Osmoprotectants exogenously supplied to a hyperosmotic culture medium are efficiently imported and amassed by stressed cells of *Escherichia coli*. In addition to their evident role in the recovery and maintenance of osmotic balance, these solutes should play an important role on the behavior of cellular macromolecules, for example in the process of protein folding. Using a random chemical mutagenesis approach, a conditional lysine auxotrophic mutant was obtained. The growth of this mutant was restored by addition of either lysine or osmoprotectants including glycine betaine (GB) in the minimal medium. The growth rate increased proportionally with the augmentation of the intracellular GB concentration. The mutation was located in the *lysA* gene and resulted in the substitution of the Ser at position 384 by Phe of the diaminopimelate decarboxylase (DAPDC), which catalyzes the conversion of meso-diaminopimelate to L-lysine. We purified both the wild type DAPDC and the mutated DAPDC-sf and demonstrated that GB was capable of activating DAPDC-sf in vitro, thus confirming the *in vivo* results. Most importantly, we showed that the activation was correlated with a conformational change of DAPDC-sf. Taken together, these results show, for the first time, that GB may actively assist *in vivo* protein folding in a chaperone-like manner.

Water availability is primordial for life of all organisms. Bacteria submitted to a severe hyperosmotic stress instantaneously lose a large amount of their intracellular water to balance the osmotic strength between intracellular and extracellular spaces. The subsequent decrease of cellular water activity together with the loss of cell turgor lead to lessen the bacterial cell expansion rate (1). Surviving such injuring conditions implies the reversion of water flux across the cell membrane; this can be achieved by amassing highly soluble compounds termed osmolytes (2, 3). Thus, *Escherichia coli* cells rapidly take up high amounts of potassium ions (4, 5) and subsequently increase their glutamate content to balance electrostatic charges. To avoid the perturbing effect of elevated ionic strength, K⁺-glutamate can be progressively replaced by organic osmolytes that behave neutral at physiological pH (6). Such compounds, termed compatible solutes (7), may be endogenously synthesized or imported from the surrounding medium (3, 8). Imported compatible solutes generally confer a high degree of osmotic tolerance to injured cells. Among these so-called osmoprotectants, glycine betaine (GB) is by far the most effective and the most commonly assayed for hyperosmotic purposes.

In addition to the obvious predominant role they play in cellular osmotic adjustment, internalized and accumulated osmoprotectants should directly participate in other intracellular processes. Protective as well as stabilizing effects of betaine and other solutes on proteins denaturation because of increased salinity or temperature have been reported (9–12). It is tempting to extrapolate these results *in vitro*; however, bacteria submitted to elevated temperature do not accumulate osmoprotectants. Similarly bacteria growing in high salinity media and in the presence of low amounts of osmoprotectant accumulate the latter at the expense of salts. Consequently *in vitro* data concerning the beneficial effect of molar range of osmoprotectants on the protection of enzymes against deleterious effects of salts and temperature could not be extrapolated *in vivo*. Furthermore we can question whether bacterial osmoprotectants are neutral, *in vivo*, as commonly admitted (1), or whether they interact with enzymes, inducing thereby structural and functional modifications. Because *in situ* monitoring of enzyme activity is rather difficult, this aspect has been scarcely studied. It was recently shown that N-trimethylamino oxide induces *in vitro* refolding of misfolded proteins (13, 14). It is therefore tempting to postulate that such effects may also occur *in vivo* with osmoprotectants; hence, mutants unable to grow unless an osmoprotectant is accumulated within the cell could be identified and used to develop an osmoprotectant sensitive enzymatic probe.

