Biochimie 118 (2015) 82–89

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Intramolecular hydrogen bonding in the polyextremophilic short-chain dehydrogenase from the archaeon *Thermococcus sibiricus* and its close structural homologs

Ekaterina Yu. Bezsudnova [a, *], Tatiana E. Petrova [b], Anna V. Popinako [a], Mikhail Yu. Antonov [c], Tatiana N. Stekhanova [a], Vladimir O. Popov [a, d]

[a] A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Leninsky pr. 33, bld. 2, 119071, Moscow, Russian Federation
[b] Institute of Mathematical Problems of Biology, RAS, Institutskaya str. 4, Pushchino, 142290, Russian Federation
[c] M.K. Ammosov North-Eastern Federal University, Belinskiy str., 58, Suite 312, Yakutsk, 677980, Republic of Sakha (Yakutia), Russian Federation
[d] Dep. "Protein Factory", NBICS Center, National Research Centre "Kurchatov Institute", Akad. Kurchatova sqr., 1, Moscow, 123182, Russian Federation

ARTICLE INFO

Article history:
Received 2 March 2015
Accepted 19 August 2015
Available online 20 August 2015

Keywords:
Protein adaptation
Short-chain dehydrogenases
Thermostability
Hydrogen bonds
Molecular dynamics

ABSTRACT

The short-chain alcohol dehydrogenase from the archaeon *Thermococcus sibiricus* (TsAdh319) exhibits adaptation to different kinds of stress: high temperature, high salinity, and the presence of organic solvents and denaturants. Previously a comparison of TsAdh319 with close structural homologs revealed an abnormally large number of charged residues on the surface of TsAdh319 tetramer. We further focused on the analysis of hydrogen bonding of TsAdh319 and its structural homologs from thermophilic and mesophilic organisms as a structural factor of adaptation to extreme environment. The calculation and analysis of the dynamics of hydrogen bonds of different kind were performed. In particular, the intramolecular hydrogen bonds of different kind according to their location and the type of a.a. residues involved in the bond were analyzed. TsAdh319 showed the greatest contribution of charged residues to the formation of surface hydrogen bonds, inner hydrogen bonding, and the bonds between different subunits compared to its structural homologs. Molecular dynamics simulations revealed that, of three enzyme molecules analyzed, TsAdh319 shows the least change in the number of hydrogen bonds of different kinds upon a temperature shift from 27 to 85 °C. The greatest changes were observed for a homologous enzyme from a mesophilic host. Only guanidine hydrochloride being a charged agent was able to deactivate TsAdh319.

We suggest that the percentage of charged residues plays a key role in the resistance of TsAdh319 to environmental stress. The analysis shows that salt bridges in TsAdh319 serve as a universal instrument of stabilization under different extreme conditions.

1. Introduction

Stabilizing intramolecular hydrogen bonds are formed in protein during folding and replace hydrogen bonds between protein atoms and solvent. The energetic effect of intramolecular hydrogen bonds is viewed as entropic because the intramolecular hydrogen bond formation in aqueous solution is accompanied by the release of two water molecules [1]. This effect is estimated to be 1.5–1.0 kcal/mol per buried intramolecular hydrogen bond [2]. If hydrogen bonds are formed by atoms of charged side groups, the additional electrostatic interactions between opposite charges significantly increase the strength of hydrogen bonds in proteins (the enthalpic effect). These hydrogen bonds are called salt bridges [3–6]. The electrostatic contribution to the free energy change upon formation of a salt bridge averages to 4.6 ± 3.2 kcal/mol [6]. Strictly speaking, the stabilizing effect of salt bridges and hydrogen

Abbreviations: TsAdh319, short-chain alcohole dehydrogenase from the hyperthermophilic archaeon *Thermococcus sibiricus*; SDRs, short-chain dehydrogenases/reductases; TmDH, short-chain dehydrogenase from *Thermotoga maritima*; cSR, sepiapterin reductase from *Chlorobium tepidum*; CAD, clavulanic acid dehydrogenase from actinomycete *Streptomyces clavuligerus*; SR, L-sorbose reductase from *Gluconobacter frateurii*.

