Posttranslational Folding of $\alpha_1$-Inhibitor 3  
EVIDENCE FOR A COMPACTION PROCESS*

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$\alpha_1$-Inhibitor 3 ($\alpha_1$I3) is a rodent-specific proteinase inhibitor of about 190 kDa belonging to the $\alpha_1$-macroglobulin family. It consists of five globular domains, three of which are connected by disulfide bridges, and contains an intramolecular thiol ester which can react with attacking proteinases. To explore the folding of newly synthesized $\alpha_1$I3, we have used rat hepatocytes and pulse-chase experiments. In one of the analyses, the radiolabeled protein was isolated from cell lysates by immunoprecipitation and its Asp-Pro bonds cleaved by treatment with formic acid. The size of the major fragment, as assessed by electrophoresis under nonreducing conditions, was found to increase from 100 to 150 kDa upon the chasing. This result, together with knowledge of the positions of the cleavage sites and the disulfide arrangement, indicates that one of the interdomain disulfide bonds is formed after the synthesis of the polypeptide. Analysis of the same material by limited proteolysis and by velocity centrifugation showed that the folded regions became larger and that the protein became more compact; the thiol ester was found to be formed after these conformational changes. These results suggest that the domains of $\alpha_1$I3 are only partially developed directly after the synthesis of the polypeptide and that they acquire their final structure as the protein condenses and the domains interact with one another.

All proteins are synthesized as linear polypeptides which subsequently fold into their unique three-dimensional structure. Studies of the renaturation of isolated proteins have shown that the information required for proper folding of a nascent polypeptide resides in the amino acid sequence alone (Anfinsen, 1973). However, folding in a living cell is faster and some acquire their final conformation more or less cotranslationally (Peters and Davidson, 1982), whereas others may require up to an hour (Lodish and Kong, 1991) facilitating analysis of the reaction.

In this study we have analyzed the folding of $\alpha_1$-inhibitor 3 ($\alpha_1$I3), a 180–200 kDa proteinase inhibitor secreted by rodent hepatocytes (Esnard and Gauthier, 1980; Geiger et al., 1987; Sottrup-Jensen et al., 1991). $\alpha_1$I3 shows sequence homology with $\alpha_2$-macroglobulin and the complement components 3 and 4 (BraCIak et al., 1988; Sottrup-Jensen, 1989). All of these proteins have two characteristic features: they contain an intramolecular thiol ester and have a sequence which is particularly sensitive to proteolysis, the bait region (Esnard et al., 1985; Sottrup-Jensen, 1989). When the polypeptide is cleaved in the bait region, the protein undergoes a conformational change, and the thiol ester becomes exposed to the exterior (Sottrup-Jensen, 1989); in this position the thiol ester may react with hydroxyl or amino groups on adjacent proteins or carbohydrates forming a covalent link (Enghild et al., 1989). $\alpha_1$I3 appears to consist of five globular domains (Rubenstein et al., 1991), and electron microscopic analysis has revealed a ring-like structure (Ikai et al., 1990). In a previous study we found that some disulfides of $\alpha_1$I3 are formed after the synthesis of the polypeptide, indicating that part of the folding occurs posttranslationally (Sjöberg et al., 1991). In the present paper we have characterized this process further. Our results indicate that the newly synthesized protein undergoes a gross conformational change upon which the globular domains acquire their final structure.

**EXPERIMENTAL PROCEDURES**

**Materials**—[35S]Methionine (>1000 Ci/mmol) and [3H]methylated molecular mass standards were purchased from Amersham International, Bucks., United Kingdom. Protein disulfide isomerase, which had been isolated from bovine liver, was kindly given to us by Margareta Ingelman (Biomedical Center University of Uppsala, Uppsala, Sweden). Saponin was from E. Merck, Darmstadt, Federal Republic of Germany, and chymotrypsin A4 from Boehringer Mannheim.