In this report we describe the development of a lysA mutant of *E. coli*, which grows only when glycine betaine is intracellularly accumulated. An enzymatic probe derived from this mutant was constructed, allowing us to ascertain that glycine betaine induces, both *in vitro* and *in vivo*, the folding of the modified enzyme. Thus glycine betaine is able to induce the transition from an inactive to an active conformation of the enzyme, thereby sharing properties somehow similar to those assigned to chaperones.
**TABLE I**

| Strains/plasmids | Relevant genotype or characteristics | References |
|------------------|--------------------------------------|------------|
| BL21 (DE3)       | E. coli B’:: dcm ompT hsdS galL (DE3), T7 polymerase gene under the lacUV5 promoter | Ref. 43 |
| K10              | His PO2A rel-1 tonA22 27R²           | R. C. Valentine |
| JM1637           | ΔlysA, lyS)                         | Ref. 29 |
| N27              | As K10 but lysA27                   | This study |
| pUN121           | AmpR, TcR, ClI                      | Ref. 44 |
| pLA17            | AmpR, lyS                          | Ref. 17 |
| pLA22            | AmpR, ClI                          | Ref. 5 |
| pET22           | AmpR, lyS                          | Novagen |
| pBluescript SK   | AmpR, lacZa                        | Stratagene |
| pK10             | pBluescript SK, lyS                 | This study |
| pN27             | pBluescript SK, lyS27               | This study |
| pK10-H6          | Derivative of pK10, lyS             | His6a |
| pN27-H6          | Derivative of pN27, lysA27          | His6a |
| pET-LA           | pET22b + lysA, His6                 | This study |
| pET-LA27         | pET22b + lysA27, His6              | This study |

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Growth Conditions**—The E. coli strains and plasmids used in this study are listed in Table I. Bacterial cells were grown aerobically in LB medium or in M63 minimal medium with glucose or lactose as carbon sources at 37 °C (15).

**Cloning and Nucleotide Sequence of lysA27—**All manipulations with recombinant DNA were carried out according to standard procedures (16) and the specifications of manufacturers. Chromosomal DNA of strains K10 and N27 was digested with HindIII and ligated with HindIII-cleaved pUN21. The ligation mixture was transformed into JM1637 (ΔlysA, lyS). Cells containing a plasmid bearing the wild type lysA gene were selected on M63 minimal medium with ampicillin and tetracycline. Transformants containing a plasmid with the lysA27 allele were selected on the same plates supplemented with 0.5 mM NaCl and 1 mM GB. All transformants contained plasmids carrying an identical 6.7-kilobase pair HindIII insert that covers the lysA region of E. coli chromosome (17) (see Fig. 1). The lysA27 gene was isolated by subcloning a 2.3-kilobase pair Hpal-HindIII fragment on pBluescript SK plasmid yielding pET22 (see Fig. 1). This plasmid was able to complement JM1637 on M63–0.5 mM NaCl–1 mM GB medium but not on M63 basal medium. The same procedure allowed the recovery of the wild type lysA gene yielding pK10 (see Fig. 1); this plasmid was able to complement JM1637 defect in M63 minimal medium. The nucleotide sequence of pK10 and pN27 inserts was determined by the dideoxy chain termination method (18).

**Construction of lysA Tagged Genes—**The 5’ part of lysA gene was amplified by polymerase chain reaction (PCR) using pK10 as a template and using primers LysNdeI (5’-CCCCCATATGGCACCATTCACT-3’) and LysEco5 (5’-CCGCTCTTCCACCGTTTG-3’). The resulting PCR product possession an engineered NdeI site at the ATG start codon and the natural EcoRI site within lysA, was digested with NdeI and EcoRI and inserted between the corresponding sites of pET22b + yielding pET-LA5. This places the 5’ part of lysA gene, with its authentic ATG start codon, under the optimized transcriptional and translational signals of the vector. The 3’ end of lysA gene carrying or not the mutation was amplified by PCR of pK10 or pN27 with the primers LysEco5 (5’-GGCATATTGTCTCTGCG-3’) and LysXho (5’-GCCCTGACGACATTC-GCA-3’). The resulting PCR products possessed the natural EcoRI site of lysA and an engineered XhoI site just before the stop codon. The amplified DNA, cleaved by EcoRI and XhoI, was inserted between the EcoRI-XhoI sites of pET-LA5, giving pET-LA (carrying the lysA) or pET-LA27 (carrying lysA 27) (see Fig. 1). These vectors contained lysA or lysA27 fused to six contiguous histidine codon at the 3’ extremity; they are expressed from the T7 Φ10 promoter in strain BL21. The nucleotide sequence of pET-LA and pET-LA27 was confirmed. The half-end of tagged genes were transferred from pET-LA to K10 yielding pK10-H6 and from pET-LA27 to pN27 creating pN27-H6 (Fig. 1). The tagged genes are transcribed under the control of the lac promoter on these plasmids.

