Chemical Physics Letters 657 (2016) 119–123

Contents lists available at ScienceDirect

Chemical Physics Letters

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

Research paper

Physical origins of remarkable thermostabilization by an octuple mutation for the adenosine A_{2a} receptor

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ARTICLE INFO

Article history:
Received 25 April 2016
In final form 26 May 2016
Available online 31 May 2016

Keywords:
Protein folding
Membrane protein
G-protein coupled receptor
Drug target
Free-energy function

ABSTRACT

It was experimentally showed that the thermal stability of a membrane protein, the adenosine A_{2a} receptor, was remarkably enhanced by an octuple mutation. Here we theoretically prove that the energy decrease arising from the formation of protein intramolecular hydrogen bonds and the solvent-entropy gain upon protein folding are made substantially larger by the mutation, leading to the remarkable enhancement. The solvent is formed by hydrocarbon groups constituting nonpolar chains of the lipid bilayer within a membrane. The mutation modifies geometric characteristics of the structure so that the solvent crowding can be reduced to a larger extent when the protein folds.

1. Introduction

Membrane proteins such as G-protein coupled receptors (GPCRs) are imperative in the life phenomena [1]. The problems caused in their functions lead to a diversity of diseases, and they construct more than 60% of current drug targets [2]. Though membrane proteins are thus crucially important, the studies on them have been hindered by their low structural stability in detergents followed by the difficulties in obtaining the three-dimensional structure by X-ray crystallography. In this Letter, we deal with human adenosine A_{2a} receptor (A_{2a}R), an important GPCR involved in the control of various physiological activities including regulation of glutamine and dopamine release in the brain [3]. Specific inhibitors of the receptor are in advanced clinical trials for the treatment of Parkinson's disease [4]. The crystallization of A_{2a}R followed by the determination of its structure has been a nontrivial task due to its low structural stability.

A method for improving the stability of a membrane protein in detergents as well as its thermostability is an amino-acid mutation [5]. Alanine (Ala) scanning mutagenesis, in which every residue is mutated to Ala (Ala is mutated to leucine (Leu)), has been applied to A_{2a}R, leading to the finding of significantly many stabilized single mutants [6]. Combining multiple stabilizing single mutations often brings more stability than a stabilizing single mutation. In particular, the thermostability is exceptionally enhanced by an octuple mutation (A54L, T88A, R107A, K122A, L202A, L235A, V239A, and S277A; seven residues are mutated to Ala): The denaturation temperature T_m of the octuple mutant (PDB ID of its structure is 3PWH) is higher than that of the wild type by ~20 °C [7]. A_{2a}R comprises the extracellular, transmembrane (TM), and intracellular regions. Five of the residues mutated are totally within the TM region and the other three are in the vicinity of the TM region (see Section 2.4). Therefore, it is probable that the structural modification occurs primarily within the TM region, leading to the remarkable stabilization. On the other hand, Murata and his coworkers [8] have succeeded in determining the structure of A_{2a}R to which a mouse monoclonal-antibody Fab-fragment is bound (PDB ID: 3VG9). In their method, the success is ascribed to the achievement of crystallization by the fragment binding to A_{2a}R. As discussed in Section 2.4 in more detail, the binding interface is at the end of the intracellular region and far from the TM region. Presumably, the structure of the TM region is not significantly modified by the fragment binding though that of the intracellular region is more or less influenced. It follows that the

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http://dx.doi.org/10.1016/j.cplett.2016.05.062
0009-2614/

physical origins of the remarkable stabilization can be investigated by comparing the TM regions of the octuple mutant of $A_{2a}R$ and $A_{2a}R$ with the fragment bound. The latter is referred to as 'wild type' hereafter, because it and the wild type should share almost the same TM-region structure. Both of the octuple mutant and the wild type are in the inactive states. Though the structures of their TM regions look almost indistinguishable in sight, there must be significant differences affecting the thermodynamic quantities governing the stability, which have not yet been understood.

In the present study, using our recently developed theory for the thermostability of a membrane protein [9], we clarify the physical factors responsible for the remarkable stabilization by the octuple mutation. For the octuple mutant and the wild type, the changes in thermodynamic quantities upon protein folding are analyzed using the structures of their TM regions. The theoretical tool is the integral equation theory (IET) [10] combined with our recently developed morphometric approach (MA) [11]: The former is a statistical-mechanical theory for fluids and the latter is introduced for treating a large, complex solute like a protein. The combined method is characterized by the concept that hydrocarbon (CH_2, CH_3, and CH) groups in nonpolar chains of lipid molecules work as 'solvent' for a membrane protein and the translational displacement of solvent particles plays a pivotal role in the protein structural stability [9,12]. It is demonstrated that we are capable of explicating the remarkable stabilization: Protein folding is accompanied by a decrease in energy related to the formation of protein intramolecular hydrogen bonds and a gain of solvent entropy, but both of the energy decrease and the entropic gain are substantially larger for the octuple mutant than for the wild type.

