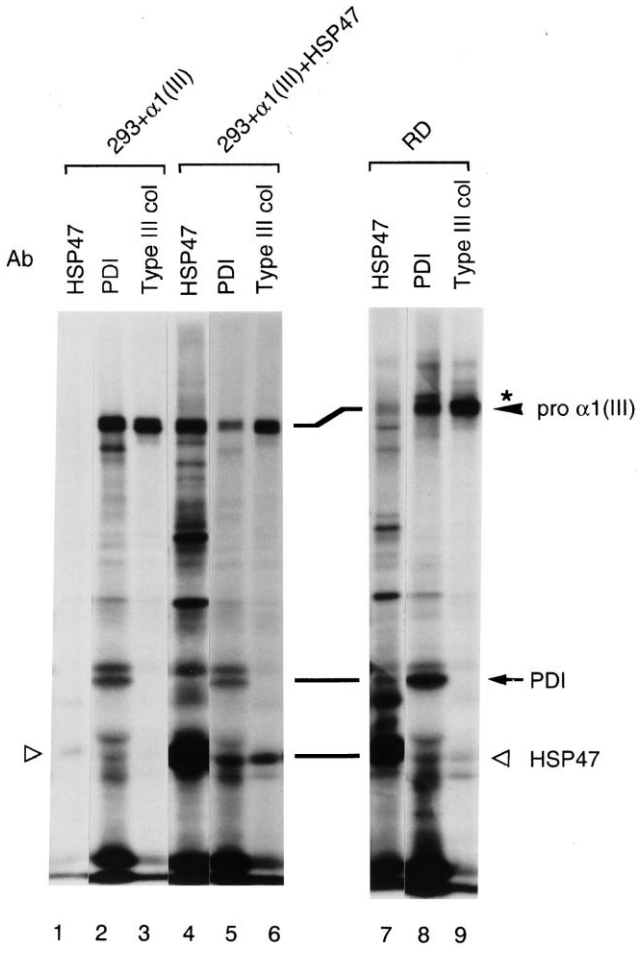

Fig. 3. Coprecipitation of type III procollagen with PDI and HSP47 in the absence of ascorbate. Cells were cultured without ascorbate, and metabolically labeled with [^{35}S]methionine as in Fig. 2. After treatment of the cells with the cross-linking reagent DSP, cell lysates were prepared and immunoprecipitation was performed. Immune complexes were analyzed by 8% SDS-PAGE. An arrow indicates PDI, and open triangles denote HSP47. Proα1(III) collagen chain is marked by an arrowhead. Film was exposed for shorter periods (approx. 1/3) when anti-HSP47 antibody was used.

3.3. Coprecipitation of type III procollagen with PDI and HSP47 in the absence of ascorbate

We have previously reported that HSP47 binds to type I or III procollagens both in the absence and in the presence of ascorbic acid [12,18] (see also Fig. 5). In this study, we compared the binding of type III procollagen to PDI and HSP47 in the absence of ascorbate (Fig. 3). In 293 cells stably transfected with both type III procollagen and HSP47 (293+α1(III)+HSP47 cells), coprecipitation of α1(III) chain was detected using either antibody against PDI or antibody against HSP47 (Fig. 3). In 293 cells transfected only with proα1(III) chain, HSP47 was expressed at very low levels and anti-HSP47 antibody did not coprecipitate detectable amounts of proα1(III) chain (Fig. 3). The amount of proα1(III) chain coprecipitated by anti-PDI antibody was much smaller in 293+α1(III)+HSP47 cells than in 293+α1(III) cells (Fig. 3, compare lanes 2 and 5). This suggests that the binding of HSP47 and PDI to procollagen is competitive in the ab-

