reduced state. Autoproteolytic cleavage and subsequent release of the fusion partner are initiated upon refolding following a monomolecular reaction with a reaction rate being only dependent on chaotrope concentration (Ueberbacher et al., Process Biochem. 44 (2009), 1217-1224).

A bottleneck step is the renaturation of the proteins. Elimination of hydrophobic intermolecular interaction during the first steps of refolding is crucial for successful renaturation at high protein concentrations and to prevent aggregation (Vallejo et al., Microb. Cell Fact. 3 (2004), 11). Several renaturation techniques are known. Due to its simplicity refolding by dilution is preferred to pressure treatment or chromatographic techniques, especially in production scale. Protein concentration, as well as chaotrop concentration are diminished in a single step preventing aggregation by intermolecular interactions. However, large volumes and low protein concentration burden downstream processing steps. (Jungbauer et al., J. Biotech. 128 (2007), 587-596).

Summary

In one aspect of the present disclosure, there is provided a method for producing a recombinant protein of interest, characterised in by the following steps:

(a) providing a fusion protein comprising an N^{pro} autoprotease moiety and a protein of interest moiety in inclusion bodies,
(b) solubilising the inclusion bodies,
(c) allowing the fusion protein to be cleaved by the N^{pro} autoprotease moiety under chaotropic conditions, wherein the chaotropic conditions correspond to a urea concentration of 2 to 5 M urea, wherein the recombinant protein of interest is cleaved from the fusion protein and wherein the recombinant protein of interest is not yet renatured or simultaneously renatured, and
(d) recovering the protein of interest, optionally including a renaturing step for the protein of interest, wherein the N^{pro} autoprotease moiety has a sequence of SEQ ID No. 1, SEQ ID No. 2 or a sequence with at least one amino acid exchange of SEQ ID No. 2, wherein the amino acid exchange is selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L.

In a further aspect of the present disclosure, there is provided a use of an N^{pro} autoprotease moiety having a sequence comprising at least one, preferably at least two, more preferably at least three, especially at least four amino acid exchanges of SEQ ID No. 2 in the method disclosed herein, wherein the amino acid exchange is selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K,

M131 to I, T135 to V, V141 to L, wherein amino acids 2 to 21 of SEQ ID NO: 2 are optionally deleted.

In another aspect of the present disclosure, there is provided an N^{pro} autoprotease moiety having a sequence comprising at least one, preferably at least two, more preferably at least three, even more preferably at least four amino acid exchanges of SEQ ID No. 2 selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L, especially wherein amino acids 2 to 21 of SEQ ID No. 2 are deleted.

In an additional aspect of the present disclosure, there is provided a use of an N^{pro} autoprotease moiety as defined herein or the N^{pro} autoprotease moiety according to SEQ ID No. 2 for the recombinant expression of proteins.

In a further aspect of the present disclosure, there is provided a fusion protein comprising a recombinant protein of interest and an N^{pro} autoprotease moiety as defined herein or the N^{pro} autoprotease moiety according to SEQ ID No. 2.

In an additional aspect of the present disclosure, there is provided a nucleic acid molecule comprising a sequence encoding an N^{pro} autoprotease moiety as defined herein or a fusion protein as defined herein.

In a further aspect of the present disclosure, there is provided an expression vector comprising a nucleic acid molecule as defined herein.

In an additional aspect of the present disclosure, there is provided an N^{pro} autoprotease moiety containing the sequence of SEQ ID No. 1.

Description

It is therefore an object of the present invention to provide an improvement in renaturation of inclusion bodies which must be renaturated, especially for autoproteolytic cleavage and preparation of recombinant proteins downstream of the process. Preferably, the invention should enable low volumes and high protein concentrations for obtaining the protein of interest and provide a method which is suitable to be established in

industrial production scale, specifically for proteins used in medicine. In addition, separation of the renaturation step of the autoprotease from that of the protein of interest would also be beneficial and would enable highly specific renaturation conditions.

