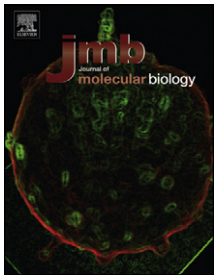

One-Dimensional Sliding of p53 Along DNA Is Accelerated in the Presence of Ca^{2+} or Mg^{2+} at Millimolar Concentrations

Agato Murata [1,2], Yuji Ito [1,2], Risa Kashima [3,5], Saori Kanbayashi [1], Kei Nanatani [4,6], Chihiro Igarashi [1,2], Masaki Okumura [1], Kenji Inaba [1], Takashi Tokino [3], Satoshi Takahashi [1] and Kiyoto Kamagata [1]

*1 - **Institute for Multidisciplinary Research for Advanced Materials,** Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan*
*2 - **Department of Chemistry,** Graduate School of Science, Tohoku University, Sendai 980-8578, Japan*
*3 - **Department of Medical Genome Sciences,** Research Institute for Frontier Medicine, Sapporo Medical University, Chuo-ku, Sapporo 060-8556, Japan*
*4 - **Department of Biomolecular Engineering,** Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan*

***Correspondence to Satoshi Takahashi and Kiyoto Kamagata:** st@tagen.tohoku.ac.jp; kamagata@tagen.tohoku.ac.jp*
http://dx.doi.org/10.1016/j.jmb.2015.06.016
Edited by S. Kowalczykowski

Abstract

One-dimensional (1D) sliding of the tumor suppressor p53 along DNA is an essential dynamics required for its efficient search for the binding sites in the genome. To address how the search process of p53 is affected by the changes in the concentration of Mg^{2+} and Ca^{2+} after the cell damages, we investigated its sliding dynamics at different concentrations of the divalent cations. The 1D sliding trajectories of p53 along the stretched DNA were measured by using single-molecule fluorescence microscopy. The averaged diffusion coefficient calculated from the mean square displacement of p53 on DNA increased significantly at the higher concentration of Mg^{2+} or Ca^{2+}, indicating that the divalent cations accelerate the sliding likely by weakening the DNA–p53 interaction. In addition, two distributions were identified in the displacement of the observed trajectories of p53, demonstrating the presence of the fast and slow sliding modes having large and small diffusion coefficients, respectively. A coreless mutant of p53, in which the core domain was deleted, showed only a single mode whose diffusion coefficient is about twice that of the fast mode for the full-length p53. Thus, the two modes are likely the result of the tight and loose interactions between the core domain of p53 and DNA. These results demonstrated clearly that the 1D sliding dynamics of p53 is strongly dependent on the concentration of Mg^{2+} and Ca^{2+}, which maintains the search distance of p53 along DNA in cells that lost homeostatic control of the divalent cations.

Introduction

The tumor suppressor p53 is the transcription factor that controls the cellular fate as a guardian of the genome [1,2]. p53 induces cell cycle arrest, apoptosis, or DNA repair upon different types of cellular stresses. p53 has four domains: N-terminal domain, core domain, tetramerization domain, and C-terminal domain. A flexible linker connects the core domain and the tetramerization domain. The core and tetramerization domains have specific folded structures, while the other domains are disordered. The core and C-terminal domains are respectively related to the binding of p53 to DNA in sequence-specific and nonspecific manners. The specific binding of p53 to the target DNA sequence causes the transactivation of gene products leading to the various cell fates. In 50% of human cancers, the mutations of p53 gene are found, which are located in the core domain and prevent the specific binding to DNA [3]. Due to its importance, the mechanism by which p53 searches for its binding sites among huge DNA has been the focus of intensive investigations [4–8].

The one-dimensional (1D) sliding of p53 along DNA is one of the essential dynamics in the search

process and has been directly investigated by single-molecule techniques. The sliding of p53 along the fixed DNA was first observed using a high-speed atomic force microscopy [4]. Subsequently, the sliding of p53 was observed using fluorescence microscopy by tracking the position of fluorescent-labeled p53 bound to DNA stretched by a flow [5,6]. The diffusion coefficient for the sliding dynamics of p53 was determined and was 0.16 $\mu m^2/s$ [5]. The C-terminal domain promotes the diffusion along the DNA, while the core domain suppresses the diffusion [5]. The diffusion coefficient does not depend on the concentration of a monovalent cation, K^+ [5,6]. The DNA sequence slightly affects the diffusion [7]. It is proposed that p53 diffuses along DNA and searches for the target sites by repeating the transient association of the core domains to DNA and their dissociation, which was supported by the course-grained molecular dynamics simulations [8]. These results demonstrate the importance of the sliding dynamics of p53 along DNA.

