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Negative and positive temperature dependence of potassium leak in MscS mutants: Implications for understanding thermosensitive channels

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ABSTRACT

Bacterial mechanosensitive channel of small conductance (MscS) is a protein, whose activity is modulated by membrane tension, voltage and cytoplasmic crowding. MscS is a homoheptamer and each monomer consists of three transmembrane helices (TM1-3). Hydrophobic pore of the channel is made of TM3s surrounded by peripheral TM1/2s. MscS gating is a complex process, which involves opening and inactivation in response to the increase of membrane tension. A number of MscS mutants were isolated. Among them mutants affecting gating have been found including gain-of-function (GOF) and loss-of-function (LOF) that open at lower or at higher thresholds, respectively. Previously, using an *in vivo* screen we isolated multiple MscS mutants that leak potassium and some of them were GOF or LOF. Here we show that for a subset of these mutants K^+ leak is negatively (NTD) or positively (PTD) temperature dependent. We show that temperature reliance of these mutants does not depend on how MS gating is affected by a particular mutation. Instead, we argue that NTD or PTD leak is due to the opposite allosteric coupling of the structures that determine the temperature dependence to the channel gate. In PTD mutants an increased hydration of the pore vestibule is directly coupled to the increase in the channel conductance. In NTD mutants, at higher temperatures an increased hydration of peripheral structures leads to complete separation of TM3 and a pore collapse.

1. Introduction

Ion channels are allosteric proteins that are able to integrate various stimuli, *e.g.*: the opening of BK K^+ channels is modulated by voltage and Ca^{2+} [1]; HCN channels are modulated by voltage and cyclic nucleotides [2] and TRP channels are modulated by voltage and temperature [3,4]. The gating of bacterial mechanosensitive (MS) channel MscS from *Escherichia coli* is modulated by membrane tension [5], voltage [6] and osmotic stress [7,8]. It is, therefore, crucial to know how various stimuli affect processes that lead to channel activation. MscS is a homoheptamer (Fig. 1A, B) of monomers whose transmembrane part is built of three helices: peripheral TM1 and TM2 that surround pore forming TM3 (Fig. 1C) [9]. The channel opens upon membrane stretch as a result of straightening of TM3a and TM3b helices (Fig. 1C) and dissociation of TM3b from ß-domain, which forms cytoplasmic part of the channel [10,11]. It was proposed that the decoupling of TM1/2 from TM3 results in the expansion of crevices between these structures and channel inactivation [6,12]. Both channel pore and the peripheral crevices are highly hydrophobic [12,13].

Ion channel gating is in many instances based on hydrophobic water repulsion. This mechanism was first recognized in simple carbon nanopores [14,15] and was postulated for several channels including MscS [16], nAchR [17], GliC [18] and K^+ channels [19]. It is supported by the existence of conserved cavities or rings of hydrophobic amino acids inside channel pores [16], which hydrophilic substitutions lead to mutants with increased open probability [20]. Such MscS mutants, called gain-of-function (GOF), exhibit low threshold pressures or spontaneous openings leading to solute leak and cell death [11,20]. This is in contrast to loss-of-function (LOF) mutants exhibiting increased threshold pressures [21,22]. It has been shown that the GOF phenotype of MscS-G98S (Fig. 1D) is a result of increased pore vestibule hydration [13]. On the other hand the LOF phenotype of MscS-V65S (Fig. 1D) is due to the separation of TM1/2 from TM3 pore helices (Fig. 1C), however, no hydration of hydrophobic peripheral crevices was observed during MD simulations [12].

In our previous work we used a K^+ uptake-deficient strain and observed its growth in various K^+ concentrations [11]. This method allowed us to isolate a number of K^+-leaky MscS mutants and determine the threshold of K^+ concentration required for the growth of a particular MscS mutant [11]. We were able to ascribe clear GOF or LOF phenotypes to a number of K^+-leaky MscS mutants (Fig. 1D), *e.g.* GOF phenotypes were exhibited by I97N and A98S pore mutants. However, general location does not seem to be a prerequisite for GOF phenotype *e.g.* A94D, one of the mutants from the pore region did not exhibit

Abbreviations: CP, specific heat capacity; GOF, gain-of-function; LOF, loss-of-function; MS, mechanosensitive; NTD, negative temperature dependent; PTD, positive temperature dependent; TM, transmembrane helix

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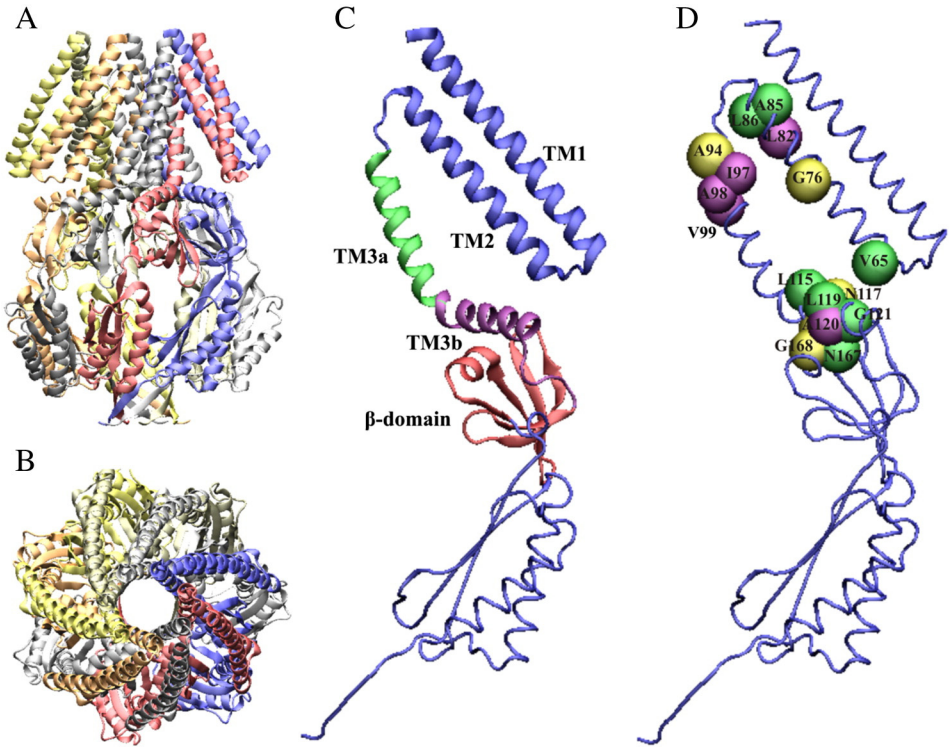

