Folding of a Mutant Maltose-binding Protein of Escherichia coli Which Forms Inclusion Bodies*

(Received for publication, September 20, 1995, and in revised form, January 16, 1996)

Jean-Michel Betton and Maurice Hofnung
From the Unité de Programmation Moléculaire et de Toxécologie Génétique CNRS-URA1444, Département des Biotechnologies, Institut Pasteur, 25, rue du Docteur Roux, 75015 Paris, France

The maltose-binding protein (MalE) of Escherichia coli is the periplasmic component of the transport system for malto-oligosaccharides. We have examined the characteristics of a MalE mutant of malE corresponding to the double substitution Gly32 → Asp/Ile33 → Pro, MalE31, previously obtained by random mutagenesis. In vivo, the MalE31 precursor is efficiently processed, but the mature protein forms inclusion bodies in the periplasm. Furthermore, the accumulation of insoluble MalE31 is independent of its cellular localization; MalE31 lacking its signal sequence forms inclusion bodies in the cytoplasm. The native MalE31 protein can be purified by affinity chromatography from inclusion bodies after denaturation by 8 M urea. The renatured protein exhibits full maltose binding affinity ($K_d = 9 \times 10^{-7}$ M), suggesting that its folded structure is similar to that of the wild-type protein. Unfolding/refolding experiments show that MalE31 is less stable ($-5.5$ kcal/mol) than the wild-type protein ($-9.5$ kcal/mol) and that folding intermediates have a high tendency to form aggregates. In conclusion, the observed phenotype of cells expressing malE31 can be explained by a defective folding pathway of the protein.

In Escherichia coli, the export of proteins to the periplasm follows the general secretion pathway (Ref. 1). Considerable genetic and biochemical information about the export machinery has accumulated (2), but little is known about the conformational state of the polypeptide chain, either during its translocation through the membrane or upon its release into the periplasm.

Maltose-binding protein (MalE or MBP), the MalE gene product, serves as the periplasmic receptor for the high affinity transport of maltose and maltodextrins (reviewed in Ref. 3). Because of its key role in maltose transport, correct export of MalE into the periplasm is essential for cells to utilize maltose as a carbon source. This feature facilitated the use of genetic selections for analyzing MalE export. Furthermore, the isolation of strains synthesizing MalE-LacZ hybrid proteins led to a novel genetic approach that identified several genes encoding the secretory machinery, the sec genes (4). As a protein model of translocation across the cytoplasmic membrane, MalE has also been extensively studied in the laboratories of the late P. Bassford and of L. Randall (5, 6). The MalE protein is synthesized in the cytoplasm as a precursor protein, pre-MalE, with an amino-terminal extension, the signal sequence. As the precursor enters the periplasm, the signal sequence is cleaved by signal peptidase. Translocation requires that the precursor exist in an export-competent conformation representing a partially unfolded state (7). Both the signal peptide (8) and the binding of SecB, the molecular chaperone involved in protein export (1), participate in the maintenance of this initial conformation. Although Randall and Hardy (7) showed that MalE folding and export are kinetically competing processes, we do not know the extent of secondary and tertiary structures of precursor proteins. Another important unsolved problem concerns the releasing and folding steps of the polypeptide chain in the periplasm. Do specific proteins or molecular chaperones catalyze or facilitate this step in the periplasm? Two integral membrane proteins, SecD and SecF, are required for efficient protein translocation (9). Interestingly, treatment of spheroplasts with anti-SecD antibodies decreased the processing of pre-MalE and increased the protease susceptibility of the mature species (10). These results would be consistent with a molecular chaperone function of SecD on the periplasmic face of the inner membrane (11).

