

Feature Article

Regulation and Dynamics of Tryptophan Import in Yeast Analyzed Using High Hydrostatic Pressure

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High hydrostatic pressure has an adverse influence in organisms that inhabit atmospheric pressure environments. In yeast *Saccharomyces cerevisiae*, pressure in the range of 15-25 MPa markedly impairs the uptake of tryptophan, and thereby it limits growth of tryptophan auxotrophic strains. The susceptibility of tryptophan import is attributable to the dynamic structural change in tryptophan permease in the plasma membrane. Studies of mutants capable of growth at high pressure reveal that tryptophan permeases Tat1 and Tat2 undergo ubiquitin-dependent degradation when cells are exposed to high pressure. We suggest that hydrostatic pressure is a unique tool for probing the dynamic function and regulation of amino acid permeases in living yeast cells.

Introduction

The effects of high hydrostatic pressure in biological systems have been mainly investigated from the following perspectives: (i) the use of pressure as a perturbation parameter of macromolecules such as proteins and lipids; (ii) microbial adaptation to high-pressure environments; and (iii) inactivation of food-spoiling microbes in foodstuff. During the past decades, an increasing number of pioneering high-pressure studies on biological processes have been performed by applying advanced techniques of genetics and molecular biology in model organisms such as bacterium *Escherichia coli* and yeast *Saccharomyces cerevisiae*^[1-6]. Recent studies in this field have revealed the potential of a broad range of microbes to adapt and develop resistance to increasing hydrostatic pressure.

The application of pressure yields a fundamental physical parameter in any reaction, that is, volume change. The following two equations describe the effect of hydrostatic pressure on the equilibrium $A \rightleftharpoons B$ and the reaction $A \rightarrow B$, respectively:

$$(\partial \ln K / \partial p)_T = -\Delta V / RT \quad \dots \dots \dots (1)$$

$$(\partial \ln k / \partial p)_T = -\Delta V^\ddagger / RT \quad \dots \dots \dots (2)$$

where K is the equilibrium constant, k the rate constant, p pressure (in megapascals [MPa]), T absolute temperature (K), and R the gas constant (ml MPa/K·mol). For the pressure unit definition, MPa is used throughout (atmospheric pressure ~ 0.1 MPa = 1 bar = 0.9869 atm = 1.0197 kg of force/cm²). ΔV is the difference between the final and initial volumes in the entire system at equilibrium (reaction volume), including the solute and the surrounding solvent. ΔV^\ddagger is the apparent volume change in activation (activation volume), representing the difference in volume between the reactants and the transition state. The direction and degree of pressure effects on any reaction are governed by the sign and magnitude of the volume change. Basically, the effects of high pressure on living cells are also dependent on the sign and magnitude of volume changes associated with any intracellular chemical reaction. Nevertheless, it is difficult to predict the outcome of increasing hydrostatic pressure in complex metabolic networks. Explorations in piezophysiology are being undertaken to discover whether the physiological outcome of living cells exposed to high pressure is relevant to their growth and survival and to identify the genes responsible. In this paper, the effects of high pressure in a model eukaryote yeast *S. cerevisiae* are described focusing on tryptophan import that is primarily important for growth under high pressure.

Tryptophan Import is Susceptible to High Pressure

In *S. cerevisiae*, the availability of tryptophan is one of the most high pressure-sensitive processes. Experimental wild-type strains usually have several nutrient auxotrophic markers such as *trp1* (tryptophan), *leu2* (leucine), *lys2* (lysine), *his3* (histidine), *ade2* (adenine), or *ura3* (uracil) for the selection of plasmid-bearing transformants, and thereby the strains require these amino acids in growth medium. Regardless of other forms of auxotrophy, the growth of *trp1* cells is arrested when cells are exposed to pressure of 15-25 MPa^[7]. This is due to reduced tryptophan import activity. Any factors that lead to increased tryptophan availability enable *trp1* cells to grow at high pressure: The addition of excess tryptophan to the medium (e.g., 1 g/L in comparison with 20-40 mg/L in normal medium), introduction of the *TRP1* gene to confer tryptophan synthesis, or overexpression of the gene encoding tryptophan permease Tat1 or Tat2 confers the ability to grow at 25 MPa on *trp1* cells^[7]. The growth of *trp1* cells is also inhibited at low temperature of 10-15 °C. In a manner analogous to high pressure, the growth defect at low temperature can be compensated for by increased tryptophan availability. It is well documented that membrane fluidity is decreased either by increasing hydrostatic pressure or decreasing temperature. In this sense, tryptophan uptake by yeast cells is sensitive to decreases in membrane fluidity caused by either high pressure or low temperature.