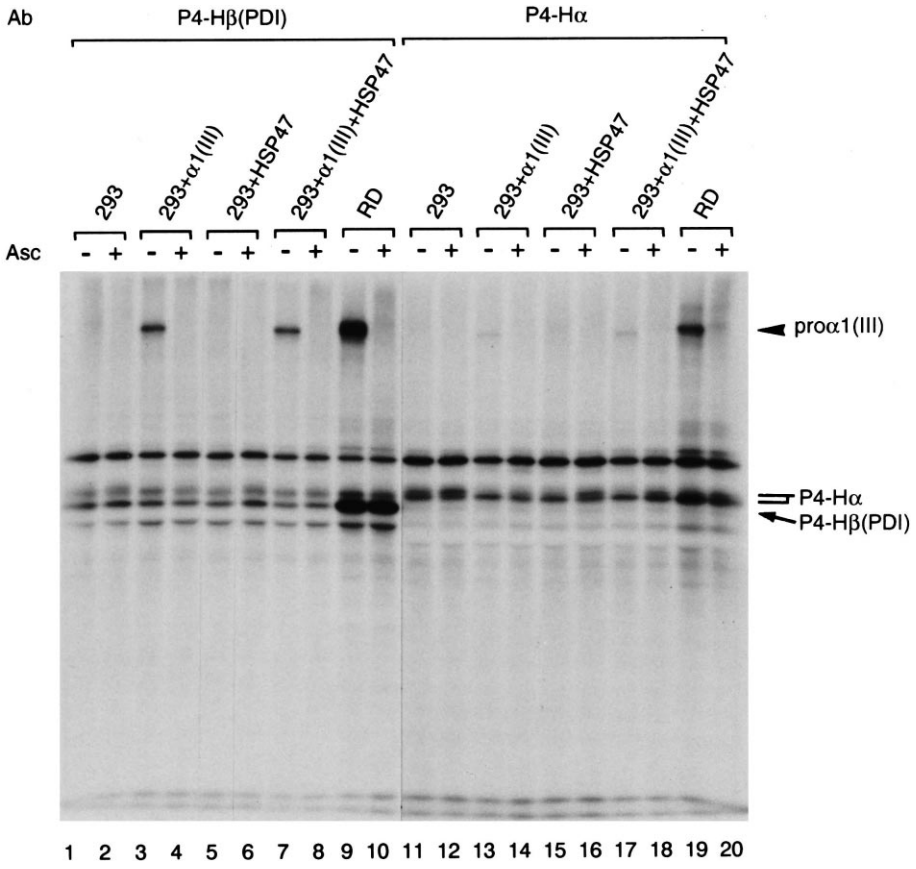

Fig. 4. Immunoprecipitation using antibodies against PDI (P4-Hβ) and P4-Hα. Cells were metabolically labeled with [^{35}S]methionine for 3 h, and immunoprecipitation was performed as described in Fig. 2. The position of PDI/P4-Hβ is indicated by arrows. Film was exposed two-fold longer when anti-P4-Hα antibody was used (lanes 11–20).

sence of ascorbate. In other words, from the point of view of chaperone activity, both PDI and HSP47 appear to be involved in retention of improperly folded procollagens within the ER.

3.4. PDI in the P4-H tetramer binds to procollagen

Because PDI is the β-subunit of P4-H (which is an $\alpha_2\beta_2$ heterotetramer), we examined whether PDI in the P4-H tetramer could bind to procollagen in the absence of ascorbate. The results of immunoprecipitation using monoclonal antibodies against P4-Hβ and P4-Hα are shown in Fig. 4. In the absence of ascorbate, coprecipitation of proα1(III) collagen chain was detected using both antibodies. A band of slightly lower mobility which coprecipitated with PDI/P4-Hβ was considered to be the α subunit of P4-H, judging by its molecular size. Thus, in the absence of ascorbate, PDI was shown to bind to procollagen, at least in part, as the β subunit of P4-H. However, it is difficult to discriminate between PDI/P4-Hβ binding to procollagen in vivo as a homodimer (PDI) and as a heterotetramer (P4-H). It is of note that almost equal amounts of PDI/P4-Hβ and P4-Hα were detected in 293 cells, which do not express procollagens (Fig. 4). This is consistent with the observation that 293 cells have P4-H activity comparable to that of RD cells (Hosokawa et al., unpublished observation; Kühn et al., manuscript submitted).