**Cell Culture**—Rat hepatocytes were isolated and cultured in 60-mm dishes for 20–24 h as described (Sjöberg et al., 1991).

**Pulse-Chase Experiments**—The cells were labeled for 10 min with 50–80 $\mu$Ci of [35S]methionine as described previously (Sjöberg et al., 1991). After different times of chasing, the plates were put on ice and the medium withdrawn. The cells were rinsed twice with 250 $\mu$l of the latter sucrose solution was added; all solutions contained 0.1 mM N-methylmaleimide. The cells were then scraped off the dishes, homogenized, and the homogenate was centrifuged at 1.6 $\times$ 10^6 x g for 10 min. The supernatant was collected and mixed with 80 $\mu$l of a solution containing 200 $\mu$l Triton X-100, 2 $\mu$l phenylmethylsulfonyl fluoride, 1 $\mu$l N-ethylmaleimide, and 20 $\mu$l iodoacetamide (sulubilizing buffer).

**Immunoprecipitation**—Normal rabbit IgG (5 $\mu$g), rabbit antibodies

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§ The abbreviations used are: ER, endoplasmic reticulum; $\alpha_1$I3, inhibitor 3; PAGE, polyacrylamide gel electrophoresis.
against human albumin (10 μg), or a rabbit serum against α1-microglobulin (1.5 μl) was added to all samples. After an overnight incubation at 4°C, 50 μl of a 10% (w/v) suspension of glutaraldehyde-fixed Staphylococcus aureus bacteria was added. The test tubes containing the mixtures were rotated end-over-end for 40–60 min, and the bacteria were removed by centrifugation. Then 1.5 μl of a rabbit serum against α1-I3 was added. After 30 min, the immunocomplexes were collected, treated with methyleamine unless indicated, and analyzed as described previously (Sjöberg et al., 1991).

Formic Acid Treatment—The immunocomplexes were subjected to SDS-PAGE under reducing or nonreducing conditions on 10% polyacrylamide gels (Mini-PROTEAN II, Bio-Rad; 0.75-mm spacers). Gel strips containing the different lanes were cut out and soaked in 75% (v/v) formic acid for 3–4 h at room temperature essentially as described (Sonderegger et al., 1982). The acid was then removed and paraffin oil (0.88 g/ml) added. After incubation at 37°C for 18 h, the gel strips were soaked twice for 1 h in 250 mM Tris/HCl, pH 8.8, 5 g/liter SDS and twice for 1 h in 125 mM Tris/HCl, pH 6.8, 5 g/liter SDS, 20% glycerol. They were then placed on top of the stacking gel of a polyacrylamide gel (20 × 25 × 0.15 cm) with a 10–15% gel gradient. Finally, the gel strips were fixed by the addition of more stacking gel and running buffer containing 1.3 g/liter SDS added. The electrophoresis was run overnight at 7 mA.

Limited Proteolysis—Immunocomplexes were collected as described above and limited proteolysis was performed essentially as described by Rubenstein et al. (1991). The washed S. aureus particles were suspended in a 10 μl of a buffer containing 50 mM NaCl, 1 mM CaCl2, 100 mM β-propiolactone, 10 mM Tris/HCl, pH 7.4 and 0.1 mM mg/ml chymotrypsin. The samples were then incubated for 15 min at 37°C. The digestion was stopped by the addition of 1.5 μl of 200 mM phenylmethylsulfonyl fluoride and 25 μl of the sample buffer used for SDS-PAGE with or without a reducing agent was added. The mixtures were then heated for 3 min at 95°C and analyzed by SDS-PAGE.

