Osmotic Shock Induced Protein Destabilization in Living Cells and Its Reversal by Glycine Betaine

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Abstract

Many organisms can adapt to changes in the solute content of their surroundings (i.e., the osmolarity). Hyperosmotic shock causes water efflux and a concomitant reduction in cell volume, which is countered by the accumulation of osmolytes. This volume reduction increases the crowded nature of the cytoplasm, which is expected to affect protein stability. In contrast to traditional theory, which predicts that more crowded conditions can only increase protein stability, recent work shows that crowding can destabilize proteins through transient attractive interactions. Here, we quantify protein stability in living *Escherichia coli* cells before and after hyperosmotic shock in the presence and absence of the osmolyte, glycine betaine. The 7-kDa N-terminal src-homology 3 domain of *Drosophila* signal transduction protein drk is used as the test protein. We find that hyperosmotic shock decreases SH3 stability in cells, consistent with the idea that transient attractive interactions are important under physiologically relevant crowded conditions. The subsequent uptake of glycine betaine returns SH3 to the stability observed without osmotic shock. These results highlight the effect of transient attractive interactions on protein stability in cells and provide a new explanation for why stressed cells accumulate osmolytes.

Introduction

Life on earth has adapted to a vast range of ionic environments, from pure water to 6 M NaCl, by regulating the concentration of solutes called osmolytes [1,2]. A large increase in extracellular salt concentration causes water efflux, which reduces the cell volume and increases the concentration of macromolecules within the already crowded cytoplasm [3,4]. This hyperosmotic shock is expected to affect protein stability. Traditional theory predicts that more crowded conditions can only stabilize proteins. Here, we test this idea by measuring protein stability in hyperosmotically stressed cells. We find that increasing the crowded nature of the cytoplasm decreases protein stability, consistent with recent studies showing that proteins can be destabilized by transient attractive interactions between the crowding molecules and the test protein [5–13].

Cells adapt to the loss of water by synthesizing or accumulating osmoprotecting solutes [14]. The bacterium *Escherichia coli* accumulates osmolytes such as glycine betaine to concentrations of nearly 1 M [15]. These solutes are known protein stabilizers *in vitro* [16], and it has been suggested that the accumulation of osmolytes by stressed cells prevents protein aggregation [14]. Here, we directly measure the effect of glycine betaine on protein stability in living *E. coli* and show that increasing the