Because P4-H is known to bind to procollagen in the ER when cells are treated with α,α′-dipyridyl [19], we compared the binding of PDI/P4-Hβ to procollagen in the absence of ascorbate and in the presence of α,α′-dipyridyl. α,α′-Dipyridyl chelates ferrous ions, one of the co-factors of P4-H, and cells treated with this drug synthesize unhydroxylated procollagen. Cells cultured with or without ascorbate, or treated with α,α′-dipyridyl, were lysed without cross-linking treatment and immunoprecipitation was performed (Fig. 5). In cells cultured without ascorbic acid or treated with α,α′-dipyridyl, coprecipitation of type III procollagen was detected by antibodies against P4-Hα or PDI/P4-Hβ. Under the conditions employed, the cell lysates contained almost equal amounts of radiolabeled procollagens. The different electrophoretic mobilities of proα1(III) collagen chains reflect the degree of hydroxylation of proline residues: partial hydroxylation occurred in the absence of ascorbate, while hydroxylation was inhibited almost completely by the addition of α,α′-dipyridyl. We detected more coprecipitated proα1(III) chain when cells were treated with the chemical cross-linking reagent DSP prior to lysis (data not shown). While HSP47 bound to procollagen under all the conditions examined, it should be noted that more procollagen bound to HSP47 when the cells were treated with α,α′-dipyridyl, which is consistent with our previous reports [14,18]. These results suggest that under conditions which inhibit procollagen hydroxylation (ascorbate deficiency or α,α′-dipyridyl treatment), the resulting improperly folded procollagens are retained within the ER through interaction with PDI/P4-H and HSP47.