**Diaminopimelic Decarboxylase Overproduction, Purification, and Activity**—The BL21(DE3)/pET-LA or pET-LA27 strains were grown in 900 ml of LB medium supplemented with ampicillin (50 μg/liter). When the Λlys of the culture reached 0.6, the synthesis of T7 RNA polymerase was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside (final concentration, 1 mM) and cells were grown for an additional 2 h at 37 °C. Proteins extractions and purification steps were performed at 4 °C using nickel-nitritrotriacetic acid-garosul column according to manufacturer recommendations (Qiagen). Both wild type and mutant diamino- pimelic decarboxylases were eluted at 150 mM imidazol. The proteins obtained contain only the diaminopimelic decarboxylase when analyzed on SDS-polyacrylamide gel electrophoresis that was performed according to Laemmli (19). Protein concentration was determined according to Bradford (20). Diaminopimelic decarboxylase assay was performed as described (21).

**Size Exclusion Chromatography—**Size exclusion chromatography was achieved by using two Superose 6 columns in series according to the manufacturer’s instruction (Amersham Pharmacia Biotech). Columns were equilibrated with 40 mM Tris-HCl (pH 7.6), 200 mM NaCl-1 mM benzamid and calibrated with α-amylose (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydride (29 kDa), and cytochrome c (12.5 kDa). 100 μl of purified protein (about 2 mg of protein) was applied onto the columns and were eluted at 0.2 ml/min with the same buffer used for column equilibration. Fractions were collected, separated on SDS-polyacrylamide gel electrophoresis, and analyzed by Coomassie Blue staining.

**Glycine Betaine Accumulation and Diaminopimelate Uptake—**Glycine betaine transport experiments were performed on purified wild type diaminopimelate decarboxylase (DAPDC) and the mutated enzyme (DAPDC-fs) were recorded with an SLM-Aminco 8100 spectrophotometer operating in the ratio mode. Excitation and emission slits were set to 4 nm. Emission spectra were collected by using an excitation wavelength of 295 nm. All emission data were corrected for instrumentation by using correction factors provided by the supplier, and the buffer contribution was removed by the proper emission subtraction.

**Tryptophan Fluorescence Quenching Measurements—**Tryptophan fluorescence quenching measurements were performed by using acrylamide, a collisional quencher having an efficiency γ equal to unity (24). As the DAPDC protein contains four tryptophanyl residues (positions 29, 140, 167, and 248), the Lehrer plot (25) was used to also determine the tryptophan-accessible fraction to acrylamide. Data treatment was essentially performed as described (26).

**Dansyl Fluorescence Anisotropy—**The steady-state fluorescence anisotropy of DAPDC labeled with the fluorescence probe dansyl was investigated as described (27).

**Infrared Spectroscopy—**Secondary structure analysis of deuterated DAPDC and DAPDC-fs was carried out by Fourier transform infrared spectroscopy of the amide I’ band. IR spectra were collected by using a Magna 460 from Nicolet equipped with a deuterated triglycine sulfate detector. The samples were placed in a CaF2 cell with a Teflon spacer of 0.05 mm. Band component analysis was achieved by firstly estimating the number and position of elementary components from the second derivative peaks in the 1600–1700 cm⁻¹ domain (28). Secondly, these parameters were introduced as first guess for band curve fitting by nonlinear regression analysis.