Protein aggregates are formed in vivo as a result of: (i) mutations that affect the folding pathway; (ii) expression of heterologous proteins; (iii) exposure of the cell to certain environmental stresses. It is generally accepted that aggregation and folding are competing processes and that aggregation is favored at elevated protein concentrations. A model suggesting that a folding intermediate is responsible for inclusion body formation has been proposed by Mitraki and King (12). Such a kinetic competition between the folding pathway and an aggregation reaction has previously been recognized as a major determinant for apparent irreversibility in refolding studies in vitro (13). Since aggregation is a second (or higher) order process, it can be much faster than first-order folding and therefore, at high protein concentrations, aggregation dominates over folding and leads to the formation of insoluble protein (14). The observation that temperature-sensitive folding (tsf) mutations in the tailspike protein of phage P22 increased the fraction of newly synthesized tailspike chains forming aggregates demonstrated the importance of folding intermediates in the mechanism of inclusion body formation (15). The tsf mutations prevent formation of the native protein at the restrictive temperature, but once folded at a lower permissive temperature, the mutant tailspike proteins have stabilities similar to that of the native protein. Intragenic suppressors of these mutations inhibit aggregation of folding intermediates and increase the folding efficiency of tsf mutants (16). Mutations influencing inclusion body formation were also identified for human interleukin-1β, expressed in the cytoplasm of E. coli (17). The mutated residues were mainly located either in a β strand or in a flexible loop. However, there was no strong correlation between the thermodynamic stability of the mutant interleukin-1β proteins and their tendency to form inclusion bodies. Another example of studies of inclusion body formation in E. coli is provided by the enzyme β-lactamase (18). In the

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In this study, we characterized a MalE mutant of the maltose binding protein (MalE31), previously isolated by random linker insertion into the malE gene (20). Our in vivo analysis revealed that the mutant MalE31 polypeptide chain forms inclusion bodies after translocation and signal peptide processing. We further report the in vitro analysis of the stability and aggregation of this mutant protein compared with the wild-type protein. These data confirmed that the observed phenotype of cells expressing malE31 can be explained by a defective folding pathway of the protein.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—E. coli K12 strain pop6499, which is sensitive to MC1010 (F ra-araD199 thiA relA thi 1 malT1) and is a derivative of MC4100, was used as the host strain for plasmids encoding MalE proteins. The strain carries a non-polar deletion of the chromosomal malE gene (21) and harbors the malT1 allele which confers constitutive expression on the maltose operon (20). Therefore maltose was not added to the growth medium.

The expression of a plon: lacZ fusion was monitored in strain SR1364, which is a derivative of MC4100.

Plasmids pPD1 and pPD364 carrying the wild-type malE gene (21) and the mutant malE31 gene (20), both under the control of the mal promoter, were designated here as pCHM and pCHM31, respectively. Plasmid pMAL79, which carries a deletion of the ribosome binding site and the first 256 codons of the malE gene (22), was used as a negative control.

Oligonucleotide-directed Mutagenesis—The EcoRI-BstEII 1107I fragment carrying the malE31 gene was isolated from plasmid pHCM31 and inserted into the EcoRI-HindIII sites of M13mp18 RF DNA (23). Oligonucleotide-directed mutagenesis was performed with the Mutagen in vitro mutagenesis kit of Bio-Rad by using the malE31 gene as a single-stranded DNA template. The following oligonucleotides were used to direct the mutagenesis: DG (AspGly), 5'-CTTCCGGCTCCTGTAGTCGT-3'; ΔΔ-2-26 (deletion 2-26), 5'-TACCTCTTCGTATCTTATATC GG-3'.

The mutations were confirmed by diodeoxyxynucleotide sequencing. The EcoRI-BglII fragments were isolated from mutant M13mp18 RF DNAs and subcloned into plasmid pHCM to construct pHCM-G32D, pHCM-M133P, and pHCM3122-26.