Ubiquitination-Dependent Regulation of Tryptophan Permeases in Response to High Pressure

We isolated mutants capable of growth at high pressure from a *trp1* strain. They are designated high-pressure growth (*HPG*) mutants^[8]. The identification of the mutation sites and phenotypic characterization allow us to elucidate the complex intracellular mechanism that regulates tryptophan permeases. The *HPG* mutants are classified into four semidominant linkage groups designated *HPG1*, *HPG2*, *HPG3*, and *HPG4*. The *HPG1* mutation sites are located in the catalytic domain of Rsp5 ubiquitin ligase^[8]. Ubiquitination is a selective degradation system of cellular proteins in eukaryotes. After ubiquitin molecules are covalently bound to target proteins, the ubiquitinated proteins are delivered to the proteasome or the vacuoles for degradation. Rsp5 plays a central role in selecting target proteins to be ubiquitinated^[9, 10]. When the wild-type cells are exposed

to pressure of 25 MPa, the tryptophan permeases Tat1 and Tat2 are degraded in a ubiquitination-dependent manner. However, both permeases become resistant in the *HPG1* mutant and are stabilized in the plasma membrane (Figure 1). Consequently, the *HPG1* mutant is capable of growth under high pressure due to stable tryptophan import^[8]. The *HPG2* mutation sites are located in the cytoplasmic tails of Tat2 protein closed to 29th and 31st lysine to be ubiquitinated^[11]. These amino acid substitutions in the *HPG2* mutant are likely to interfere with ubiquitination on the lysines by Rsp5 ubiquitin ligase. Consequently, the mutant forms of Tat2 are stabilized, leading to high-pressure growth (Figure 1). We assume that high pressure impairs the structure and activity of tryptophan permeases in living yeast cells. In this sense, tryptophan permeases undergo ubiquitination-mediated quality control system when cells are exposed to high hydrostatic pressure.

Probing for Dynamics of Tryptophan Import Using Hydrostatic Pressure

The effects of hydrostatic pressure on an enzymatic reaction are interpreted within the framework of the simplest kinetic mechanism in which the transition state presents the highest energy barrier, and the chemical transformation of substrate to product is considered to be a single rate-limiting step. Equation 2 represents a quantitative estimation of the effects of pressure in chemical reactions. The activation volumes associated with tryptophan import through the tryptophan permeases Tat1 and Tat2 are relatively large, at 89.3 ml/mol and 50.8 ml/mol, respectively^[8]. This means that increasing hydrostatic pressure dramatically impairs the permease-mediated tryptophan import. There is a significant difference in the activation volume for tryptophan import between Tat1 and Tat2, which means that the defect is more severe with Tat1 than with Tat2. In other words, Tat1 undergoes a more dramatic conformational change than Tat2 upon tryptophan import. The remarkable difference between the two permeases in the activation volume is accounted for by the difference in the volume of the initial states, *i.e.*, the initial volume of Tat1 is likely to be smaller than that of Tat2. This explanation agrees well with the finding that Tat1 is localized in the highly ordered lipid phase in which the volume is likely to be small, whereas Tat2 is localized in the disordered phase in which the volume is likely to be large^[8]. To elucidate membrane protein dynamics in living cells, hydrostatic pressure is an attractive tool for probing lipid microdomains and the proteins resident in membranes by measurement of their activation volumes.

