Expression of Wild-Type and Modified Proα Chains of Human Type I Procollagen in Insect Cells Leads to the Formation of Stable \([\alpha(1)I]_2\alpha(2)I]_3\) Collagen Heterotrimers and \([\alpha(1)I]_3\) Homotrimers but Not \([\alpha(2)I]_3\) Homotrimers*

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Insect cells coinfecte\(d\) with a baculovirus coding for the pro\(1(I)\) chain of human type I procollagen and a double promoter virus coding for the \(\alpha\) and \(\beta\) subunits of human prolyl 4-hydroxylase produced homotrimeric \([\text{pro}\(1(I)\)]_3\) procollagen molecules. The use of an additional virus coding for the pro\(2(I)\) chain led to the formation of a heterotrimeric molecule with the correct 2:1 ratio of pro\(1\) to pro\(2\) chains of \(\alpha\) type I procollagen \([\text{pro}\(1(I)\)] and pro\(2(I)\)] chains, respectively), unless the pro\(1(I)\) chain was expressed in a relatively large excess. Replacement of the sequences coding for the signal peptide and the N propeptide of the pro\(1(I)\) chain with those of the pro\(1(III)\) chain increased level of expression of the pro\(1(I)\) chain, whereas no similar effect was found when the corresponding modification was made to the virus coding for the pro\(2(I)\) chain. Molecules containing such modified N propeptides were found to be processed at their \(N\) terminus more rapidly than those containing the wild-type propeptides. The \(T_m\) of the type I collagen homotrimer was similar to that of the heterotrimer, both values being about 42–43 °C when determined by circular dichroism. The wild-type pro\(2(I)\) chain formed no homotrimers. Replacement of the C propeptide of the pro\(2(I)\) chain with that of the pro\(1(I)\) chain or pro\(1\) chain of type III procollagen \([\text{pro}\(1(III)\)] chain) led to the formation of homotrimers, but the \(\alpha(2)I\) chains in such molecules were completely digested by pepsin in 1 h at 22 °C. The data thus suggest that, in addition to control at the level of the C propeptide, other restrictions may exist at the level of the collagen domain that prevent the formation of stable homotrimeric \([\text{pro}\(2(I)\)]_3\) molecules in insect cells.

The collagen superfamily now includes at least 19 proteins formally defined as collagens and more than 10 additional proteins with collagen-like domains (for reviews, see Refs. 1–6). Type I collagen is the most abundant member of this family and was the first to be characterized. Its molecule is a heterotrimer consisting of two identical \(\alpha(1)I\) chains and a slightly different \(\alpha(2)I\) chain that are coiled around one another into a triple-helical conformation. The molecule is synthesized in the form of a precursor in which the pro\(1(I)\) and pro\(2(I)\) chains have propeptide extensions at both their N- and C-terminal ends. In addition to this heterotrimer, several tissues contain small amounts of a molecule with a chain composition of \([\alpha(1)I]_2\alpha(2)I\] known as the type I collagen homotrimer (1). In fact, early renaturation experiments with individual \(\alpha\) chains of type I collagen indicated that they are able to form both \([\alpha(1)I]_2\alpha(2)I\] heterotrimers and \([\alpha(1)I]_3\] homotrimers although the former were favored, and the \(T_m\) of the latter was slightly lower than that of the former (7). Homotrimers with the structure of \([\alpha(2)I]_3\] were also obtained at low temperatures, but their yield was much lower and the \(T_m\) was only about 20–24 °C (7).

Type I collagen is now used in many medical applications as a biomaterial and as a delivery system for certain drugs (8–10). The collagen used in these applications has been isolated from animal tissues and is liable to cause allergic reactions in up to 3% of human subjects (11). It is obvious, therefore, that an efficient large-scale recombinant system for the production of type I collagen would have many practical applications.

We recently reported that insect cells provide an excellent system for the large-scale expression of native triple-helical human type III collagen, a homotrimer consisting of three identical \(\alpha(1)I\) chains (12). Nevertheless, coexpression with the \(\alpha\) and \(\beta\) subunits of human prolyl 4-hydroxylase, an \(\alpha_2\beta_2\) tetramer (6, 13, 14), was required for the production of molecules with stable triple helices (12). The properties of the recombinant type III collagen were very similar to those of the corresponding nonrecombinant protein, and the highest expression levels obtained in suspension cultures were up to 40 mg/liter type III collagen, corresponding to 60 mg/liter type III procollagen.