Cell Fractionation—Suspensions of cultures were grown in LB medium supplemented with 0.1 mg/ml ampicillin at 30°C. After 3 h, cells were harvested by centrifugation and fractionated by spheroplast preparation. The cell pellets, normalized to the same absorbance reading at 600 nm (5 × 10^9 cells/ml), were suspended in 10 mM Tris-HCl (pH 7.5) containing 0.7 M sucrose and 1 mM phenylmethylsulfonyl fluoride. Lysosome (0.2 mg/ml) and EDTA (10 mM) were added, and the suspensions were incubated for 20 min at 4°C. Then, the samples were centrifuged for 5 min at 10,000 rpm in an Eppendorf microcentrifuge, and the supernatants (periplasmic fraction) were removed. The pellets were resuspended, washed, freeze-thawed twice, and centrifuged as described above. Supernatants were kept (cytoplasmic fraction) and the pellets resuspended in Tris-HCl buffer and washed (membrane fraction). The three fractions were mixed with an equal volume of 2% SDS sample buffer (0.1 M) and heated to 100°C for 3 min before being analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were stained with Coomassie Blue and scanned with a MasterScan I integrative densitometer (Scanalytics).

Radiolabeling and Immunoprecipitation—Cells were grown at 30°C in M6381 medium supplemented with 40 mg/ml amino acids minus methionine, containing 0.1 mg/ml ampicillin and 0.4% glycerol. During the mid-expansion phase of growth, cells were labeled with 60 mCi of [35S]methionine/ml for 15 s, then chased with 4 mg/ml nonradioactive methionine for 10, 30, and 60 s. In experiments in which export was blocked, sodium azide was added to a final concentration of 5 mM, 5 min before labeling. All the samples were precipitated with 2% cold trichloroacetic acid and solubilized in 50 mM Tris-HCl buffer (pH 8) containing 1% SDS, 1 mM EDTA, and 1% phenylmethylsulfonyl fluoride. MalE-wt and MalE31 were immunoprecipitated using rabbit anti-MalE serum as described previously by Itt et al. (24). Immunoprecipitates were analyzed by SDS-PAGE and autoradiography.

Protein Purification—The wild-type MalE protein was purified by osmotic shock procedure as described previously (20). The MalE31 protein was purified from inclusion bodies. Cultures were grown to late exponential phase at 30°C in LB broth (2 liters) with 0.1 mg/ml ampicillin. Cells were harvested and suspended in 50 ml of 25 mM Tris-HCl buffer (pH 7.5) (buffer A) containing 0.1 mg/ml DNase I and lysed in a French press at 12,000 p.s.i. The lysate was centrifuged at 14,000 × g for 15 min, and the pellet (inclusion bodies and membranes) was suspended in 20 ml of buffer A containing 2% Triton X-100. After centrifugation at 14,000 × g for 15 min, the pellet was solubilized by 8 M urea in buffer A and incubated 30 min at room temperature. Renaturation was achieved by dilution (20-fold) into buffer A at 4°C. The cloudy renatured mixture was centrifuged at 14,000 × g for 15 min, and the supernatant was loaded onto a cross-linked amyllose column at 4°C (25).

In order to take into account the effect of the solvent on the fluorescence signal, the following equation was used:

\[ \Delta G_{Gdm} = \Delta G_{Gdm} + \text{m} \times \text{eq} \]

where \( Y_a \) and \( Y_d \) are the signal of the native and denatured forms, respectively, at the same denaturant concentration; \( M_n \) and \( M_d \) are the solute effects on the native and denatured protein signal, respectively. Experimental data were fitted by using a simplex procedure based on the

1 S. Raina, unpublished results.
2 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; GdnHCl, guanidine hydrochloride.
3 J. M. Betton, unpublished result.
TABLE I

| Plasmid      | Phenotype        | Mal (min) | Maltose transport | Periplasmic yield |
|--------------|------------------|-----------|-------------------|------------------|
| pHCME        | 116 ± 4          | + +       | 5.5 ± 0.1         | 0.2 ± 0.1        |
| pHCM32       | 247 ± 8          | –         | 1.2 ± 0.1         | 23 ± 4           |
| pHCM32-133P  | 195 ± 7          | +/-       | 2.2 ± 0.1         | 6 ± 1            |
| pHCM32-G32D  | 121 ± 2          | + +       | 6.0 ± 0.5         | 22 ± 3           |

a Doubling time in liquid 63B maltose minimal medium at 37°C.
b Growth on MacConkey dextrin plates after overnight incubation at 37°C. Dark red colonies were scored + + and white colonies were scored --; colors showing decreased redness were scored + and +/-.
c The initial rates of maltose uptake were measured at 1.2 μM maltose.