2. Model and theory

2.1. Two essential physical factors

Following our earlier work [9], we assume that the thermostability of a GPCR is governed by that of the TM region. For it, nonpolar chains of lipid molecules act as 'solvent' [9,12]. Insertion of a protein into the membrane causes an entropic loss because it reduces translational, rotational, and vibrational freedoms of the nonpolar chains. However, the reduction of translational freedom is the most serious, because it reaches all the nonpolar chains coexisting with the protein, whereas the reduction of rotational and vibrational freedoms occurs only in the close vicinity of the protein surface. Therefore, we take account of only the effect of the translational displacement of hydrocarbon (CH_2, CH_3, and CH) groups which are treated as if they were not connected with one another, so that a tractable statistical-mechanical theory can be applied (more details are described in Section 2.2). A gain of protein intramolecular van der Waals (vdW) attractive interactions upon protein folding is significantly cancelled out by the loss of protein-solvent vdW attractive interactions accompanied. This is not the case for protein intramolecular hydrogen bonds (IHBs) because there are no solvent-protein hydrogen bonds (HBs) [12]. The importance of IHBs is reflected, for instance, on the high helical content of a GPCR. We assume that the wild type and the octuple mutant, which are both quite compact, share the same protein conformational entropy.

We take account of the solvent-entropy effect and the protein intramolecular hydrogen bonding as two essential factors which most influence the structural stability of a membrane protein [9,12]. The validity of accounting for only the above two factors and of employing the simplified solvent model has been confirmed by our success in discriminating the native structure from ~15000 non-native structures generated by a computer simulation for glycophorin A (GpA) [9,12].

2.2. Entropic excluded-volume effect by solvent

Hydrocarbon (CH_2, CH_3, and CH) groups in the nonpolar chains are referred to as 'solvent particles'. Upon protein folding, the excluded volume (EV) (i.e., the volume of the space which the centers of solvent particles cannot enter) decreases to a large extent, which is followed by a corresponding increase in the total volume available to the translational displacement of solvent particles in the system [13]. The effect of the close packing of side chains is particularly important: A cartoon is shown in Fig. 1. A notable point is that the presence of a solvent particle also generates an EV for the other solvent particles. In this sense, all of the solvent particles are entropically correlated. This correlation is referred to as 'solvent crowding' [14,15]. The increase in the total volume available mentioned above reduces the solvent crowding. Primarily through this effect, protein folding leads to a large gain of solvent entropy. The solvent-entropy gain originating from this EV effect is dependent on not only the EV but also the solvent-accessible surface area (SASA) and surface curvatures (see Section 2.5).

Note that a membrane is immersed in aqueous solution. When the protein structure becomes less compact and generates a larger EV, for instance, the membrane also generates a larger EV for water molecules. Thus, water also acts as the solvent. It has been shown in experiments that many membrane proteins fold and function in nonpolar environments which bear little similarity to the membrane [16]. It follows that correct folding of a membrane protein is realized only if it is immersed in nonpolar environment where the EV effect is present and the intramolecular hydrogen bonding is essential. The details of specific characteristics of the nonpolar chains are not relevant.

2.3. Solvent model

We have found that water can be modeled as 'neutral hard spheres' in a theoretical treatment focused on the entropic EV effect at ambient temperature and pressure [13–15]. On the basis of this finding and the discussion in Section 2.2, the solvent is modeled as an ensemble of neutral hard spheres whose diameter and packing fraction are set at those of water at 298 K and 1 atm

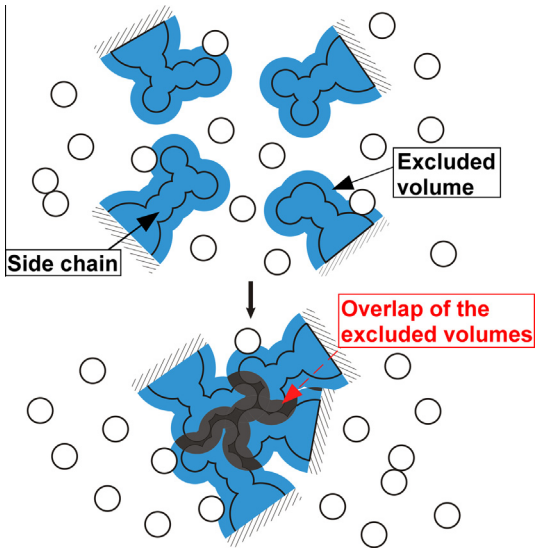

Fig. 1. Close packing of side chains upon protein folding in solvent. Overlap of excluded volumes occurs, and the total volume available to the translational displacement of solvent particles increases by the overlapped volume. A sphere corresponds to a solvent particle.