**RESULTS**

**Isolation of Conditional Auxotrophic Mutants—**A 1-methyl-3-nitro-1-nitrosoguanidine mutagenesis was undertaken on E. coli K10. Conditional auxotrophic mutants able to grow only in the presence of 0.5 mM NaCl and 1 mM GB were isolated. Among them, a stable lysine conditional auxotroph (N27) required lysine for its growth in M63 medium. Alternatively, the growth of N27 was restored by the addition of 1 mM GB in M63 medium containing 0.5 mM NaCl, 0.5 mM KCl, or 0.9 mM sucrose; these three
media generate the same osmotic strength of 1.2 osmol/kg H₂O. However, in the presence of the free permeant glycerol (0.9 M), GB was unable to promote growth. Thus, growth restoration resulting from GB addition is linked to osmolarity rather than to ionic strength or water activity. When 1 mM of the osmoprotectants ectoine, proline, dimethylsulfoniopropionate, or dimethylsulfoniopropionate were added to 0.5 M NaCl M63 medium, the growth rate of N27 was similar to that observed in the presence of 1 mM GB. Hence the reversal of N27 auxotrophy is not GB specific but seems to be dependent on the presence of any osmoprotectant.

Identification of the Mutation—Addition of diaminopimelate, a biosynthetic precursor of bacterial lysine, to the medium was unable to promote the growth of N27, although it was actively taken up by the cells. Thus N27 seems affected in the last step of lysine biosynthesis, i.e. in DAPDC activity. Indeed, the enzyme assay confirmed that DAPDC activity was absent from the crude extract of N27. The phage P1-mediated transduction experiment revealed 20% cotransduction linkage of the mutation with argA, which suggests that the mutation was located in the region of lysA-lysR (29). Further complementation experiments of N27 by pLA17 (lysA−) or pLA40 (lysR−) revealed that N27 has a mutation in lysA gene. The corresponding allele was named lysA27. The wild type lysA and the mutated lysA27 genes were cloned and sequenced. The comparison of the two sequences allowed identification of a single mutation corresponding to a C-T transversion in lysA sequence, which induced the substitution of Ser384 by Phe at the protein level. This mutated enzyme was designated as DAPDC-sf.

Growth of lysA27 Strain Is Correlated to Intracellular GB Level—To study the dependence of lysA27 mutant upon the intracellular GB level, the growth of the strains K10 and N27 was followed in M63 medium of increasing salt content added or not with 1 mM GB (Fig. 2). In the absence of GB, the growth rate of K10 slowed down when NaCl concentration was increased and was abolished over 0.5 M NaCl. The addition of 1 mM GB to the hyposmotic medium allowed improvement of salt tolerance of bacterial cells. The intracellular GB concentration augmented in parallel to the NaCl content in growth medium. However, despite GB accumulation, the growth rate of K10 decreased when the amount of salt was raised (Fig. 2).

The mutant N27 was unable to grow in the medium deprived of GB and supplemented or not with NaCl. However, in the presence of GB, the growth rate of N27 augmented proportionally with increasing concentration of NaCl that stimulates the uptake and intracellular accumulation of GB. Therefore, the growth rate of N27 was in direct proportion to intracellular GB content and became identical to that of K10 at concentrations of NaCl higher than 0.5 M (Fig. 2).

Purification of the Mutated and the Wild Type DAPDC and in Vitro Activation of the Mutated DAPDC—The above mentioned results indicate an in vivo activation of the mutated DAPDC. To assess the activation mechanism, the wild type and mutated DAPDC were purified. To facilitate the purification, a His₆ codon tag was introduced at the 3’ extremity of the lysA and lysA27 genes on the plasmids pK10-H6 and pN27-H6, respectively. Growth parameters of the strains JM1637 (ΔlysA-lysR) containing pK10-H6 or pN27-H6 were similar to those observed with K10 and N27 (Fig. 2) or with JM1673 carrying pK10 or pN27 (data not shown), respectively. Therefore, the addition of the His₆ tag at the C-terminal of wild type DAPDC or mutated DAPDC-sf did not affect the enzymatic properties. The cellular
content of the two His tag enzymes was quantified after partial purification using Ni²⁺-NTA agarose columns. The values obtained were identical for the two strains in all growth conditions. These results clearly showed that the restoration of the growth of N27 does not result from an increase of enzyme synthesis but rather from the activation, by GB, of the enzymatic activity of DAPDC-sf.