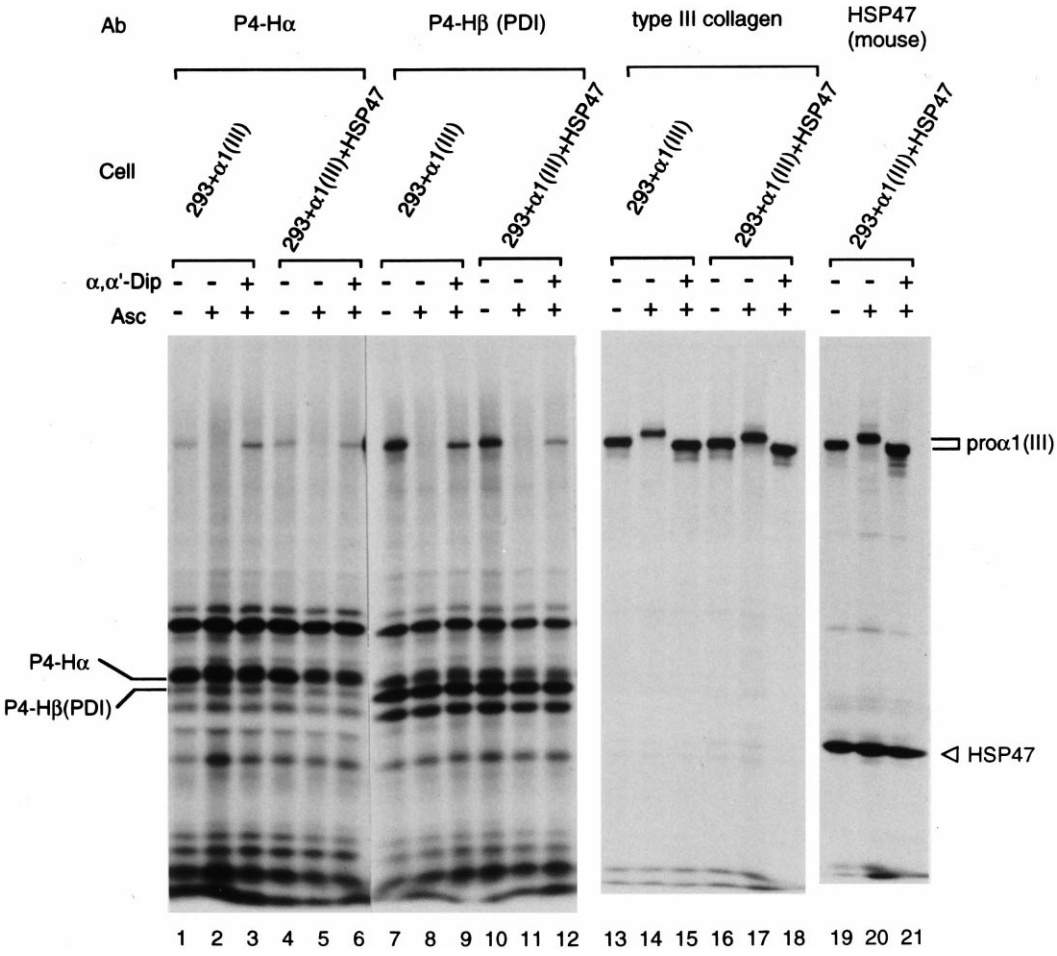

Fig. 5. Binding of type III procollagen to P4-H and HSP47 in cells treated with α,α′-dipyridyl. Cells were cultured in the absence or presence of ascorbate, or treated with α,α′-dipyridyl. After metabolic labeling for 3 h, cells were lysed without cross-linker, and immunoprecipitation was performed.

3.5. *In vitro translation-translocation of type X procollagen using semipermeabilized cells*

We next looked for differences in procollagen synthesis in the presence or absence of HSP47, using an in vitro translation-translocation system in reticulocyte lysates. We prepared semipermeabilized cells using digitonin, and synthesized bovine type X procollagen RNA by in vitro transcription with SP6 RNA polymerase. We used type X collagen as a model for procollagen trimer formation, because the translation-translocation efficiency was very poor when long procollagen RNAs such as proα1(III) or proα1(I) were used. Parental 293 cells and 293 cells stably transfected with mouse HSP47 (293+HSP47 cells) were used to compare the effect of HSP47. The translation and translocation efficiencies were almost identical in these two cell lines (data not shown). When the efficiency of triple helix formation was examined by pepsin digestion, pepsin-resistant type X procollagen trimer was detected in both cell lines, and there was no difference in the time course of trimer formation between the cell lines (Fig. 6). We also examined earlier time points (15 and 30 min), but could not detect any difference between the parental and HSP47-transfected cell lines (data not shown). These results suggest that HSP47 does not facilitate trimer formation of type X procollagen.

4. Discussion

We examined the involvement of ER chaperone proteins in the retention of improperly folded type III procollagen within the ER under conditions of ascorbate deficiency. We found that both PDI and HSP47 bound to procollagen in the absence of ascorbic acid, and that PDI was the main chaperone protein which bound to the procollagen chain in cells lacking HSP47 (Figs. 2 and 3). The observation that type III procollagen was coprecipitated by antibody against P4-Hα (Fig. 4) suggests that PDI bound to procollagen, at least in part, in the form of P4-H. P4-H has been reported to bind to unhydroxylated procollagen treated with α,α′-dipyridyl [19]. The binding of the α and β subunits of P4-H to type III procollagen was similar in cells treated with α,α′-dipyridyl and cells cultured without ascorbate (Fig. 5).

We could not detect binding of type III procollagen to BiP/GRP78 in the absence of ascorbate (data not shown). This is consistent with the observation that in cells from osteogenesis imperfecta patients, type I procollagen with a point mutation in the C-propeptide region binds to BiP/GRP78, while procollagen with a point mutation in the triple helical region binds to PDI/P4-H but not to BiP/GRP78 [21–23]. From the point of view of quality control, BiP/GRP78 may be mainly in-