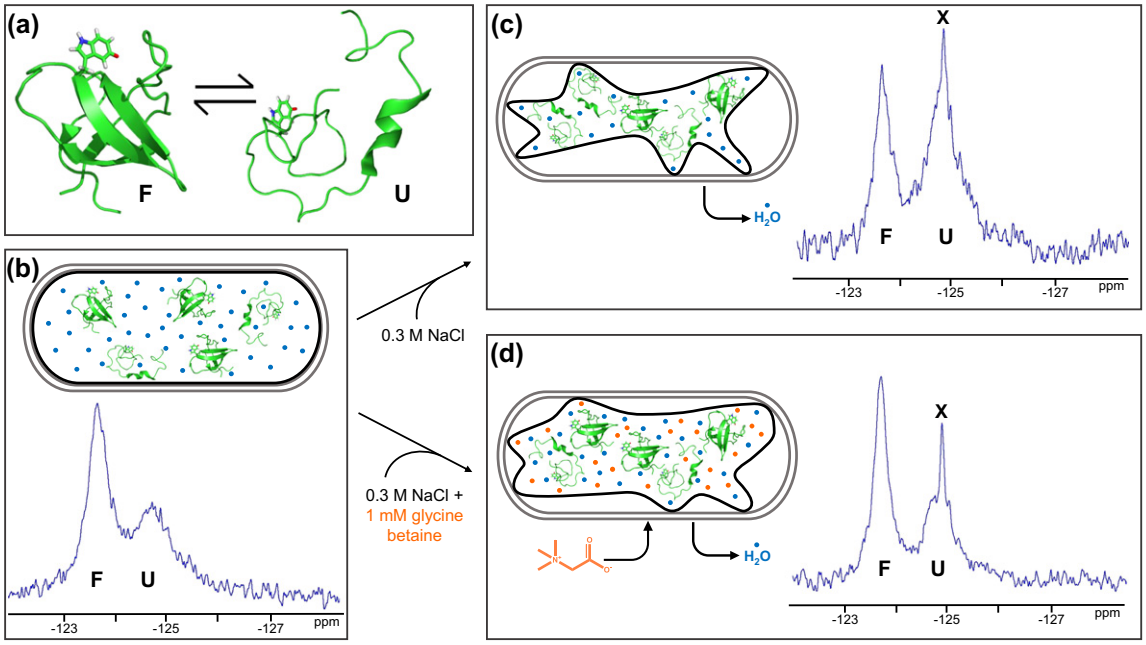

Fig. 1. Glycine betaine reverses the destabilizing effect of hyperosmotic shock. (a) SH3 exists in an equilibrium between its folded state (PDB ID: 2A36) and an unfolded ensemble with a free energy of unfolding near zero under non-denaturing conditions. Tryptophan 36 with fluorine at position 5 is highlighted in red. Protein stability was measured in live *E. coli* cells under three conditions at 298 K. (b) Both the unfolded and folded forms are populated in cells under normal osmotic conditions. Gray outlines represent the cell wall. Black outlines represent the cytoplasmic membrane. Blue circles represent H_2O. (c) Hyperosmotic shock caused by adding 0.3 M NaCl to the media destabilizes SH3. (d) Adding 1 mM glycine betaine to the 0.3 M NaCl causes the uptake of glycine betaine, returning SH3 to the stability observed without osmotic shock. Orange circles represent glycine betaine. Leakage of fluorine-containing metabolites (X) occurs upon hyperosmotic shock (Fig. S1).

glycine betaine concentration in cells returns the stability lost due to hyperosmotic stress. These results provide a new explanation for why stressed cells accumulate osmolytes.

The protein used in these experiments is the 7-kDa N-terminal SH3 domain of *Drosophila* signal transduction protein drk (SH3). This metastable protein exists in a simple, reversible two-state equilibrium between its folded state and its unfolded ensemble [17] such that both forms are present at comparable concentrations under non-denaturing conditions. SH3 can be labeled with a fluorine atom on its sole tryptophan at position 36, allowing the application of ^{19}F NMR spectroscopy [10,18]. Exchange between the folded form and the unfolded ensemble is slow compared to the difference in the NMR frequencies of the fluorine label in the two states, enabling the quantification of the modified standard-state free energy of unfolding by integrating the resonances: $\Delta G^{\circ}_{U} = -RT\ln(\frac{\text{population unfolded}}{\text{population folded}})$. The ability to measure SH3 stability both *in vitro* and in living cells [10] makes this protein useful for studying protein folding under stressed conditions in cells in the presence and absence of glycine betaine.

Results and discussion

Quantification of protein stability in cells

Increasing the external osmolarity of *E. coli* by adding 0.3 M NaCl to the external media causes water efflux, reducing the cell volume by ~35% and increasing the concentrations of macromolecules [3]. The qualitative conclusions that hyperosmotic stress destabilizes SH3 are easy to see by examining the areas under the peaks marked F and U in Fig. 1. When osmotically stressed *E. coli* cultures are provided with betaines, the stressed cells rapidly accumulate these compounds to maintain the turgor pressure and prevent dehydration [19]. More specifically, 1 mM glycine betaine in the media under hyperosmotic conditions results in a cytoplasmic concentration of 0.68 ± 0.07 m [20]. The qualitative conclusion that the accumulation of glycine betaine restores the stability is also easy to see by inspecting Fig. 1.

SH3 aggregation does not complicate our analysis because the protein aggregates only under highly acidic (pH 2) conditions [21,22]. For experiments