The purpose of the present work was to study whether coexpression of the pro\(1(I)\) and pro\(2(I)\) chains of human type I procollagen in insect cells that also express human prolyl 4-hydroxylase will lead to the formation of heterotrimeric molecules with the correct 2:1 chain ratio. The association of type I procollagen chains begins with interactions among the C

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¶ The abbreviations used are: pro\(1(I)\) and pro\(2(I)\) chain, pro\(1\) and pro\(2\) chain of type I procollagen, respectively; pro\(1(III)\) chain, pro\(1\) chain of type III procollagen; PAGES, polyacrylamide gel electrophoresis; M0I, multiplicity of infection; CD, circular dichroism; \(\alpha\) chain, a chain produced from procollagen by the cleavage of the N and C propeptides; telopeptide, a short region between a N or C propeptide and the collagen domain; \(\pi\)N propeptide, a polypeptide chain produced by the cleavage of the C propeptide; bp, base pair(s); PCR, polymerase chain reaction.
propeptides, and it has been regarded as likely that their structural features favor the formation of heterotrimeric rather than [proOl1(III)] homotrimers and prevent the formation of [proOl2(III)] homotrimers (1, 6, 15). We therefore also studied whether proo2(1) chains in which the C propeptide has been replaced with that of the proOl1(1) or proOl1(III) chain will form homotrimeric molecules or whether additional restrictions exist at the level of the collagen domain, as suggested by the early renaturation experiments with isolated o2(1) chains (7).

**MATERIALS AND METHODS**

**Construction of Baculovirus Transfer Vectors and Generation of Recombinant Viruses**—A full-length cDNA for the human proOl1(1) chain (16) was digested with XbaI and ligated to pVL1392 (Invitrogen). A full-length cDNA HpH2010 (17) for the human proo2(1) chain was cloned as a blunt-ended fragment into the EcoRI site of pSp72 (Promega), generating pSp72-C1A2. A BglII site was created 9 bp upstream of the translation initiation codon by PCR, and the full-length cDNA was digested with BglII and BamHI and ligated to pVL1392. The pVL constructs were cotransfected into Spodoptera frugiperda SF9 cells with a modified Autographa californica nuclear polyhedrosis virus DNA using the BaculoGold transfection kit (Pharmingen), and the resultant constructs were termed pSp72-C1A1 and pSp72-C1A2, respectively.

**Expression of Human Type I Collagen**

The expression of recombinant human type I collagen was analyzed by SDS-PAGE using a double-promoter virus 4PHab(23) coding for the α(19) and β(20) subunits of human prolyl 4-hydroxylase (21). The collagen-coding viruses were used in a 5–10-fold excess over the collagen-domain virus (12). L-Amino acids (mg/ml) (Wako) was added to the culture medium daily. The cells were harvested 72 h after infection, washed with a solution of 0.15 M NaCl and 0.02 M phosphate, pH 7.4, homogenized in a solution of 0.3 M NaCl, 0.2% Triton X-100, and 0.07 M Tris, pH 7.4, and centrifuged at 10,000 × g for 20 min. The Triton X-100 soluble proteins were analyzed by SDS-PAGE, followed by staining with Coomassie Brilliant Blue or Western blotting with a polyclonal antibody against the N propeptide of type I (PINP) or type III procollagen (PIIINP) (Farms Diagnostica) or a monoclonal antibody 95D1A recognizing the collagenous regions of various collagen chains. 2 The aliquots of the Triton X-100 supernatants were incubated with pepsin (0.2 mg/ml) for 1 h at 22 °C (22), and some samples were subsequently digested with a combination of trypsin (0.1 mg/ml) and chymotrypsin (0.25 mg/ml) for 2 min at various temperatures (22).

**Quantification and Purification of the Collagen**—Two assays were used to measure the level of expression of the wild-type or modified type I collagen. The first was based on measurement of the 4-hydroxyproline content, assuming that all the hydroxylysylatable proline residues in the α(11) and o2(1) chains had been converted to 4-hydroxyproline. Aliquots of the Triton X-100 supernatants were hydrolyzed at 110 °C for 16 h and studied by a colorimetric method for 4-hydroxyproline (23). The second assay was based on densitometry of the Coomassie-stained collagen α chains bands in SDS-PAGE using known amounts of type I collagen (Chemicon) as a standard. The amounts of the α(11) and o2(1) chains were estimated by densitometry of the Coomassie-stained bands using a Bioimage instrument (Millipore Corp.).

