

学位論文要旨

Halophilic mechanisms of the structure, stability and function of
a halophilic dihydrofolate reductase from *Haloarcula japonica* strain TR-1
(*Haloarcula japonica* TR-1 株由来ジヒドロ葉酸還元酵素の
構造、安定性、機能における好塩性のメカニズム)

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To elucidate how salt ions affect the structure, stability, and function of enzymes, a novel dihydrofolate reductase (DHFR) from an extremely halophilic archaeon *Haloarcula japonica* strain TR-1 (HjDHFR P1) was overexpressed and purified. Salt concentration dependence of the circular dichroism and fluorescence spectra suggested that the addition of 500 mM NaCl induced structural formation around the substrate-binding site in HjDHFR P1. However, its structural stability for thermal and urea-induced unfolding increased depending on NaCl concentration regardless of this structural change, and the halophilic mechanism is suggested as the contribution of preferential interactions between the protein and salt ions.

On the other hand, HjDHFR P1 showed moderately halophilic characteristics for enzymatic activity at the acidic to neutral pH region, although there are no significant effects of NaCl on its structure. From a comparison of the activation effects of inorganic and organic cations and anions, binding of inorganic anions enhance the enzymatic activity of HjDHFR P1. Furthermore, rapid-phase ligand binding experiments showed that the fluorescence quenching caused by the rapid binding of DHF to HjDHFR P1 increased with increasing NaCl concentration at pH 6.0. In addition, the THF-releasing rate decreased with increasing NaCl concentration, consistent with the decrease of k_{cat} value. These results suggested that the activation mechanism of HjDHFR P1 by salt is the population change of anion-unbound and anion-bound conformers, which are DHF-unboundable and -boundable forms, respectively. Conversely, the salt-inactivation mechanism is via deceleration of the THF-releasing rate, which is the rate-determining step at the neutral pH region. Such activation mechanisms of structure, stability, and function may also be possible for other two halophilic DHFRs from *Haloferax volcanii*, and the inactivation mechanism in its function may be a common feature of non-halophilic DHFR from *Escherichia coli*.