* Corresponding author.
E-mail address: eubez@inbi.ras.ru (E.Yu. Bezsudnova).

http://dx.doi.org/10.1016/j.biochi.2015.08.010
0300-9084/

bonds depends on the cost of the dehydration of a.a. residues, the stringent geometric restraints introduced by hydrogen-bonding interactions, the environment, and the intrinsic propensity of interacting atoms etc.; that is, it depends on the location and type of a.a. residues [3,6,7].

In 1994–1996, by using different methods, Makhatadze, Privalov, and Pace came independently to a conclusion that the contribution of hydrogen bonds to protein stability and folding is as important as the hydrophobic effect [2,8]. Salt bridges initially were considered to play a more significant role in molecular recognition and catalysis rather than in protein folding and stabilization. However, it has been consistently observed that salt bridges occur more frequently in proteins from thermophiles than from mesophiles [5,9–11]. It has been shown that salt bridges and other stabilizing ion pairs provide added resilience and resistance to denaturation by increasing the kinetic barrier to unfolding [3,12]. The stabilizing effect of ion pairs drastically increases in the network [13]. Moreover, recently the increase in the number of salt bridges with temperature was shown by MD simulation for thermostable hemoglobin and SAICAR synthetase [14,15]. The effectiveness of the contribution of hydrogen bonds and salt bridges to the thermal stabilization of proteins is achieved by the optimization of amino acid composition followed by an increase in the packing density, a change in the length of surface loops, the size and position of alpha-helices and beta-sheets, etc. These changes do not necessarily lead to a more compact structure but generally provide a balance between integrity and flexibility at a certain temperature [10,16,17]. In addition, it was noted that the thermal adaptation in proteins often leads to the polyextremophilicity, including the resistance to proteolysis, organic solvents, and high salinity [18].

In the present work, we continued the investigation of the relationship between the stability and hydrogen bonding in proteins. We focused on short-chain dehydrogenases/reductases (SDRs) – proteins with well organized tertiary structures and their highly thermostable representative – alcohol dehydrogenase from the archaeon *Thermococcus sibiricus* (TsAdh319).

Earlier, we characterized biochemically and determined the crystal structure of TsAdh319 [19,20]. The comparison of the amino acid composition of homologs of TsAdh319 from thermophiles and mesophiles showed a much higher percentage of Arg, Glu and Leu in SDRs from thermophiles, with TsAdh319 having the largest percentage of charged residues. The enzyme demonstrated the highest thermostability (6 h at 90 °C and 20 h at 80 °C) among SDRs studied [19]; the temperature optimum of activity with 2(S),5(S)-hexanediol and 2-propanol was 83 °C and 85 °C, respectively [21]. The enzyme was resistant to NaCl concentrations up to 4 *M*; in the presence of 1 *M* NaCl an increase in TsAdh319 thermostability was detected (calculated half-life values were 37 vs. 20 h at 80 °C and 8 vs. 6 h at 90 °C). The preincubation of TsAdh319 for 4 h at 55 °C in the presence of 50% [v/v] DMSO, DMFA, methanol, acetonitrile, chloroform and n-hexane prior to the activity assay did not result in the decrease in the enzyme activity. The solution of the crystal structure of TsAdh319 (PDB code 3TN7) revealed that the TsAdh319 subunit consists of the highly structured compact Rossmann fold (1–190 a.a.) and a low-structured region (191–234 a.a.), which form active center, the whole construction being typical for SDRs. A comparison of the structure of TsAdh319 with the nearest structural homologs (RMSD between 1.02 and 1.60 Å) from thermophilic and mesophilic organisms showed that charged residues in all the counterparts are located on the surface of subunits, with TsAdh319 having the highest percentage of charged residues in the accessible surface area. Moreover, nearly 70% of charged residues are involved in ion-pair interactions with the formation of isolated ion pairs or an ion-pair network.