The recombinant type I collagen was purified as described previously (12), with the exception that the collagen was precipitated with 4 M NaCl.

**Other Assays**—Amino acid analyses of the homotrimeric and heterotrimeric recombinant type I collagen were performed in an Applied Biosystems 421 or Beckman system 6300 amino acid analyzer. The melting curves were determined in a Jasco J-500 spectropolarimeter with a temperature-controlled quartz cell of path length of 1 cm (Gillford) (12).

**RESULTS**

**Expression of a Recombinant Human Type I Procollagen Homotrimer in High Five Cells**—A recombinant virus coding for the proOl1(1) chains was generated and used to infect High Five cells together with a double-promoter virus coding for the α and β subunits of human prolyl 4-hydroxylase. The cells were cultured either as monolayers or in suspension, harvested 48 and 72 h after infection, homogenized in a buffer containing 0.2% Triton X-100, and centrifuged. The Triton X-100 soluble proteins of the cell homogenates were then digested with pepsin at 22 °C for 1–4 h. Samples were analyzed by SDS-PAGE under reducing conditions followed by Coomassie staining or Western blotting. Two bands corresponding to the proOl1(1) and pNol1(1) chains were seen in the Coomassie-stained gel of non-pepsinized samples 48 h after infection (Fig. 1A, lane 2) while only the pNol1(1) chains were seen at 72 h (Fig. 1A, lane 3). The presence of the proOl1(1) and pNol1(1) chains was confirmed by

2 This virus was generated using a double promoter baculovirus transfer vector p2Bac (Invitrogen) coding for the α(19) and β(20) subunits of human prolyl 4-hydroxylase under the p10 and polyhedrin promoters of p2Bac, respectively.

3 T. Helaakoski, M. Nokelainen, J. Myllyharju, H. Nothbom, T. Pihlajaniemi, P. F. Fietzek, and K. I. Kivirikko, unpublished observations.

4 This monoclonal antibody was generated using a collagenous fragment of recombinant human type XIII collagen as an antigen. It was found to recognize the collagen domains of various denatured collagen chains (A. Snellman and T. Pihlajaniemi, unpublished observations).
Western blotting using the 95D1A antibody against the collagenous regions of various collagens (Fig. 1A, lane 1) and the PINP antibody against the N propeptide of human type I procollagen (data not shown). In the case of the pepsin-digested samples, a major band corresponding to the α1(I) chains was seen in the Coomassie-stained gel (Fig. 1A, lane 4), and the same band was identified in the Western blot using the 95D1A antibody (Fig. 1B, lane 2). The level of expression of the human type I collagen homotrimer was about 10–20 mg/liter, which is lower than the figure of up to 40 mg/liter obtained for type III collagen in the same cells (12). As in the case of type III collagen expression (12), only a minor fraction of the total type I collagen homotrimer produced in High Five cells was found in the culture medium (data not shown).

Sequences at the 5’ ends of DNA constructs influence the expression level of many polypeptides in the baculovirus system (18). Because the expression level obtained for type I homotrimer was less than that obtained for type III collagen, we decided to study whether the level of expression of the former can be increased by replacing the sequences coding for the signal peptide and the N propeptide of the proα1(I) chain with those of the proα1(III) chain. A new virus, C1A1NproIII, was generated and High Five cells were infected and analyzed as above. A faint band corresponding to the hybrid proα1(I) chains and a major band corresponding to the hybrid proα1(N) chains were seen in Coomassie-stained SDS-PAGE 48 h after infection (Fig. 1A, lane 5), and these bands were also stained in Western blotting using the 95D1A (Fig. 1B, lane 3) and PINNP (data not shown) antibodies. A major band corresponding to the hybrid proα1(N) chains and a minor band with the mobility of fully processed α chains were seen 72 h after infection (Fig. 1A, lane 6). The latter band could not be stained by the PINNP antibody, suggesting that it indeed represented fully processed α chains. In the case of the pepsin-digested samples, a major band corresponding to the α1(I) chain was seen both in the Coomassie-stained SDS-PAGE (Fig. 1A, lane 7) and in the Western blot stained by the 95D1A antibody (Fig. 1B, lane 4). About a 3-fold increase was obtained in the level of expression of the type I collagen homotrimer using the C1A1NproIII virus, the level ranging up to about 60 mg of collagen/liter, which corresponds to about 90 mg of procollagen.