DAPDC and DAPDC-sf proteins were purified to homogeneity. Analysis of these proteins by size exclusion chromatography revealed that both DAPDC and DAPDC-sf were located in fractions corresponding to the molecular mass marker of 50 kDa. Because the molecular mass of DAPDC deduced from its sequence is 46.177 kDa, the DAPDC must function as a monomer. This result also rules out the possibility that the loss of enzyme activity of DAPDC-sf would result from a failure of oligomerization.

The activity of purified DAPDC and DAPDC-sf were measured in vitro in presence or absence of GB (Fig. 3). DAPDC activity was poorly affected by increasing GB concentration. Its $K_m$ increased from 2 to 3.5 mM, and $V_{max}$ increased from 48 to 66 μmol of lysine formed/min/mg of protein in the presence of 1 mM GB compared with that from medium deprived of betaine. In contrast, GB exhibited a strong stimulating effect on DAPDC-sf activity. This mutated protein was almost completely inactive in the absence of GB, and its activity increased proportionally to GB concentration in the assay medium (Fig. 3). In the absence of GB, the $V_{max}$ value of DAPDC-sf was less than 0.01 μmol of lysine formed/min/mg of protein, and its $K_m$ could not be calculated. In the presence of 1 mM GB the $V_{max}$ increased to 4.4 μmol of lysine formed/min/mg of protein, and its $K_m$ value was 62 mM. Although these values are much different from those of the wild type enzyme, the 400-fold increase of $V_{max}$ of the mutated DAPDC-sf is still significant. Therefore, GB is capable of activating the mutated DAPDC-sf, a result consistent with its capacity to restore the prototrophic phenotype of N27. The partial activation might result from the lack of pyridoxal phosphate cofactor in the mutated DAPDC-sf as observed by UV-visible absorption spectra analysis. Our attempt to improve the in vitro activation by adding crude extracts in the reaction solution was not successful. Furthermore, GB-dependent activation curves were the same for DAPDC-sf present in the crude extracts of JM1637/pN27 or JM1637/pN27-H6 (data not shown). Therefore, the addition of the His tag did not affect the activation of DAPDC-sf by GB.

**Structural and Dynamic Defects of DAPDC-sf**—To understand the mechanism of GB-assisted in vivo activation of the mutated DAPDC-sf, the relationship between the structure and activity of DAPDC-sf was investigated in vitro by various approaches. UV-visible absorption spectra of both DAPDC and DAPDC-sf molecules (data not shown) indicated that the aromatic absorption band of DAPDC-sf remained roughly unaltered compared with the one of DAPDC. Upon selective excitation of tryptophan residues (Fig. 4), the fluorescence emission spectrum of DAPDC-sf presented a total fluorescence intensity about 2-fold higher than that of DAPDC, whereas a maximum emission wavelength at 328 nm was observed for both enzymes. Such a $\lambda_{max}$ of fluorescence emission indicates either that the Trp residues are deeply buried into the protein bulk (30) or that the indol rings interact with hydrophobic residues. The large enhancement of Trp fluorescence in DAPDC-sf may not arise from an altered exposure of Trp residues to the solvent. To assess this point, the secondary structures of DAPDC and DAPDC-sf enzymes were investigated through infrared spectroscopy in the amide I band domain. DAPDC spectrum (Fig. 5) is composed of two major peaks located at 1642 and 1652 cm⁻¹, which can be assigned to random coil and $\alpha$-helix, respectively. The $\alpha$-helix component features 76% of the total amide I band area. The DAPDC-sf IR spectrum (Fig. 5) is dominated by a large random coil component (1641 cm⁻¹), which features as much as 84% of the total band area. A weaker band, absent in DAPDC spectrum, is located at 1671 cm⁻¹ and may feature $\beta$-sheets or, more likely, turns and represents 16% of the band area. It should be emphasized that, opposite to DAPDC, no helical structure is apparent in DAPDC-sf. Hence, as a result of the mutation, the DAPDC secondary structure shifts from a mainly helical protein to a rather unordered protein displaying a low $\beta$-sheet or turns content.