The amount of MalE in soluble periplasmic fraction was determined by immunosassay and normalized to protein content of whole cells determined by the Bradford procedure using bovine serum albumin as a standard.

RESULTS

We previously described a set of MalE mutant proteins generated by random insertion of an oligonucleotide linker into the malE gene (20). Among this collection, six mutations prevented both growth on maltose as a carbon source and the periplasmic release of the corresponding proteins by cold osmotic shock (32). In all cases but one, a deletion of several amino acid residues from the mature region of the protein could explain their defective phenotypes. However, in one case, malE31, the linker insertion did not modify the length of the protein, but changed six nucleotides, resulting in Gly→Asp and Ile→Pro substitutions at position 32 and 33 of the mature sequence, respectively (20).

Properties of MalE31 and Its Derivatives—To identify the particular codon in malE31 responsible for the Mal− phenotype, derivatives carrying either the single mutation at position 32 (Mal-E-G32D) or at position 33 (Mal-E-I33P) were constructed. The biological properties of the corresponding mutants are indicated in Table I. Although bacteria carrying malE31 had altered growth properties, as indicated by their doubling time in maltose minimal medium or their phenotype on MacConkey dextrin plates (Table I), production of MalE31 did not affect growth in rich medium (data not shown). The single substitution G32D led to a mutant with biological properties similar to those of cell expressing wild-type MalE. In contrast, the Ile33→Pro substitution affected both growth and maltose transport, but the defects were less severe than in malE31. While the level of total expression in cells producing these various proteins was similar, as assessed on SDS-PAGE, the amount of MalE31 and its derivatives in the periplasmic fraction correlated with their ability to grow on maltose minimal medium (Table I). The cellular localization of the MalE31 protein and its derivatives was determined after spheroplast preparations (Fig. 1). The periplasmic and cytoplasmic localization of wild-type MalE expressed from pHCM31 or from pHCM31Δ2-26, coding for signal sequence deleted MalE protein, confirmed that the cell fractionation procedure was correct. However, the pHCM31-encoded MalE31 protein was entirely recovered in the subcellular membrane fraction. This result suggested that export of MalE31 protein was blocked somewhere in the membrane or, alternatively, that MalE31 formed inclusion bodies either in the cytoplasm from the precursor, before translocation, or in the periplasm from the mature species, after translocation. Interestingly, a large fraction of pHCM31Δ2-26-encoded MalE31 (73%) was also found in the membrane fraction. Close inspection of the gel revealed the presence of increased amounts of two proteins of about 60 and 70 kDa, in the cytoplasmic fraction of cells carrying pHCM31Δ2-26. These proteins correspond to the major heat-shock proteins GroEL and DnaK (see below). While the MalE-G32D protein was localized entirely in the periplasmic fraction (98%), most of MalE-I33P (63%) was in the membrane fraction. Although, some synergistic effect was observed with the double substitution, these data confirmed that the Ile33→Pro substitution is mainly responsible for the defective phenotype of cells expressing malE31.

MalE31 Forms Inclusion Bodies—In the experiment described above, MalE31 sediments with the membranes, irrespective of whether or not it was made as a signal peptidase-bearing precursor. To determine whether these proteins were in fact periplasmic or cytoplasmic, respectively, or were indeed membrane-associated, we performed two kinds of experiments.