The thermal stability of the type I collagen homotrimer was studied by digestion with a mixture of trypsin and chymotrypsin at various temperatures. These experiments indicated that the $T_m$ of the type I collagen homotrimer was about 40 °C, a value which remained unaffected by replacement of the type I N propeptide (data not shown).

Purification of the Recombinant Type I Collagen Homotrimer—The type I procollagen homotrimer was expressed in High Five cells in suspension in shaker flasks using either the C1A1 or the C1A1NproIII virus together with the 4PHβ virus. The recombinant type I collagen was purified as described previously for the recombinant type III collagen (12), with minor modifications. The purified type I collagen homotrimer was studied by amino acid and CD spectrum analyses. The amino acid composition agreed with that reported for the human α1(I) chains (24), except that the hydroxylysine content was about 80% of that in the nonrecombinant protein (Table I). The $T_m$ of the recombinant type I collagen homotrimer was 42.8 ± 1.2 °C (determined from four individual samples) (Fig. 2).

Expression of Recombinant Human Type I Procollagen Heterotrimer in High Five Cells—To study whether collagens consisting of more than one type of α chain can be assembled in insect cells, a recombinant virus C1A2 coding for the proα2(I) chain was generated and used to infect High Five cells either with or without the C1A1 virus but in the presence of the 4PHβ virus. Equal MOI amounts of the C1A1 and C1A2 viruses were used in a 5–10-fold excess over the 4PHβ virus. The cells were cultured and homogenized as above, and the samples were analyzed by SDS-PAGE under reducing conditions followed by Coomassie staining. When the proα1(I) and proα2(I) chains were coexpressed, bands corresponding to the α1(I) and α2(I) chains with an approximate ratio of 2 to 1 (1.89 ± 0.20 to 1, determined from the integrated densitometry values of four individual samples) were seen in Coomassie-stained SDS-PAGE of pepsinized samples (Fig. 3, lane 2). When the proα2(I) chains were expressed alone, no pepsin-resistant band was seen (Fig. 3, lane 3). Thus, the pepsin-resistant α2(I) chains seen in lane 2 must have been present in heterotrimeric molecules. As the ratio of the α1(I) to α2(I) chains was about 2:1, essentially all the α1(I) chains must likewise have been present in heterotrimers. The highest expression levels obtained for the type I collagen heterotrimer were found to be about 20 mg/liter.

To study whether the level of expression of the type I procollagen heterotrimer can be improved by replacing the signal sequence and N propeptide of the proα2(I) chain with those of the proα1(III) chain, a new virus C1A2NproIII was generated. Several experiments were performed to express the type I
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Amino acid analysis of the purified recombinant human type I collagen homotrimer and heterotrimer

| Amino acid | Recombinant human type I collagen homotrimer | Recombinant human type I collagen heterotrimer | Nonrecombinant human type I collagen homotrimer | Nonrecombinant human type I collagen heterotrimer |
|------------|--------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Residues/1000 |                                             |                                             |                                             |                                             |
| Aspartic acid | 44 ± 0                                      | 42                                          | 49 ± 4                                      | 43                                          |
| Glutamic acid | 73 ± 14                                     | 73                                          | 71 ± 2                                      | 71                                          |
| 4-Hydroxyproline | 96 ± 4                                      | 108                                         | 91 ± 5                                      | 103                                         |
| Serine       | 35 ± 5                                      | 333                                         | 29 ± 4                                      | 32                                          |
| Glycine      | 334 ± 5                                     | 333                                         | 334 ± 5                                     | 333                                         |
| Histidine    | 3 ± 1                                       | 6 ± 1                                       | 6 ± 1                                       | 6                                           |
| Arginine     | 50 ± 1                                      | 51 ± 1                                      | 50                                          |                                              |
| Threonine    | 16 ± 2                                      | 18 ± 4                                      | 17                                          |                                              |
| Alanine      | 112 ± 8                                     | 112 ± 2                                     | 112 ± 2                                     | 111                                         |
| Proline      | 126 ± 12                                    | 124                                         | 123 ± 7                                     | 120                                         |
| Valine       | 22 ± 2                                      | 29 ± 3                                      | 26                                          |                                              |
| Methionine   | ND                                          | 7                                           | ND                                          | 6                                           |
| Tyrosine     | ND                                          | 1                                           | ND                                          | 2                                           |
| Isoleucine   | 6 ± 0                                       | 6                                           | 11 ± 3                                      | 9                                           |
| Leucine      | 29 ± 4                                      | 29 ± 4                                      | 3 ± 1                                       | 10                                          |
| Hydroxylsine | 7 ± 3                                       | 9                                           | 12                                          |                                              |
| Phenylalanine| 12 ± 0                                      | 12 ± 1                                      | 12                                          |                                              |
| Lysine       | 35 ± 5                                      | 30 ± 2                                      | 23                                          |                                              |