To probe whether these structural alterations have repercussions on protein fluctuations, molecular dynamics was probed by collisional quenching experiments in the presence of increasing acrylamide concentrations. Fig. 6 displays the Lehrer plots of DAPDC and DAPDC-sf. Linear regression of the data yields similar accessible fractions of 1 for both DAPDC and DAPDC-sf molecules. Assuming that each Trp residue has the same quantum yield (which is likely because the Stern-Volmer plot yields a straight line), this finding allows to assess that all...
of the four Trp residues are accessible to acrylamide in both the wild type and mutated enzymes. However, the different slopes indicate that the quenching constant $K$ is largely altered as a consequence of the mutation. As estimated from data, the DAPDC exhibits a quenching constant $K$ of 3.51 M$^{-1}$ versus 2.08 M$^{-1}$ for the DAPDC-sf. This may indicate that the protein fluctuations in the Trp environments are more constrained in DAPDC-sf compared with DAPDC. As a whole, this in vitro study clearly states that the loss of biological activity arises from large structural and dynamic alterations of the enzyme, which indicates that the mutated enzyme is not properly folded.

To study the relation between activation and conformational change, the effect of 1 M GB on the DAPDC-sf fluorescence emission spectrum was investigated (Fig. 4). The presence of GB drastically affects the Trp fluorescence of the DAPDC-sf in the sense that it restores the emission spectrum observed with the DAPDC. Indeed 1 M GB induces a 2-fold decrease in total fluorescence intensity without any Stokes shift variation. Thus, the fluorescence emission properties of Trp in DAPDC and DAPDC-sf with 1 M GB are very similar. A kinetic study of this transconformation has been performed by monitoring both the GB-induced Trp fluorescence decrease at 340 nm (Fig. 7) and the global protein tumbling (data not shown) as a function of time. Data fitting of the Trp emission decay leads to the conclusion that the transconformation is best described by a single exponential; this implies that the protein folding is ruled by a two-state equilibrium with relaxation time $\tau = 9.8 \pm 0.7$ min (95% confidence interval). Dansyl labeling experiments, which permit probing the global enzyme tumbling through fluorescence anisotropy, further supports the proposed mechanism of action of GB on the enzyme folding. The observed anisotropies were 0.206 ± 0.007 ($n = 15$) for DAPDC versus 0.1721 ± 0.001 ($n = 15$) and 0.1474 ± 0.0017 ($n = 15$) for the DAPDC-sf in the absence and in the presence of 0.5 M GB, respectively. Clearly, these data indicate that the molecular shape of the wild type and mutated proteins is different (they exhibit rotational correlation time $\tau_c$ of 16.5 and 10.9 ns, respectively) and that GB addition results in a further decrease of $\tau_c$ from 10.9 to 8.1 ns, which fits well with a folding process. Because dansyl anisotropy is only sensitive to the global protein tumbling and, hence, probe global events, attempts were made to correlate the dansyl anisotropy decay to that of the Trp fluorescence emission previously observed. Fitting of the data, once again, yields a single exponential with a relaxation time $\tau = 10.1 \pm 1.9$ min (95% confidence interval). This decay matches the one observed when probing Trp emission intensity. In conclusion, it can be assessed that these independent experiments actually reflect the same phenomenon, i.e., the DAPDC-sf folding driven by GB. However, it should be outlined that the rather low dansyl anisotropy value of DAPDC-sf observed in the presence of GB
as compared with DAPDC indicates that although the Trp environments appear fully recovered, the molecular shape (and hence the protein global conformation) of the mutated protein still differs from that of the wild type; this finding well agrees the partial restoration of the activity observed upon GB addition.