Velocity Centrifugation—The cells in two dishes were pulse-labeled as described above and the cells in one dish were then chased for 20 min. After rinsing in phosphate-buffered saline, the cells were incubated for 60 min at +4°C with 1 ml of phosphate-buffered saline containing 1.0 mg/ml of saponin, 0.1 mM N-ethylmaleimide, and 2 mM iodoacetamide on a rocking device; this treatment releases all secretory proteins from the ER (Wassler et al., 1987). The saponin extracts from both dishes were collected, mixed, and 400 μl was layered on a 5–30% (w/w) sucrose gradient containing 25 mM Hepes/NaOH, pH 7.4, 150 mM KCl, and 0.1 mM N-ethylmaleimide. After centrifugation at 112 × 80°C for 60 min, the bottom of the tube was punctured and 250-μl fractions collected. A sample containing aldolase was treated in the same way.

Cell-free Folding—The cells in two dishes were pulse-labeled, homogenized, and centrifuged as described above except that the sucrose-containing solutions lacked N-ethylmaleimide and were supplemented with 10 mM Tris/HCl, pH 7.5. The resulting supernatant was aliquoted, frozen in liquid nitrogen, and stored at −70°C. After thawing, the sample was centrifuged in an Airfuge (Beckman) at maximum speed for 10 min. The supernatant was withdrawn and the pellets membranes suspended to their original volume in a buffer containing 20 mM NaCl, 100 mM KCl, 5 mM MgCl2, 1.0 mM NaH2PO4, 25 mM Hepes, 2.0 mM EDTA, pH 7.4, with or without 10 mM dithiothreitol was added, and the reaction mixture was placed on ice. After 5 min, the samples were transferred to a 30°C water bath and then to an 80°C water bath for 2 min. Then 5 μl of 50 mM dithiothreitol was added, and the samples were heated at 95°C for 3 min.

RESULTS

Electrophoretic Mobility Shift—Most proteins migrate more rapidly upon SDS-PAGE in the oxidized than in the reduced form presumably, because the presence of disulfides makes the proteins more compact. α1-I3 (in its secreted, mature form) is unusual in that it has a lower mobility under nonreducing than reducing conditions; the apparent molecular masses of secreted α1-I3 upon SDS-PAGE in the absence and presence of a reducing agent are 210 and 184 kDa, respectively (Saito and Sinohara, 1985; Sjöberg et al., 1991). Examples of other proteins behaving in the same way are complement component 4 (Karp, 1983) and the insulin receptor (Olson et al., 1988). Apparently, the disulfides in these proteins are arranged in such a way that they disturb the SDS binding. Analysis of cellular α1-I3 from a pulse-chase experiment by SDS-PAGE under nonreducing conditions shows that the mobility decreases stepwise (Fig. 1), the apparent molecular mass increasing from 162 to 210 kDa. Four different bands can be detected, denoted α and β1–β3 in Fig. 1, indicating that at least three new disulfides are formed posttranslationally. As α disappears, β1 and β2 appear, apparently simultaneously, and finally, β3 becomes the major band. Under reducing conditions, α and β1–β3 all have the same mobility, which is intermediate to those of α and β1 (Sjöberg et al., 1991). Thus, the mobility of pulse-labeled α1-I3, in contrast to that of later forms, is higher under nonreducing than reducing conditions. This fact shows that at least one disulfide is formed cotranslationally. In our previous study, a substantial part of the radiolabeled α1-I3 remained in the cells even after a prolonged chase (Sjöberg et al., 1991). We later found that the inefficiency of the secretion was due to the presence of cytoheximide which was added to stop further incorporation of the labeled amino acid; this drug was therefore not used in the present study.