Therefore, the present invention provides a method for producing a recombinant protein of interest, characterised in by the following steps:

(a) providing a fusion protein comprising an N^{pro} autoprotease moiety and a protein of interest moiety in inclusion bodies,

(b) solubilising the inclusion bodies,

(c) allowing the fusion protein to be cleaved by the N^{pro} autoprotease moiety under chaotropic conditions, wherein the recombinant protein of interest is cleaved from the fusion

Claims:

1. Method for producing a recombinant protein of interest, characterised in by the following steps:
(a) providing a fusion protein comprising an N^{pro} autoprotease moiety and a protein of interest moiety in inclusion bodies,
(b) solubilising the inclusion bodies,
(c) allowing the fusion protein to be cleaved by the N^{pro} autoprotease moiety under chaotropic conditions, wherein the chaotropic conditions correspond to a urea concentration of 2 to 5 M urea, wherein the recombinant protein of interest is cleaved from the fusion protein and wherein the recombinant protein of interest is not yet renatured or simultaneously renatured, and
(d) recovering the protein of interest, optionally including a renaturing step for the protein of interest, wherein the N^{pro} autoprotease moiety has a sequence of SEQ ID No. 1, SEQ ID No. 2 or a sequence with at least one amino acid exchange of SEQ ID No. 2, wherein the amino acid exchange is selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L.

2. Method according to claim 1, characterized in that the N^{pro} autoprotease moiety sequence comprises at least two, preferably at least three, especially at least four amino acid exchanges of sequence of SEQ ID NO. 2, wherein the amino acid exchange is selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L.

3. Method according to claim 1 or 2, characterized in that amino acids 2 to 21 of the N^{pro} autoprotease moiety of SEQ ID No. 2 are deleted.

4. Method according to any one of claims 1 to 3, characterized in that the inclusion bodies were generated in a recombinant production system, preferably in a prokaryotic host cell, especially in E. coli host cells.

5. Method according to any one of claims 1 or 4, characterized in that the conditions in step (b) correspond to a urea concentration of more than 5 M, preferably more than 6 M, especially more than 7.5 M.

6. Method according to any one of claims 1 to 5, characterized in that the N^{pro} autoprotease moiety has a cleavage rate at 2.5 M urea of at least 20%, preferably at least 30%, especially at least 40%.

7. Method according to any one of claims 1 to 6, characterized in that the N^{pro} autoprotease moiety has a sequence selected from the group consisting of SEQ ID nos. 1 or 2,

preferably wherein the interferon regulating factor 3 binding site is deleted.

8. Method according to any one of claims 1 to 7, characterized in that step (b) is performed in the presence of NaOH or KOH, preferably of more than 5 mM NaOH or KOH, preferably more than 25 mM NaOH or KOH, more preferred of more than 50 mM NaOH or KOH, especially more than 100mM NaOH or KOH.

9. Use of an N^{pro} autoprotease moiety having a sequence comprising at least one, preferably at least two, more preferably at least three, especially at least four amino acid exchanges of SEQ ID No. 2 in the method according to any one of claims 1 to 8, wherein the amino acid exchange is selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L, wherein amino acids 2 to 21 of SEQ ID NO: 2 are optionally deleted.

10. N^{pro} autoprotease moiety having a sequence comprising at least one, preferably at least two, more preferably at least three, even more preferably at least four amino acid exchanges of SEQ ID No. 2 selected from the group consisting of N28 to V, L or I; A30 to T; T39 to V or I; L81 to V, F82 to Y, V83 to I, K84 to E, P85 to L, P87 to A, V88 to I, Q91 to K; S94 to I, L or V; Q105 to L; P110 to V; N127 to K, M131 to I, T135 to V, V141 to L, especially wherein amino acids 2 to 21 of SEQ ID No. 2 are deleted.

11. Use of an N^{pro} autoprotease moiety according to claim 10 or the N^{pro} autoprotease moiety according to SEQ ID No. 2 for the recombinant expression of proteins.

12. Fusion protein comprising a recombinant protein of interest and an N^{pro} autoprotease moiety according to claim 10 or the N^{pro} autoprotease moiety according to SEQ ID No. 2.

13. Nucleic acid molecule comprising a sequence encoding an N^{pro} autoprotease moiety according to claim 10 or a fusion protein according to claim 12.

14. Expression vector comprising a nucleic acid molecule according to claim 13.

15. N^{pro} autoprotease moiety containing the sequence of SEQ ID No. 1.