First, the kinetics of signal sequence processing was determined using a pulse-chase analysis. Processing of the precursor, a late step in export, is a reliable indicator that the protein has, at least partially, crossed the inner membrane. Cells were pulse-labeled with [35S]methionine and then chased with nonradioactive methionine for different times. The labeled MalE proteins were analyzed by immunoprecipitation. Fig. 2 shows that the MalE31 precursor is processed very rapidly, and that an export defect cannot be observed at the earliest time point (10 s). In conclusion, there were no differences in the processing kinetics observed for pre-MalE31 species compared with those of wild-type pre-MalE. Furthermore, amino-terminal microsequence analysis of purified MalE31 protein (see below) confirmed that pre-MalE31 was processed at the normal cleavage site.

Second, transmission electron microscopic studies of cells harboring pHCM31 revealed the presence of electron dense material between the inner and outer membranes of the bacteria (Fig. 3A). Periplasmic inclusion bodies of MalE31 were small and frequently included more than one per cell. In contrast, cells producing the signal sequence deletion MalE31 protein (pHCME31Δ2-26) had rare inclusion bodies that were clearly localized within the cytoplasm and that had blurred boundaries (Fig. 3B). By analogy with other proteins which form inclusion bodies in E. coli (33), the amount of insoluble MalE31 was temperature-dependent, with higher growth temperatures promoting inclusion body formation (data not shown). These results indicated: (i) that the MalE31 precursor was correctly processed and thus that the mature protein is localized in the periplasm, (ii) that the malE31 mutation causes MalE aggregation whatever its cellular localization, and (iii) that inclusion body formation in the periplasm arose from the association of processed MalE31 protein.

Heat-shock Response Induced by MalE31 Production—From Fig. 1 it is evident that cytoplasmic accumulation of MalE31 (pHCME31Δ2-26) increased the synthesis of two proteins which correspond to the GroEL (60 kDa) and DnaK (70 kDa) proteins. In contrast, no such elevated level of heat-shock proteins was visible with periplasmic MalE31 aggregates (pHCME31). To determine quantitatively the extent of heat-shock

4 F. Trincard and J.-M. Betton, unpublished results.
gene induction caused by MalE31, we used a plon-lacZ promoter fusion construct transcribed by the sigma factor \( \sigma^{32} \), the rpoH gene product (strain SR1364). This strain was transformed with plasmids pHCMEM and its derivatives, grown at 30°C, and \( \beta \)-galactosidase activities were determined according to Miller (34). The same level of \( \beta \)-galactosidase induction was observed in all cases, except for pHCMEM D2–26 (Fig. 4). In this case, the synthesis of \( \beta \)-galactosidase directed from the lon promoter was 2-fold higher than for strains containing the other plasmids. This result confirmed that periplasmic inclusion bodies of MalE31 did not induce the \( \sigma^{32} \)-dependent heat-shock response.

In Vitro Folding and Aggregation of MalE31—The production of misfolded proteins that could form inclusion bodies is one of the main factors responsible for induction of heat-shock response in E. coli (35, 36). To explore this possibility, we studied the in vitro folding characteristics of purified MalE31. We purified soluble MalE31 protein to homogeneity from periplasmic inclusion bodies by a simple denaturation-renaturation step and affinity chromatography (Fig. 5). The maltose binding affinity for purified wild-type and MalE31 proteins were assayed by fluorescence quenching experiments. As indicated in Table II, the two proteins display the same maltose binding affinity (\( K_d = 9 \times 10^{-7} \) M).

To investigate the folding properties of MalE31, the GdnHCl-induced unfolding transitions of the wild-type and MalE31 proteins were determined using tryptophanyl fluorescence as a probe of the tertiary structure (Fig. 6). At the final protein concentration of 0.2 mM, both transitions were found to be reversible (see below) and symmetrical. The concentration of GdnHCl at the midpoint of the transition (\( C_m \)) for MalE-wt was 0.92 ± 0.02 M, whereas the midpoint occurred at 0.62 ± 0.02 M GdnHCl for MalE31 (Table I). This reflects the lower stability of the mutant MalE31; the \( \Delta G^{H_2O} \) value obtained by curve fitting using Equation 2 is only −5.5 kcal/mol, compared with −9.5 kcal/mol for MalE-wt (Table II).