Chemical Cleavage—Fig. 2 shows the domain structure as well as the disulfide arrangement of mature α1-I3, as proposed by Rubenstein et al. (1991) and Braciack et al. (1988), respectively. To characterize the posttranslationally formed disulfide bonds of α1-I3, we cleaved the radiolabeled protein at specific sites with formic acid and analyzed the products by SDS-PAGE under nonreducing conditions. The triangles in Fig. 2 indicate the positions of the amide bonds that are sensitive to treatment with formic acid, those between aspartyl and prolyl residues (Landon, 1977). Only two of the acid-sensitive bonds are located outside disulfide-linked cysteine residues, and these are both near the N terminus. Therefore, treatment of secreted α1-I3 with formic acid followed by SDS-PAGE under nonreducing conditions should yield a relatively large fragment. It should be added here that cloning of cDNA for rat α1-I3 has revealed that there are at least four different isoforms of this protein (Braciack et al., 1988). The complete nucleotide sequence is known for only one of them but the partial amino sequence obtained from proteolytic fragments of α1-I3 isolated from serum (Rubenstein et al., 1991) indicates that this is the major form.

For the experiment shown in Fig. 3A, α1-I3 was isolated by immunoprecipitation from the medium of hepatocytes labeled with [35S]methionine or from a lysate of pulse-labeled cells. Cofractionating proteins were then separated from α1-I3 by electrophoresis in a short polyacrylamide gel. The gel strips
containing the different electrophoretic lanes were cut out and treated with formic acid. They were then placed along the upper edge of an electrophoresis gel, and the protein fragments were separated. When the electrophoresis was performed under nonreducing conditions, secreted \( \alpha_1 I_3 \) (lane 1) yielded two major bands: the intact protein of 200 kDa and a 150-kDa fragment (arrow). Cellular, pulse-labeled \( \alpha_1 I_3 \) (lane 2) yielded both these fragments as well as smaller ones, in particular in the range 90–110 kDa (bracket). Under reducing conditions, identical fragments were obtained from both secreted and pulse-labeled \( \alpha_1 I_3 \) (lanes 3 and 4, respectively); the major bands had apparent molecular masses of about 95 and 55 kDa. As judged from the amino acid sequence of \( \alpha_1 I_3 \), the largest fragments formed by mild acid hydrolysis should be of 43 and 44 kDa (cf. Fig. 2). The presence of the 95 kDa band therefore implies that the cleavage reaction was incomplete; longer incubation was not beneficial, however, because it resulted in substantial loss of fragments. In a time course study we found that the relative amount of polypeptides larger than 110 kDa decreased upon chasing (Fig. 3B); densitometric analysis showed that this amount increased from 38 to 86% during 40 min of chase, the half-maximal increase occurring after 10–20 min.

Limited Proteolysis—Incomplete proteins are more sensitive to proteolytic degradation than mature ones, and a decrease in protease sensitivity can therefore be used as an indicator of folding (Scheele and Jacoby, 1982). To see if this technique would reveal conformational changes in \( \alpha_1 I_3 \), we isolated the protein by immunoprecipitation from cells that had been pulse-labeled and chased for different times and incubated it briefly with chymotrypsin; the protease inhibiting activity of \( \alpha_1 I_3 \) had been blocked by the addition of \( \beta_2 \)-propionitrile (Rubenstein et al., 1991). The cleavage products were then detected by SDS-PAGE under reducing conditions. Since artifactual bands have been reported to occur with this technique, the results should be treated with some caution (Rüchel et al., 1974). With pulse-labeled \( \alpha_1 I_3 \) (Fig. 4; 0-min chase), three protease-resistant fragments of 77, 50, and 37 kDa were obtained. Upon chasing, the relative amount of the 77- and 50-kDa bands decreased and concurrently, polypeptides of 97, 89, 86/84, and 65 kDa appeared. Densitometric analysis of the fluorograms showed that the half-life of the former bands was 10–20 min. When \( \alpha_1 I_3 \) from the cell medium was treated with chymotrypsin under the same conditions, three major bands of 90, 69, and 43 kDa were formed (data not shown).