The sliding dynamics of p53 might be regulated by the cellular conditions under various cell phases. For example, the cell death is deeply linked to Ca^{2+} signaling [9,10]. Under some stress, the expression level of p53 is elevated and the Ca^{2+} concentration is increased [11,12]. The elevated concentration of Ca^{2+} in nucleus induces chromatin unfolding and promotes the gene transcriptions regulated by Ca^{2+}-binding proteins, as well as the digestion of nuclear DNA by Ca^{2+}- and Mg^{2+}-dependent endonucleases. In addition, Mg^{2+} deficiency induces the elevated expression of p53, resulting in cell cycle arrest [13]. Since these divalent cations bind tightly and specifically to DNA [14–19], the sliding dynamics of p53, dictated by the interaction between p53 and DNA, would also be modulated by the divalent cations. In fact, early investigations of the lac repressor suggested that Mg^{2+} alters the 1D sliding along DNA and the binding ability to DNA [20,21]. Accordingly, we hypothesize that the loss of homeostatic control for divalent cations such as Ca^{2+} and Mg^{2+} might regulate the target search of p53.

In this study, we investigated the effect of the divalent cations on the sliding dynamics of p53 along DNA. To this end, we prepared a stable tetrameric mutant of p53 [the pseudo-WT (*w*ild *t*ype)] that can maintain the specific binding ability to DNA sufficient for the single-molecule fluorescence measurements. Using the pseudo-WT, we examined the nonspecific binding ability relevant to the sliding dynamics. We found that the dissociation rate constant was increased as the increase in the concentration of Mg^{2+} or Ca^{2+}. We observed the sliding of p53 along DNA at the single-molecule level at different concentrations of Mg^{2+} and Ca^{2+} and found that the sliding of p53 was significantly accelerated by adding the millimolar concentration of the divalent cations. The analysis of the sliding trajectories further suggests that p53 possesses two sliding modes with different diffusion coefficients. We also prepared a coreless mutant of p53, in which the core domain and the N-terminal domain were deleted, and found that a single diffusion mode was observed in the coreless mutant. We propose that the increased diffusion of p53 in the presence of the divalent cations occurs as the result of a weakening of the DNA–p53 interaction upon the binding of the divalent cations to DNA.

Results

Construction of the stabilized mutant of p53 with a single exposed cysteine

In our early trials, we prepared the human WT p53 by expressing the protein with GST (*g*lutathione *S*-*t*ransferase) tag in *Escherichia coli*, followed by the cleavage of the tag and the purification. The oligomeric state of the purified protein was examined by gel filtration, SDS-PAGE for the cross-linked p53 and native PAGE. In addition, the DNA binding activity was examined by electrophoretic mobility shift assay. We succeeded in the preparation of the tetrameric p53 having the specific binding ability toward the target DNA; however, it was difficult to reproduce the activity especially after frozen storages. This is likely due to the aggregation-prone property and/or the incomplete folding of the core domain of the WT p53 [22]. In addition, the labeling of the WT p53 by fluorophore for the single-molecule measurements likely affected its activity because some of the four solvent-exposed cysteines are located in the binding site for DNA (Supplementary Fig. 1 and the supplementary text). We concluded that the WT p53 is not suitable for the investigation based on the single-molecule fluorescence measurements. The instability of the WT p53 was similarly reported in previous investigations [5,22,23].

To obtain reproducible results, we prepared a thermostable mutant having a single solvent-exposed cysteine for the labeling. The mutations introduced are C135V, C141V, W146Y, C182S, V203A, R209P, C229Y, H233Y, Y234F, N235K, Y236F, T253V, and N268D, which are based on thermostable mutations reported as ST7 [23]. C176, C238, and C242 are left intact due to their involvement in coordination to Zn^{2+}, which is required for the binding of p53 to DNA. C124A, C275A, and C277A are introduced to remove the rest of the cysteines, and K292C is introduced as the single labeling site. We name the thermostable mutant pseudo-WT. The pseudo-WT p53 with GST tag expressed in *E. coli* was purified after cleavage of the tag. The yield of the purified mutant was 1.7 mg per 1 L of culture, which was improved significantly compared with the yield, 0.4 mg per 1 L of culture, of the WT p53. The increased yield is likely the result of