Light scattering and apparent irreversibility were detected when the MalE31 concentration was increased in the unfolding experiments described above. To test the effects of protein concentration on renaturation, we performed a two-step dilution experiment (37). Starting from the native state, MalE31
was incubated in various GdnHCl concentrations for 6 h at 25 °C and then diluted into the renaturation buffer (Fig. 7). Two different protein concentrations in the first step were explored, and we used a quantitative immunoassay to monitor the recovery of native MalE31 because of its high sensitivity (31). The renaturation became partially irreversible when the protein had been exposed to concentrations of GdnHCl corresponding to the end of the unfolding transition zone. However, the aggregation of MalE31 folding intermediates is correlated with the formation of inclusion bodies.

**DISCUSSION**

In vivo and in vitro analyses of MalE31 folding revealed that the variant polypeptide chain had a high tendency to aggregate. The three-dimensional structure of MalE consists of two globular domains separated by a cleft in which maltose and maltdextrins bind (38). Each of the two domains is constructed from secondary structural elements belonging to both the amino- and carboxyl-terminal halves of the protein, thereby forming a central β-sheet framed by α-helices. The structural location of amino acid substitution corresponding to the malE31 mutation is shown in Fig. 8. Positions 32 and 33 are located in a turn connecting helix I to strand B forming the first ββ supersecondary structure of the amino-terminal domain. In other proteins, similar structural locations were also found for amino acid substitutions which promote aggregation (17, 31).
The altered turn could be the formation of a supersecondary structure localized in the amino-terminal domain. Intragenic suppressors of export-defective signal peptide, MalE31 refolds more slowly than MalE-wt in vitro. However, this slowest refolding step could not be explained by a slow isomerization step in the unfolded state of MalE31.5

Inclusion body formation and in vitro aggregation are similar phenomena, originating from the failure of folding intermediates to achieve final folding. The putative cellular pathway for export and folding of precursors is shown in Fig. 8. As the precursor protein elongates, it begins to acquire secondary structure. This newly synthesized precursor may face many competing pathways, including binding molecular chaperones, folding, degradation, aggregation, and export. All of these possible outcomes depend on the intrinsic folding properties of the precursor being synthesized (or its folding rate relative to the rate of other processes). In this schematic drawing, molecular chaperones play an essential role in determining the choice between folding and export, but they may also participate in proteolysis by maintaining proteins in an unfolded state accessible to proteases. MalE belongs to a subset of precursors that interact preferentially with SecB, which seems to be entirely dedicated to export (45). However, heat-shock proteins can substitute for SecB during protein export. Indeed, overproduction of DnaK and DnaJ improved the export of wild-type pre-MalE in a secB::Tn5 mutant (46). Although periplasmic molecular chaperones involved in general protein folding have not yet been identified, we assume that such molecules could exist in this compartment. Another class of periplasmic proteins is enzymes that can either directly catalyze disulfide bond formation or maintain an appropriate oxidized environment in the periplasm. The lack of Cys residues in MalE eliminates the possible complication of intra- and/or extramolecular disulfide bond formation.

Our results suggest that the step at which productive folding of MalE31 is blocked is likely to be a folding intermediate localized in the periplasm. These data do not fit the model of alternative pathways open to protein precursors within the cell, as defined by Randall and Hardy (48). Indeed, they proposed that the kinetic partitioning between folding and aggregation depends strongly on binding to SecB in the cytoplasm. Although direct experiments on the interactions between SecB and MalE31 have not been performed, we have independently confirmed by several approaches that the MalE31 precursor did not accumulate in the cytoplasm. This behavior would indicate that the altered turn of the MalE31 precursor affects neither its export-competent conformation nor its ability to bind SecB. Structurally, a nascent precursor in an export-competent conformation may be similar to misfolded proteins. Indeed, Wild et al. (49) showed that accumulation of secretory protein precursors in strains lacking the SecB chaperone generates a signal for induction of heat-shock proteins. Obviously, the absence of increased σ32 activity in bacteria producing pre-MalE31 eliminates this possibility. However, the formation of periplasmic inclusion bodies of MalE31 specifically triggers the σ32-dependent stress.6 This alternative heat-shock sigma factor is involved in response to stress occurring in extracyto-