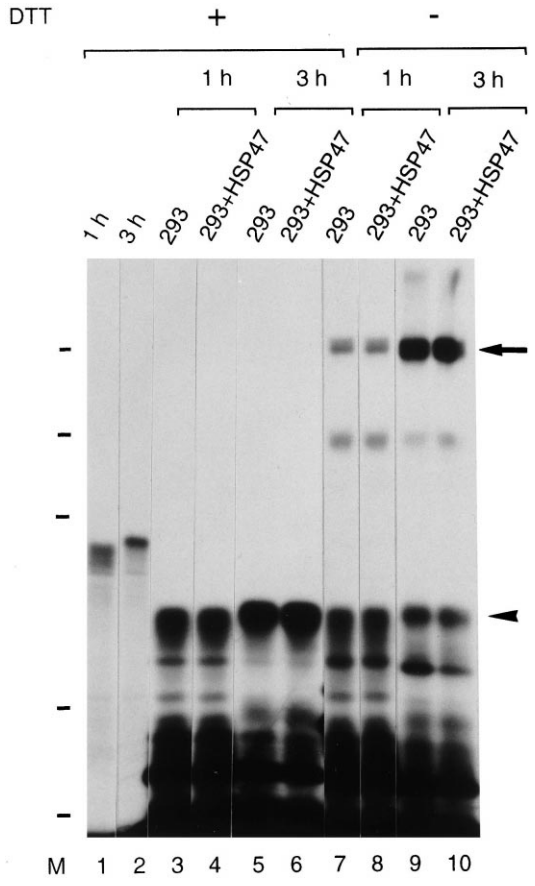

Fig. 6. In vitro translation-translocation of type X procollagen using semipermeabilized cells. Semipermeabilized cells were prepared using digitonin, and in vitro translation-translocation was performed in rabbit reticulocyte lysates using in vitro transcribed type X procollagen RNA. Reactions were performed at 30°C and at the times indicated, aliquots (1/25) of the sample were digested with proteinase K for 30 min at 4°C (lanes 1,2). For pepsin digestion, cells were pelleted and resuspended in the digestion buffer, and treated with pepsin for 14 h at 4°C. Samples were separated by 8% SDS-PAGE in the presence (lanes 3–6) or absence (lanes 7–10) of DTT. An arrow indicates the monomer and an arrowhead shows the trimer position of pepsin-resistant type X collagen. Molecular mass markers (209, 130, 85, 43.9 and 32.1 kDa) are shown in lane M.

volved in regulating abnormality in the C-propeptide region, while PDI/P4-H monitors the triple helical region.

For this study, we used 293 cell lines which were stably transfected with the proα1(III) collagen chain, with or without HSP47. Using these cell lines, we could separate the expression of procollagens and HSP47. A transfection-based model was necessary because, among the cell lines we have examined, all vertebrate cell lines which express some type of collagen also express HSP47 at the same time [2,12]. Our results suggest that the binding of HSP47 and PDI/P4-H to procollagen in the absence of ascorbate is competitive, and that in cells lacking HSP47, PDI/P4-H is the main chaperone protein that binds to hypohydroxylated procollagen (Fig. 3). The binding of PDI/P4-H and HSP47 to procollagen was different in the presence of ascorbate. Although the binding of PDI/P4-H to procollagen was transient in the presence of ascorbate, HSP47 bound stably to procollagen either in the presence or absence of ascorbate (Figs. 2 and 4). Several studies, including expression of collagens in yeast [24], insect cells [25,26] and 293 cells [12], suggest that it is possible for various cells to synthesize and secrete 'roughly normal' collagen trimer without HSP47. In the present study, HSP47 was not necessary for procollagen trimer formation in an in vitro translation-translocation system (Fig. 6). In contrast, we have shown that disruption of the HSP47 gene was embryonic lethal in mice as a result of abnormal procollagen processing, suggesting that HSP47 plays an essential role in normal procollagen biosynthesis in vertebrates (Nagai et al., manuscript submitted). Although the precise mechanism by which HSP47 acts as a molecular chaperone remains to be clarified, these data suggest that HSP47 is essential for procollagen biosynthesis as a molecular chaperone in addition to the chaperone activity of PDI/P4-H.

While we were preparing this article, N. Bulleid's group published a manuscript demonstrating that procollagen is retained within the ER in the absence of ascorbate by P4-H, using a 'minicollagen' which lacks most of the triple helical region of proα1(III) [27]. While they showed that HSP47 did not bind to the 'minicollagen', we could clearly show the binding of HSP47 with type III procollagen in the absence or presence of ascorbic acid. This discrepancy is probably due to the the fact that the Bulleid group used 'minicollagen' for their experiments, whereas we used natural full-length procollagen. Further experiments will be needed to clarify the function of HSP47.