Fig. 1. The crystal structure of MscS heptamer (A., B.) and its monomer (C., D.) (PDB ID: 2OAU). The side (A.) and the top (B.) views of MscS with each monomer shown with a different color. C. MscS monomer with its domains marked: TM1/2 (blue), TM3a\b (green\purple), ß-domain (red). D. MscS monomer, the positions of the amino acids altered in K^+-leaky mutants isolated in (11) are shown as balls. The substitutions that result in the GOF or LOF phenotypes are marked by purple and green, respectively. The amino acids that cause K^+ leak without any additional phenotype are shown in yellow.

GOF. Interestingly GOF phenotype was exhibited by the mutants: V99E on TM3 located slightly outside of the pore, and L82E on the peripheral TM2, indicating rotation of the TM3 helix during the opening of the channel [23]. On the other hand, LOF phenotype, was a result of changes of the following amino acids: V65D, A85T, L86N, V96D, L115Q and L119D located in the peripheral crevices. However, no LOF phenotype was observed in the G76D mutant even though G76 is located in these crevices.

In general, the transition from the closed to the open channel might be the result of minute conformation changes involving movement of amino-acid side chains [24]. Consequently, channel gating is influenced by protein thermal motions and an increase in temperature can shift the equilibrium towards the open channel state [25]. For most channels changes in the gating evoked by heat within the physiological range are insufficient to open them. Transient receptor potential (TRP) channels are those of exception. The temperature coefficient Q_{10} of TRP channels exceeds 100, which translates to the enthalpy in the order of 100 kJ/mol [26]. Enthalpy of this magnitude is comparable to the total heat of unfolding of small proteins like lysozyme [27], but not consistent with the relatively small conformational changes typical for the ion channel gating. It was speculated therefore, that the thermal sensitivity of TRP channels can arise from changes in the temperature-induced membrane fluidity [28]. However, other numerous facts support the hypothesis that thermosensitivity is an intrinsic property of the channel proteins. It has been shown that certain extramembranous domains within TRPV and TRPA channels are responsible for the thermal sensitivity, and the transfer of these domains into non-thermosensitive homologs is sufficient for the recovery of heat sensitivity [29,30]. Moreover, thermosensitivity has been detected in structurally related but functionally distinct voltage-activated Kv channels and certain mutations in voltage sensing domains increase dramatically the temperature dependence of these channels [31,32].

We wondered whether the MscS channel activity is modulated by temperature and if so, whether mutations that change properties of the channel pore can influence the temperature sensing of MscS. Here we report that some MscS mutants that change mechanosensitivity of the channel modulate also its thermosensitivity.

2. Materials and methods

2.1. Media

Growth assays were performed in the defined media. The K115 media consisted of the following: K_2HPO_4, 46 mM; KH_2PO_4, 23 mM; $(NH_4)_2SO_4$, 8 mM; $MgSO_4$, 0.4 mM; $FeSO_4$, 6 μM; sodium citrate, 1 mM; thiamine hydrochloride, 1 mg/l; and glucose, 0.2% (w/v) [33]. For K0 medium, equimolar sodium phosphate was used to replace potassium phosphate. Suitable mixed proportions of K0 and K115 were used to create a medium with intermediate K^+ concentrations. In all media, ampicillin was used at 100 μg/ml. Solid media contained 1% [w/v] agar.

2.2. Strains

E. coli strain deficient in potassium transport systems TK2446 (*F − thi rha lacZ nagA Δ(kdp FAB)5 trkD1 trkG(kan) trkH(cam)Δ(trkA-mscL)*) and LB2003 (*trkAkup1 (trkD1) kdpABC5 rpsL metE thi rha gal*) were kindly provided by Professor Brad S. Rothberg (Temple University School of Medicine, Philadelphia) and Professor Evert P. Bakker (University of Osnabruck, Germany). *E. coli* strains MJF429 (*ΔmscK::kan ΔmscS*) and MJF465 (*ΔmscK::kan ΔmscS ΔmscL::Cm*) were kindly provided by Professor Ian R. Booth (University of Aberdeen, Aberdeen, UK).

2.3. Phenotypic analysis

The construction of plasmids carrying K^+ leaky mutants of MscS was described earlier [11]. For phenotypic analysis the plasmids expressing mutated MscS were transformed into TK2446 or LB2003 strain and grown overnight in K115 media at 37 °C in 96-well plates. The individual