---

5 V. Laurent and J.-M. Betton, unpublished results.
6 J.-M. Betton and D. Missiakas, unpublished results.
plasmic compartments (50). It is worth noting that the soluble fraction of MalE31 that is periplasmic (2%) is less than the soluble fraction of MalE31 that is cytoplasmic (27%). This observation suggests that in the latter case, the increased level of GroEL and DnaK, that results from induction of the heat-shock response by MalE31, can partially suppress the cytoplasmic aggregation of MalE31. It appears that under these experimental conditions, there are insufficient molecular chaperones in the periplasm to prevent or reduce aggregation of MalE31.

Finally, this work on the aggregation of MalE31 opens the way to the selection of mutants affected in protein folding and/or aggregation in the E. coli periplasm. Indeed, MalE31 carried by plasmid pHCE31 was not able to complement the deletion ΔmalE444 of strain pop6499. If general molecular chaperones exist in the periplasm of E. coli, they might modulate the aggregation reaction from the defective folding intermediate of MalE31 as they do in the cytoplasm. Thus, mutations that improve protein folding in the periplasm could be selected by their ability to promote growth on maltose.

Acknowledgments—We are greatly indebted to S. Raina for strain SR1364, F. Traincart for the gift of the monoclonal antibody 56.5, P. Gounon, and B. Chavniier-Jove for electron microscopy facilities. We thank T. Pugsley and E. Johnson for carefully reading the manuscript.