Velocity Centrifugation—We wished to see if the posttranslational folding of \( \alpha_1 I_3 \) could be detected also as a change in the sedimentation properties of the protein. A sample was therefore prepared containing both early and late forms of radioactively labeled \( \alpha_1 I_3 \) and subjected to velocity centrifugation. Fig. 5 shows that \( \alpha_1 I_3 \) of the \( \beta_3 \) form sedimented faster than both the \( \alpha \) and the \( \beta_2 \) forms, suggesting that \( \alpha_1 I_3 \) becomes more spherical and/or compact shortly after its synthesis; the difference between the sedimentation coefficients of the slowest and fastest forms can be estimated to be about one S unit. The binding of a small protein would also account for the increase in the sedimentation rate of \( \alpha_1 I_3 \). However, the fact that the sedimentation rate seemed to increase stepwise with time of chase makes this possibility unlikely. The sedimentation rate of the \( \alpha \) form was similar to that of aldolase (data not shown), which has a sedimentation coefficient of 7.4 S (Stellwagen and Schachman, 1962); that of secreted \( \alpha_1 I_3 \) is 8.6 S (Esnard and Gauthier, 1980).

Disulfide Formation In Vitro—To be able to biochemically analyze the process underlying the folding of \( \alpha_1 I_3 \), we attempted to obtain the reaction in a cell-free system. For this purpose, microsomes were prepared from pulse-labeled hepatocytes and incubated at 37°C for up to 30 min. \( \alpha_1 I_3 \) was then
analyzed by SDS-PAGE under nonreducing conditions. Fig. 6 shows that within 15 min of incubation, the initial form of \( \alpha_1I_3 \) was isolated by immunoprecipitation, treated with chymotrypsin, and the resulting fragments were separated by SDS-PAGE under reducing conditions. Fig. 6 shows that upon incubation of pulse-labeled cells, solubilized them with Triton X-100, and incubated at 37 °C; predominantly the intermediate forms, \( \beta_1 \) and \( \beta_2 \), were formed (Fig. 6, TX). If, however, the medium was made less oxidizing by the addition of reduced and oxidized glutathione (both of 0.6 mM), a minor increase of \( \beta_3 \) occurred (Fig. 6, TX + G); the calculated redox potential of this solution is \( -0.14 \) V (Hwang et al., 1992). Addition of protein disulfide isomerase to the incubation mixture (TX + G + PDI) enhanced the rate of transition several times, yielding band \( \beta_3 \) as the end product.

Thiol Ester Formation—If thiol ester-containing proteins are heated under denaturing conditions, an amide bond adjacent to the thiol ester is cleaved (Harpel et al., 1979; Howard et al., 1980). When the secreted form of \( \alpha_1I_3 \) is treated in such a way, analysis by SDS-PAGE yields two fragments of about 150 and 60 kDa (Esnard et al., 1985; Sjöberg et al., 1991) (see Fig. 7A, bands denoted b' and c'). The cell samples in Fig. 7A show the time course for the formation of the thiol ester. As reported previously (Sjöberg et al., 1991), temperature-sensitive \( \alpha_1I_3 \) appears with a lag of 10–30 min. The relative amount of cleaved \( \alpha_1I_3 \) was lower in the cell than in the medium samples, indicating that the formation of the thiol ester occurs immediately before or upon secretion. Since our results showed that the thiol ester is formed after the disulfide bond, we were interested to see if this structure would also form in a cell-free system. Fig. 7B shows that upon incubation of pulse-labeled microsomes, heat-sensitive \( \alpha_1I_3 \) was rapidly formed; densitometric analysis of the fluorogram showed that the \( t_{1/2} \) of the reaction was about 15 min and that the maximum level was 25–35% of the total amount. When the microsomes were first solubilized and then incubated under the conditions that we had found to be optimal for the formation of the disulfides, the thiol ester was formed with the same efficiency as in the microsomes.