In this work, we compared the hydrogen bond scaffolds of TsAdh319 and its close structural homologs from thermophilic and mesophilic hosts selected previously on the basis of high structural similarity [20]: TmDH, the short-chain dehydrogenase from *Thermotoga maritime* (pdb code 1VL8); cSR, the sepiapterin reductase from *Chlorobium tepidum* (pdb code 2BD0); CAD, the clavulanic acid dehydrogenase from actinomycete *Streptomyces clavuligerus* (pdb code 2JAH); and SR, L-sorbose reductase from *Gluconobacter frateurii* (pdb code 3AI2). All structures were solved at a resolution higher than 2.0 Å and had no breaks in polypeptide chains. All counterparts in solution are tetramers formed in a similar way through contacts between neighboring subunits [22]. For each structure, we analyzed the intramolecular hydrogen bonds of different kind according to their location and the type of a.a. residues involved in the bond. We estimated and compared the effects of urea and guanidine hydrochloride, agents affecting hydrogen bonds, on the activity and stability of TsAdh319. We took into account the specific effect of guanidine hydrochloride as a denaturant due to its ionic properties (because of protonation of GuHCl in solutions with neutral pH) and its ability to mimic the interactions mediated by the guanidine moiety of the arginine side chain [23,24]. To gain further insight into the role of hydrogen bonds of different kind, we performed dynamic studies. *In silico* molecular dynamics (MD) simulations revealed an increase in the number of salt bridges and the destruction of hydrogen bonds between neutral residues on a temperature shift from 27 to 85 °C in all analyzed SDRs. Therefore, we suggest that the highest percentage of charged residues is a crucial factor for the polyextremophilicity of TsAdh319.

2. Materials and methods

2.1. Procedure for the calculation and analysis of hydrogen bonds

First, for each protein analyzed, a model from the Protein Data Bank (PDB) was taken, and a file (in the pdb format) was prepared, which contained only those monomers that form a protein molecule in solution. Water molecules and ligands were removed from the model. Second, all potential hydrogen bonds in the model were determined using the program HBOND (http://cib.cf.ocha.ac.jp/bitool/HBOND). The maximal distance between a donor and an acceptor atom was 3.5 Å. Third, the accessible surface area (ASA) for each atom in the model was calculated using the program AREAIMOL of CCP4 package [25]. Atoms with ASA values equal to 0.0 were considered as inner atoms, while the remaining atoms were considered as being on the surface. Forth, for a protein model that contained some residues in multiple conformations, both files (output of HBOND and AREAMOL) were corrected. These residues are: His43, Met 46, Lys62, Glu64, Ile166, Asp202, Cys210, Met224 for TsAdh319; Mse122 for clavulanic acid dehydrogenase from *S. clavuligerus*, and Ser203 for dehydrogenase from *T. maritime*. Fifth, both files (output of HBOND and AREAMOL) were merged into one single file. Depending on the ASA value and the side group of each residue, all bonds were categorized into several groups. We determined the number of hydrogen bonds between inner atoms (**Inside**–**Inside**), between atoms which both were on the surface (**Surface**–**Surface**), between atoms one of which either belonged to a side group of a neutral residue or was N or O atom of the main chain and the other belonged to a side group of a charged residue (**Charged-Neutral**), between atoms of the side group of neutral residues and/or N or O atom of the main chain (**Neutral**–**Neutral**), and between atoms of the side groups of charged residues (**Charged**–**Charged**). The difference between the total number of hydrogen bonds and the sum of Inside–Inside and Surface–Surface hydrogen bonds is the number of **Inside-Surface** hydrogen bonds formed between a solvent-accessible atom and an atom buried