*The values are given as mean ± S.D., n = 3 for the homotrimer and n = 4 for the heterotrimer.

Fig. 2. Circular dichroism analysis of denaturation of the purified recombinant type I collagen homotrimer and heterotrimer. Melting profiles for representative samples of recombinant type I collagen homotrimer (dotted line) and heterotrimer (solid line) in 0.05% acetic acid measured with circular dichroism. Normalized first derivatives of the ellipticities are plotted versus temperature. The Tm values determined from the maxima of the differentiated curves are 44.2 °C for the homotrimer and 41.6 °C for the heterotrimer.

Fig. 3. Analysis of expression of the recombinant human type I procollagen heterotrimer by SDS-PAGE under reducing conditions. High Five cells were infected with the C1A2 virus or with different combinations of the C1A1, C1AINproIII, C1A2, and C1A2NproIII viruses, all in the presence of the 4PHb virus. They were harvested 72 h after infection, and the cell extracts were digested with pepsin for 1 h at 22 °C. The samples were electrophoresed on 8% SDS-PAGE under reducing conditions and analyzed by Coomassie staining. Lane 1, molecular weight markers; lanes 2–7, pepsinized samples from cells infected with the C1A1 and C1A2 (lane 2), C1A2 (lane 3), C1AINproIII and C1A2 (lane 4 and 5), C1A1 and C1A2NproIII (lane 6), and C1AINproIII and C1A2NproIII (lane 7). The arrows indicate the α1(I) and α2(I) chains.

pepsin-resistant α2(I) chains were detected in all the samples by Coomassie staining of SDS-PAGE, but significant differences were found in the α1(I) to α2(I) chain ratios upon densitometry (Fig. 3, lanes 4–7). When insect cells were coinfected with the C1A1 and C1A2 viruses coding for the wild-type proα1(I) and proα2(I) chains, the ratio of pepsin-resistant α1(I) to α2(I) chains was consistently 2 to 1 (Fig. 3, lane 2). However, when the C1A2 virus was used together with the C1AINproIII virus, an excess of the pepsin-resistant α1(I) chains was found in several experiments and the α1(I) to α2(I) chain ratio varied, being about 2–5 to 1 (3.11 ± 1.09 to 1, determined from the integrated densitometry values of four individual samples) (Fig. 3, lanes 4–5). The level of expression of type I collagen obtained by coinfection with the C1AINproIII and C1A2 viruses was about 40 mg/liter. When insect cells were coinfected with the C1A1 or C1AINproIII and C1A2NproIII viruses, the formation of heterotrimeric type I collagen appeared to be very inefficient, the ratio of the pepsin-resistant α1(I) to α2(I) chains being about 5–10 to 1 (Fig. 3, lanes 6–7).

In further experiments, the ratio of viruses coding for the proα1(I) and proα2(I) chains was varied by keeping the amount of C1A2 virus constant but using different amounts of either the C1A1 virus (Fig. 4, lanes 2–5) or C1AINproIII virus (Fig. 4, lanes 6–9) so that the MOI ratio of the C1A1 or C1AINproIII virus to the C1A2 virus was 0.25 (Fig. 4, lanes 2 and 6), 0.5 (Fig. 4, lanes 3 and 7), 0.75 (Fig. 4, lanes 4 and 8), or 1.0 (Fig. 4, lanes 5 and 9). Pepsin-digested samples were then studied by SDS-PAGE followed by Coomassie staining and densitometry of the bands. The amount of pepsin-resistant α1(I) chains increased with increasing amounts of the C1A1 or C1AINproIII virus as could be expected. In addition, the amount of pepsin-resistant α2(I) chains increased in a similar manner even though the amount of C1A2 virus was kept constant (Fig. 4). The ratio of pepsin-resistant α1(I) to α2(I) chains varied with the original C1A1 and C1A2 viruses, from about 3:1 at a virus ratio of 0.25 (Fig. 4 lane 2) to about 2:1 with ratios of 0.75 and 1.0 (Fig. 4,
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DISCUSSION