**DISCUSSION**  
In this study we obtained evidence that at least one of the interdomain disulfides of \( \alpha_1I_3 \) is formed after the synthesis of the polypeptide. This conclusion is based on the analysis of pulse-chase experiments in which \( \alpha_1I_3 \) was isolated by immunoprecipitation and cleaved with formic acid. Subsequent detection of the cleavage products by SDS-PAGE under nonreducing conditions showed that the size of the major fragment shifted from 100 to 150 kDa upon chasing (Fig. 3B). With the assumption that cleavage occurred at the same rate at all Gly-Pro bonds, the simplest explanation for this finding is that the disulfide that links domains III and IV (Fig. 2), which spans half of the cleavage sites, is formed during the chase.

Another method we used to monitor the folding of \( \alpha_1I_3 \) is
method had earlier been used to identify the domains of ma-
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*α*1-Inhibitor 3 Folding

Fig. 7. Thiol ester formation of *α*1I3 in cells and in cell-free systems. A, hepatocytes were pulse-labeled and chased for different times and *α*1I3 was isolated from the cells and the media by immuno-
precipitation. Thiol ester-dependent cleavage was then induced by heat-
ing the samples, and cleavage was assessed by SDS-PAGE under re-
ducing conditions, a and a' denoting intra- and extracellular forms of intact *α*1I3. In the cells, only the larger fragment of *α*1I3 (band b) is
apparent, whereas in the medium, both the larger and the smaller cleavage products can be seen (bands b' and c', respectively). B, microsomes were prepared from pulse-labeled cells and incubated at 37 °C for
different times either in the presence of reduced/oxidized glutathione (Microsomes) or with detergent, reduced/oxidized glutathione, and pro-
tein disulfide isomerase (Free solution). *α*1I3 was then isolated by immu-
noprecipitation and assayed for thiol ester-dependent cleavage; only the upper part of the gel is shown.

Based on the observation that tightly folded regions or domains
are more resistant to proteolytic degradation than extended polypeptide segments (Porter, 1959; Fontana et al., 1986). This method had earlier been used to identify the domains of ma-
ture, secreted *α*1I3 (Rubenstein et al. 1991; Fig. 2). In that investigation, the protein was isolated from rat serum and treated with increasing concentrations of chymotrypsin. The cleavage products were then separated by SDS-PAGE and the obtained polypeptide bands identified by amino acid sequenc-
ing. The initial cleavage products were two bands of about 100
kDa, which were found to be formed by scission of the bait region (see Fig. 2), the upper and lower bands representing the C- and N-terminal halves, respectively. At higher chymotryp-
sin concentrations, the C-terminal fragment gave rise to two bands of 64 and 43 kDa (domains IV and V, respectively; see Fig. 2).

In the present study we found that when radiolabeled *α*1I3 from cells chased for 20 min or more was treated with a low concentration of chymotrypsin, three major fragments of 86/84, 65, and 37 kDa were obtained (Fig. 4). The similarity of the sizes of these fragments to those obtained from secreted *α*1I3 suggests that they represent the N-terminal half of the mole-
cule and domains IV and V, respectively. (In intracellular forms usually have lower apparent molecular masses due to incom-
plete carbohydrate processing.) Chymotrypsin treatment of pulse-labeled *α*1I3 also yielded the 37-kDa band, but the other major fragments, of 77 and 50 kDa, were clearly different from those obtained from chased cells; however, comparison of the peptide patterns of the 77- and the 86/84-kDa fragments after cleavage with formic acid indicated that these fragments were partially identical. A simple explanation for these results is

that the C-terminal region of *α*1I3 (domain V) is fully folded in
the pulse-labeled protein, whereas the central region is not and is therefore partially degraded upon treatment with chymo-
trypsin. The 77- and 50-kDa fragments would then represent truncated forms of domains I–III and domain IV, respectively. Regardless of whether this interpretation is correct or not, our results show that the proteinase resistant regions become larger after the synthesis of the polypeptide. Furthermore, they indicate that the protein occurs in two distinct conformations, since the fragments of *α*1I3 obtained from pulse-labeled and chased cells were markedly different and there seemed to be no intermediates.