**DISCUSSION**

Although in vitro studies showed for a long time that osmoprotectants could influence protein stability and folding, such an effect was never ascertained in vivo mainly because of the difficulty of monitoring such processes. To overcome the difficulty, we have developed a genetic approach that allowed us to obtain osmoprotectants-dependent conditional auxotrophic mutants. The DAPDC-sf of the mutant N27 contains a Ser–Phe substitution at the amino acid 384, which results in total loss of the enzyme activity and explains the lack of growth of this mutant in minimal medium in the absence of lysine. Addition of osmoprotectants restores the growth of this mutant, indicating the recovery of the DAPDC activity. We clearly showed that the recovery of the activity of the mutated DAPDC is proportional to the intracellular availability of glycine betaine. These results suggest an in vivo osmoprotectant-assisted refolding of the DAPDC-sf. This hypothesis was further confirmed by in vitro studies. GB significantly restores the activity of the purified DAPDC-sf, which is in parallel with refolding of the mutated enzyme toward the conformation of the wild-type enzyme as monitored by various approaches.

The intrinsic Trp fluorescence emission spectra show that there are no major changes in the solvent exposure of DAPDC-sf tryptophan residues. These Trp residues may be involved in hydrophobic nucleation domains, which are not exposed in DAPDC-sf. This protein proved to be stable and not submitted to hydrophobic aggregation by providing a hydrophilic environment (34–36), whereas the former forces the protein to decrease its exposure to solvent by drastically decreasing the water activity (33). In vivo, chaperones and GB could exert a synergistic protection; nevertheless we have shown that N27 (lysA27) growth strictly depends on GB (or other osmoprotectants) accumulation. So GB seems essential for the folding of DAPDC-sf, whereas chaperones were unable to suppress the deficiency of folding. Inefficiency of chaperones could be explained by two hypotheses: (i) Chaperones do not interact with DAPDC-sf. This protein proved to be stable and not submitted to proteolysis, suggesting that it is not recognized as aberrant by the bacterial proteases. Chaperones recognize the extended hydrophobic chains present in loosely folded proteins (37, 38). Our results showed that such hydrophobic domains are not exposed in DAPDC-sf. By contrast, osmoprotectant efficiency does not rely on the recognition of a particular protein domain. (ii) Chaperones are present in limiting amounts in osmotically stressed cells. Chaperones are dramatically overproduced in response to a temperature upshift (36) and, to a lesser extent, to other stresses such as osmotic constraint in animal cells (39, 40) and bacteria (41). Osmoprotectants are able to revert only the osmotic induction of Hsp (40), probably because they are only accumulated under osmotic stress; their accumulation in the absence of an osmotic constraint would be pernicious for the cell. So, in a case where osmoprotectants would accumulate in the cell, chaperones are produced at a low amount, and the protection of destabilized proteins is mainly ensured by osmoprotectants.

Why did two processes of protein protection arise during evolution? Growth in high osmolarity media increases the amount of misfolded proteins. A large population of proteins interacting with chaperonins for extended periods of time may be highly detrimental to the organism. The capacity of glycine betaine to take part of the refolding task allows chaperones to assist in protein synthesis and export, thus maintaining an optimal growth rate. This phenomenon could exert a strong influence on cellular metabolism, not only by maintaining normal enzymatic activities but also by participating in the global regulation of cellular metabolism when it concerns regulatory metabolic steps and regulatory proteins. This latter point is of particular interest because the search for a regulatory protein specifically involved in osmoadaptation in E. coli remains unsuccessful (1). In bacteria, osmoprotectants could participate in the global regulatory processes by affecting the folding of central regulatory proteins as RpoS (42) allowing a global regulation of metabolism without the involvement of a specific regulator.

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