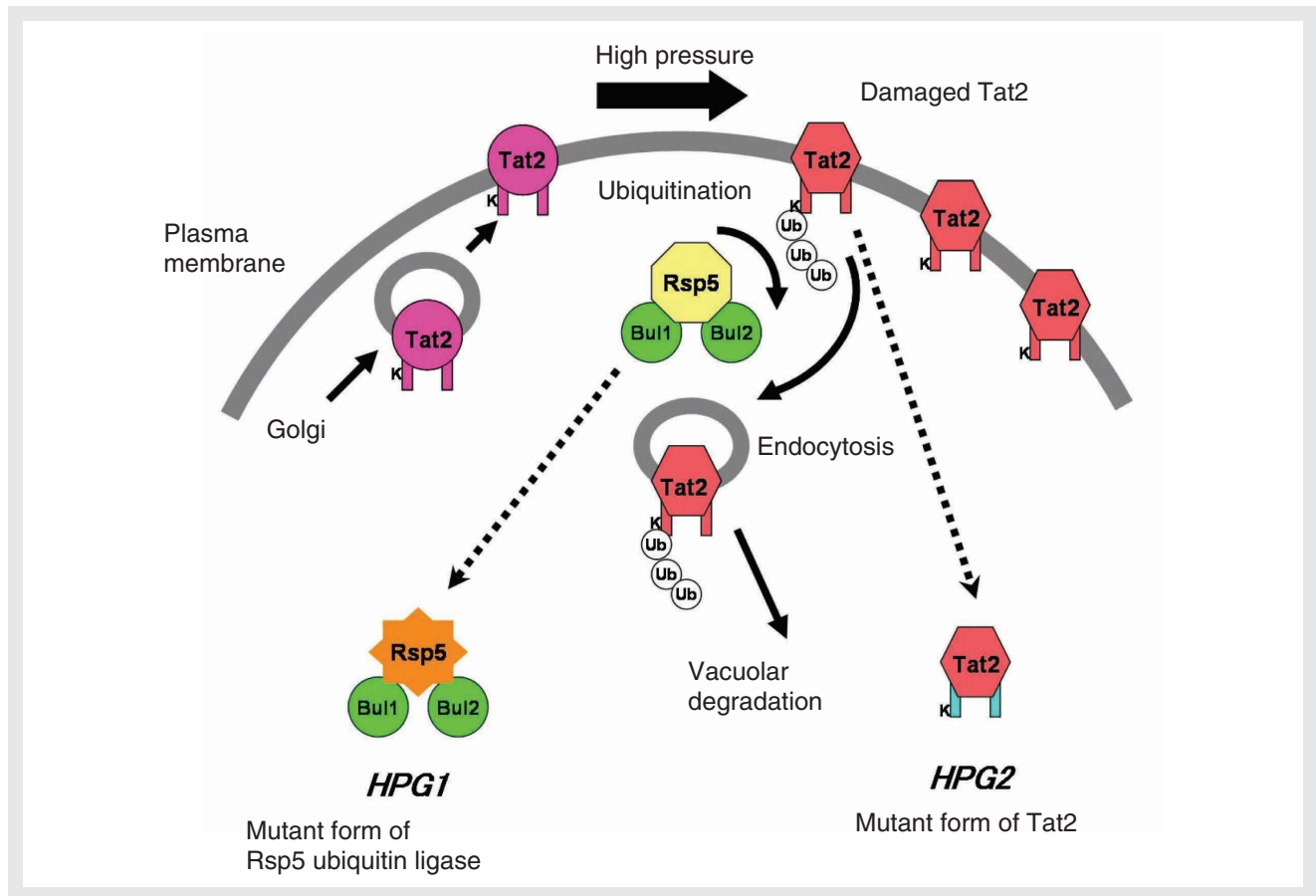


Figure 1 Pressure-induced degradation of tryptophan permease Tat2 under high hydrostatic pressure. High pressure is assumed to impair the structure and activity of tryptophan permease Tat2 in living yeast cells. In the wild-type cells, damaged Tat2 is ubiquitinated by Rsp5 ubiquitin ligase followed by vacuolar degradation. The *HPG1* mutation sites are located in the catalytic domain of Rsp5, and the *HPG2* mutation sites are located in the cytoplasmic tails of Tat2. Consequently, a large amount of the less active form of Tat2 proteins are accumulated in the plasma membrane. Enhanced tryptophan import enables the tryptophan auxotrophic strain to grow under high pressure. In the same way, the other tryptophan permease Tat1 is regulated by ubiquitination.

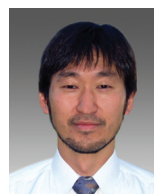
Conclusion

Utilizing advanced techniques for molecular biology, studies of the yeast *S. cerevisiae* have improved our fundamental understanding of the effects of high hydrostatic pressure in living cells. However, actual structure-function relationship in intracellular proteins and lipids remains to be answered. More biophysical approaches will provide evidence for the question. Breakthroughs will come based on detailed descriptions of experimental conditions and results, and cooperative interaction among investigators in high-pressure biosciences regardless of their objectives or types of target organism.

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