The data reported here indicate that coexpression of the proα1(I) and proα2(I) chains of human type I procollagen in insect cells leads to the formation of heterotrimeric molecules with the correct 2:1 chain ratio. The data further indicate that expression of proα1(I) chains without proα2(I) chains effectively leads to the formation of homotrimeric molecules. The formation of the heterotrimers was nevertheless clearly favored, as the ratio of pepsin-resistant α1(I) to α2(I) chains remained at 2:1 in the coexpression experiments unless the proα1(I) chains were expressed in a relatively large excess. Replacement of the sequences coding for the signal peptide and the N propeptide of the proα1(I) chain with those of the proα1(III) chain increased the level of expression of the proα1(I) chain about 3-fold, whereas no corresponding effect was seen when a similar modification was made to the proα2(I) chain. The highest expression level obtained for the type I collagen homotrimer with the modified construct was 60 mg/liter, thus being slightly higher than that previously obtained for type III collagen in High Five cells (12).

The heterotrimetric and homotrimeric procollagen molecules produced in High Five cells were found to be processed with time, as partial conversion of the wild-type proα1(I) and proα2(I) chains to pNα1(I) and pNα2(I) chains (i.e., cleavage of the C propeptides) was seen in all the expression experiments (although not shown for the proα2(I) chain in the coexpression experiments). Although the N propeptides of the wild-type proα1(I) and proα2(I) chains appeared to be stable, the N propeptide of the proα1(III) chain artificially transferred into the proα1(II) chain was less so, as some of the modified proα1(I) chains were processed to α(I) chains even in the case of homotrimeric molecules in which the three type III N propeptides should be able to form a correctly folded trimer. Similar processing of the N propeptide of the proα1(III) chain in insect cells was also found when this propeptide was transferred to the proα1(II) chain, whereas the wild-type N propeptide of the proα1(II) chain and the N propeptide of the proα1(III) chain...
The present data differ from recent results obtained in trans-
fection experiments in a rabbit reticulocyte lysate system.

It thus seems more likely that synthesis and assembly of
recombinant heterotrimeric and homotrimeric type I procollagen
molecules with stable triple helices did not require the presence of any recom-
binant Hsp-47. The possibility is not excluded that the cells
may have marked up-regulated expression of an insect
Hsp-47, and that this protein assisted folding of the human procollagen molecules. This possibility does not seem very
likely, however, as baculovirus infection interferes with the
synthesis of cellular proteins (30). It is thus more likely that
assembly of the type I procollagen heterotrimers and homotrimers did not require Hsp-47.

The present data indicate, however, that formation of the
heterotrimeric and homotrimeric type I procollagen
molecules involved in the assembly and/or secretion of collagens (28,
29). The present data indicate, however, that formation of the
type I procollagen heterotrimer and homotrimer with stable
triple helices did not require the presence of any recom-
binant Hsp-47. The possibility is not excluded that the cells
may have marked up-regulated expression of an insect
Hsp-47, and that this protein assisted folding of the human procollagen molecules. This possibility does not seem very
likely, however, as baculovirus infection interferes with the
synthesis of cellular proteins (30). It is thus more likely that
assembly of the type I procollagen heterotrimers and homotrimers did not require Hsp-47. The failure to obtain
[heterotrimers] or homotrimers with stable triple helices from
procollagen molecules having modified C propeptides in insect cells
and in renaturation experiments starting from individual
2(I) chains in vitro might be due to the lack of Hsp-47 if this
chaperone was especially important for folding of the
2(I) chains. The current data on Hsp-47 (28, 31) does not
suggest any such specificity, however. The failure of insect
cells to secrete most of the type I and type III (12) procollagen
molecules might likewise be due to lack of Hsp-47, but it has
been reported previously that insect cells are also poor at
secreting many other recombinant secretory proteins (32–
34). It thus seems more likely that synthesis and assembly of
recombinant heterotrimeric and homotrimeric type I procollagen
molecules in insect cells does not require Hsp-47.

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