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References

[1] Hammond, C. and Helenius, A. (1995) Curr. Opin. Cell Biol. 7, 523–529.
[2] Nagata, K. (1996) Trends Biochem. Sci. 21, 23–26.
[3] Nagata, K. (1997/1998) Matrix Biol. 16, 379–386.
[4] Wang, C-C. and Tsou, C-L. (1998) FEBS Lett. 425, 382–384.
[5] Gilbert, H.F. (1997) J. Biol. Chem. 272, 29399–29402.
[6] Bächinger, H.P., Fessler, L.I., Timpl, R. and Fessler, J.H. (1981) J. Biol. Chem. 256, 13193–13199.
[7] Kivirikko, K.I., Myllylä, R. and Pihlajaniemi, T. (1992) in: Post-Translational Modifications of Proteins (Harding, J.J. and Crabbe, M.J.C., Eds), pp. 1–51, CRC Press, London.
[8] Kivirikko, K.I. and Myllyharju, J. (1997/1998) Matrix Biol. 16, 357–368.
[9] Kellokumpu, S., Suokas, M., Risteli, L. and Myllylä, R. (1997) J. Biol. Chem. 272, 2770–2777.
[10] Wilson, R., Lees, J.F. and Bulleid, N.J. (1998) J. Biol. Chem. 273, 9637–9643.
[11] Nischt, R., Pottgiesser, J., Krieg, T., Mayer, U., Aumailley, M. and Timple, R. (1991) Eur. J. Biochem. 200, 529–536.
[12] Hosokawa, N., Hohenadl, C., Satoh, M., Kühn, K. and Nagata, K. (1998) J. Biochem. 124, 654–662.
[13] Takechi, H., Hosokawa, N., Hirayoshi, K. and Nagata, K. (1994) Mol. Cell. Biol. 14, 567–575.
[14] Nakai, A., Satoh, M., Hirayoshi, K. and Nagata, K. (1992) J. Cell Biol. 117, 903–914.
[15] Wilson, R., Allen, A.J., Oliver, J., Brookman, J.L., High, S. and Bulleid, N.J. (1995) Biochem. J. 307, 679–687.
[16] Middleton, R.B. and Bulleid, N.J. (1993) Biochem. J. 296, 511–517.
[17] Bulleid, N.J. and Freedman, R.B. (1988) Biochem. J. 254, 805–810.
[18] Satoh, M., Hirayoshi, K., Yokota, S.-i., Hosokawa, N. and Nagata, K. (1996) J. Cell Biol. 133, 469–483.

[19] Juva, K. and Prockop, D.J. (1969) J. Biol. Chem. 244, 6486–6492.
[20] Prockop, D.J. and Kivirikko, K.I. (1995) Annu. Rev. Biochem. 64, 403–434.
[21] Chessler, S.D. and Byers, P.H. (1992) J. Biol. Chem. 267, 7751–7757.
[22] Chessler, S.D. and Byers, P.H. (1993) J. Biol. Chem. 268, 18226–18233.
[23] Lamandé, S.R., Chessler, S.D., Golub, S.B., Byers, P.H., Chan, D., Cole, W.G., Sillence, D.O. and Bateman, J.F. (1995) J. Biol. Chem. 270, 8642–8649.
[24] Vuorela, A., Myllyharju, J., Nissi, R., Pihlajaniemi, T. and Kivirikko, K.I. (1997) EMBO J. 16, 6702–6712.
[25] Tomita, M., Ohkura, H., Ito, M., Kato, T., Royce, P.M. and Kitajima, T. (1995) Biochem. J. 312, 847–853.
[26] Lamberg, A., Helaakoski, T., Myllyharju, J., Peltonen, S., Notbohm, H., Pihlajaniemi, T. and Kivirikko, K.I. (1996) J. Biol. Chem. 271, 11988–11995.
[27] Walmsley, A.R., Batten, M.R., Lad, U. and Bulleid, N.J. (1999) J. Biol. Chem. 274, 14884–14892.