REFERENCES

1. Pugsley, A. T. (1993) Microbiol. Rev. 57, 50–108
2. Wickner, W., Driessen, A. J. M., and Hartl, F. U. (1991) Annu. Rev. Biochem. 60, 101–124
3. Shuman, H. A., and Panagiotidis, C. H. (1993) J. Biol. Chem. 268, 513–620
4. Schultz, P. J., and Beckwith, J. (1990) Annu. Rev. Genet. 24, 215–248
5. Bassford, P. J. (1990) J. Bacteriol. 169, 43–50
6. Randall, L. L., Hardy, S. J. S., and Thom, J. R. (1987) Annu. Rev. Microbiol. 41, 507–541
7. Randall, L. L., and Hardy, S. J. S. (1986) Cell 46, 921–928
8. Park, S., Liu, G., Topping, T. B., Cover, W. H., and Randall, L. L. (1988) Science 239, 1033–1035
9. Poglian, J. A., and Bedwith, J. (1994) EMBO J. 13, 554–561
10. Matsuyama, S. I, Fujita, Y., and Mizuchima, S. (1993) EMBO J. 12, 265–270
11. Wulffing, C., and Plückthun, A. (1994) Mol. Microbiol. 12, 685–692
12. Mitra, A., and King, J. (1989) Biotechnology 7, 690–697
13. Janiakc, R. (1987) Prog. Biophys. Mol. Biol. 49, 117–237
14. Kiefhaber, T., Rudolph, R., Kohler, H. H., and Buchner, J. (1991) Bio/Tech nology 9, 825–829
15. Haase-Pettingill, C. A., and King, J. (1988) J. Biol. Chem. 263, 4977–4983
16. Mitra, A., Fane, B., Haase-Pettingill, C., Sturtevant, J., and King, J. (1991) Biochim. Biophys. Acta 1054, 18053–18061
17. Chruny, B. A., Evans, J., Lillquist, J., Young, P., and Wetzel, R. (1993) J. Biol. Chem. 268, 18053–18061
18. Georgiou, G., Valax, P., Ostermeier, M., and Horowitz, P. (1994) Protein Sci. 3, 653–660
19. Bowden, G. A., and Georgiou, G. (1990) J. Biol. Chem. 265, 16760–16766
20. Duplay, P., Szmolec, S., Bedouelle, H., and Hofnung, M. (1987) J. Mol. Biol. 194, 669–673
21. Duplay, P., Bedouelle, H., Fowler, A., Zabin, I., Saurin, W., and Hofnung, M. (1984) J. Biol. Chem. 259, 10606–10613
22. Betton, J. M., and Hofnung, M. (1994) EMBO J. 13, 1226–1234
23. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Ito, K., Bassford, P. J., and Bedwith, J. (1991) Cell 24, 707–717
25. Ferenci, T., and Klotz, U. (1978) FEBS Lett. 94, 213–217
26. Szmolec, S., and Hofnung, M. (1975) J. Bacteriol. 124, 112–118
27. Miller, D. M., III, Olson, J. S., Pfugra, J. W., and Quiocio, F. A. (1983) J. Biol. Chem. 258, 13685–13672
28. Nosaki, Y. (1970) Methods Enzymol. 26, 43–50
29. Pace, C. N. (1986) Methods Enzymol. 131, 266–280
30. Press, W. H., Flannery, B. P., Teukolsky, S. A., and Vetterling, W. T. (1988) Numerical Recipes: The Art of Scientific Computing, Cambridge University Press, Cambridge
31. Martin, P., Guillet, J. G., Lederer, C., and Hofnung, M. (1992) Gene (Amst.) 113, 35–46
32. Duplay, P., and Hofnung, M. (1998) J. Bacteriol. 170, 4445–4450
33. Schein, C. H. (1991) Curr. Opin. Biotechnol. 2, 746–750
34. Miller, J. H. (1992) A Short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
35. Goff, S. A., and Goldberg, A. L. (1983) Cell 41, 578–595
36. Parsell, D. A., and Sauer, R. T. (1989) Genes & Dev. 3, 1226–1232
37. Mitra, A., Betton, J. M., Desmadir, M., and Yon, J. M. (1987) Eur. J. Biochem. 163, 29–34
38. Spurino, C. J., Lu, G. Y., and Quiocio, F. A. (1991) J. Biol. Chem. 266, 5202–5219
39. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
40. Knappik, A., and Plückthun, A. (1995) Protein Eng. 8, 3–9
41. Ufer, R., and Kirschner, K. (1992) Protein Sci. 1, 31–45
42. Schimmel, P. R., and Flory, P. J. (1968) J. Biol. Chem. 243, 4977–4983
43. Chun, S. Y., Strobel, S., Bassford, P. J., and Randell, L. L. (1993) J. Biol. Chem. 268, 20855–20862
44. Liu, G., Topping, T. B., Cover, W. H., and Randell, L. L. (1991) J. Biol. Chem. 266, 5202–5219
45. Wild, J., Walter, W. A., Gross, C. A., and Altman, E. (1993) J. Biol. Chem. 268, 1165–1172
46. Deleted in proof
47. Chun, S. Y., Strobel, S., Bassford, P. J., and Randell, L. L. (1993) J. Biol. Chem. 268, 1165–1172
48. Wild, J., Altman, E., Yura, T., and Gross, C. A. (1992) Genes & Dev. 6, 2165–2172
49. Deleted in proof
50. Mestas, J., Rouviere, P., Erickson, J. W., Donohue, T. J., and Gross, C. A. (1993) Genes & Dev. 7, 2618–2628

Fig. 9. Model for protein export and folding in E. coli. This schematic representation illustrates the present discussion and tries to emphasize the different kinetic partitioning between folding, binding to chaperone, aggregation, degradation, and export. The outcome of the pathway depends on the folding parameters of the protein being exported.
Folding of a Mutant Maltose-binding Protein of Escherichia coli Which Forms Inclusion Bodies
Jean-Michel Betton and Maurice Hofnung

J. Biol. Chem. 1996, 271:8046-8052.
doi: 10.1074/jbc.271.14.8046

Access the most updated version of this article at http://www.jbc.org/content/271/14/8046

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 18 of which can be accessed free at http://www.jbc.org/content/271/14/8046.full.html#ref-list-1