Concurrent with the shift in the sizes of the proteinase-
resistant fragments and the formation of new disulfides, *α*1I3 became more compact as shown by the fact that its sedimenta-
tion rate increased (Fig. 5). It is possible that these confor-
mational changes reflect a transition from an open, extended
structure to the ring-like shape of the mature protein (Ikai et al., 1990). We also found that these changes preceded the
appearance of the thiol ester, suggesting that the formation of this structure requires an almost completely folded protein.

This notion is supported by the finding that mutated forms of complement component 3, in which the thiol ester could not be
formed because one of the necessary amino acid residues was
missing, had a native-like conformation (Isaac and Jensen, 1992). When the bait region of *α*1I3 and related proteins is proteolytically cleaved, the thiol ester becomes exposed to the exteri-
or of the molecule making linkage to proteases possible
(Enghild et al., 1989). Simultaneously, the conformation of the C-terminal part changes so that the proteins will bind to cell
surface receptors effecting their removal from the blood stream
(Van Leuven et al., 1986; Law and Dodds, 1990). Under the
electron microscope, *α*1I3 appears to consist of one large and
two small elements forming an asymmetric ring: upon cleav-
age of the bait region, the molecule acquires a more open, C-like shape (Ikai et al., 1990). Presumably, the bait region is located in the larger, central domain, and upon cleavage, this part of the molecule changes its conformation so that the flanking
structures are forced apart. Clearly, this reaction must require
a close association between neighboring domains; the presence
of interdomain disulfides (Fig. 2) indicates that this is indeed the
case.

The redox potential of the interior of the ER has been found
to be intermediate between that of the cytoplasm and the exter-
cellular space (Hwang et al., 1992). The physiological signi-
ficance of this observation has been borne out by studies on
the folding of proteins in cell-free systems which have shown
that the formation of correct disulfides is optimal under weakly
oxidizing conditions (Scheele and Jacoby, 1982; Huth et al.,
1994). Consistent with these observations, we found that when pulse-labeled *α*1I3 in free form was incubated in the presence of a mixture of reduced and oxidized glutathi-
one, a substantial part of the protein acquired the correct disul-
fides as judged by its mobility upon SDS-PAGE under nonreducing conditions (Fig. 6, TX+G); under more oxidizing
conditions, in the absence of glutathione, the formation of the disulfides was incomplete and/or incorrect (Fig. 6, TX).

In agreement with previous studies on other proteins (Bulleid and Freedman, 1988; Huth et al., 1993; Creighton et al., 1993), we
found that the efficiency of the folding reaction was greatly
enhanced in the presence of protein disulfide isomerase (Fig. 6,
TX+G+PDI); in addition to mediating the formation of disulf-
fides, this protein may also act as a chaperone (Nowina and Lena-
zar, 1992). We also measured the formation of the thiol
ester of *α*1I3 in the cell-free systems. This analysis showed that
the in vitro folding was only partially complete (Fig. 7B). It is

2 M. Wassler, F. Esnard, and E. Fries, unpublished observations.
Braciak, T. A., Northemann, W., Hudson, G. O., Shiels, B. R., Gehring, M. R., and Anfinsen, C. B. (1973) higher folding rate, as judged by the rate of disulfide formation, was too oxidizing. This explanation is supported by the fact that the incubation medium was higher (−0.14 V) than what has been reported to be optimal for other in vitro folding systems (−0.18 to −0.31 V; Huth et al. 1993; Marquardt et al. 1993). It should also be noted that in the living cell, the thiol ester formation is less efficient than the formation of the disulfides (cf. Figs. 1 and 7A).

In summary, our results suggest that α1I3 directly after its synthesis has an only partially developed domain structure. It then undergoes a conformational change that appears to bring the domains together, whereby they acquire their final structure. Such an interactive folding process has previously been described for oligomeric proteins in which the conformation of the subunits has been found to change upon assembly (Huth et al., 1992; Hurtley and Helenius, 1989).

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