Biological Science

15A/2003G322

DNA-binding properties of human NF-κB p50 subunit and its mutant form R59E studied by synchrotron small-angle x-ray scattering

Alexander Timchenko[1], Maria Timchenko[2], Viktoria Shyp[1], Kazumoto Kimura[3], Hiroshi Kihara[4*]
[1] Institute of Protein Research, Pushchino, Russia, 142290;
[2] Institute of Theoretical and Experimental Biophysics, Pushchino, Russia, 142290;
[3]Department of Biochemistry Dokkyo University, Mibu, Tochigi 321-02, Japan
[4] Department of Physics, Kansai Medical University Uyamahigashi, Hirakata Osaka 573, Japan

Introduction

The human transcription factor NF-κB plays an important role in tumorigenesis participating in the expression of oncogenes. NF-κB is presented in a tumor cell mainly as heterodimer p50-p65, where p50 subunit is responsible for DNA binding and p65 - for transcriptional activity [1]. For prevention of tumor progression it's necessary to inhibit NF-κB activity. Therefore the problem of NF-κB inhibition is intensively studied now [2]. One of the possible way to inhibit the NF-κB is the usage of mutant forms of NF-κB incapable for DNA binding but keeping the stability and dimerization properties close to those of wild type of protein. Here we present the DNA-binding properties of wild type p50 subunit of NF-κB and its mutant form R59E with considerably reduced DNA-binding activity.

Experimental

Wild type (WT) and mutant form R59E of human p50 subunit of NF-κB (replacement of Arg59 for Glu) were isolated from the *E.coli* BL21(DE3) carried the plasmids encoding corresponding gene. For the construction of gene encoding mutated p50 molecules a plasmid pEt-14b encoding wild type of p50 protein (kindly provided by A.Israel) was used as template. The buffer conditions are: 7.5mM HEPES (pH8.0), 34mM NaCl, 1mM $MgCl_2$, 0.5mM DTT, 0.05mM EDTA. Protein concentrations were in the range 1.5-2.0 mg/ml. 50mkM concentrations of synthetic specific DNA duplexes were used. Synchrotron X-ray measurements were done on the small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q= 0.008-0.15 nm^{-1}.

Results

One can see in Fig. 1 and Fig. 2 that Kratky plots ($I*Q^2$ versus Q) exhibit bell shape for the studied protein and its complexes with DNA-duplexes, indicating a compact structure of particles. The position of maximum corresponds to a dimer of protein. Guinier plots (log I versus Q^2) have nonlinear behavior (data not shown) pointing out some association of protein. The evaluation of radius of gyration and molecular mass from these plots gives values about 4 nm and 90 kD, respectively. These meanings correspond to the dimer of protein. Addition of DNA-duplexes shifts the position of maximum on Kratky plot toward higher Q values indicating some compactization of protein-DNA complexes. At the same time some shift to smaller Q-values is observed for R59E complexes only at the excess of DNA over protein that likely demonstrates the unspecific binding of DNA to protein. This fact correlates with high dissociation constant for R59E found by us in gel-shift experiments. It permits to consider the R59E mutant as perspective one for NF-κB inhibition in a cell.

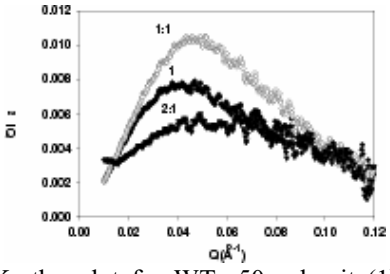

Fig. 1 Kratky plot for WT-p50 subunit (1) and its complex with DNA at the indicating p50:DNA ratio.

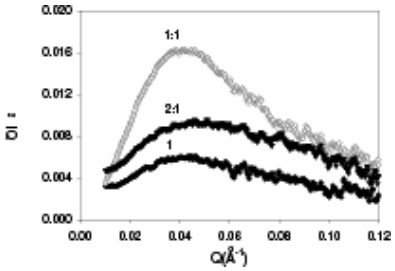

Fig. 2 Kratky plot for R59E (1) and its complex with DNA at the indicating p50:DNA ratio.

References

[1] Perkins, TIBS, **25**, 434, 2000
[2] Huang et al. Oncogene, **20**, 4188, 2001

kihara@makino.kmu.ac.jp