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Influence of point mutations in the NF-κB p50 subunit region interacting with DNA minor groove on the protein stability studied by SAXS

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Introduction

The eukaryotic transcription factor NF-κB takes part in the activation of many cellular oncogenes and viral replication [1]. The key moment of these processes is the interaction of NF-κB with the κB-site of recognition in chromosomal or viral DNA. Therefore the search for NF-κB selective inhibitors is one of the main goals of pharmacotherapy. We suggested a novel approach based on the use of mutant forms of the NF-κB p50 subunit responsible for specific DNA-binding which are unable to bind with DNA. Such mutant proteins have to keep stability and should be able to form dimers with another subunit of NF-κB.

Here we present the study of conformation and stability of the obtained mutant forms at different urea concentration.

Experimental

Human p50 subunit of NF-κB in the form p50-His6 (p50WT, M=44.7kDa) and its mutant forms R57A, R59E, R59A, C62W and K147W were isolated from the BL21(DE3) strain of *E .coli* carried the pEt-14B plasmid kindly provided by A. Israel (France). The buffer conditions are: 7.5mM HEPES (pH8.0), 34mM NaCl, 1mM $MgCl_2$, 0.5mM DTT, 0.05mM EDTA. Protein concentration was 2-3 mg/ml. 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= 0.008-0.2 $Å^{-1}$.

Results

For all studied proteins in native conditions Kratky plot exhibited a bell shape indicating compact structure of proteins existing preferentially in dimeric form. In Fig.1 such plot is presented, as example, for wild type of p50 and R59E in comparison with that for globular protein BSA. From Guinier plot the molecular masses of proteins were evaluated. It was found that at even 3M of urea all proteins (excluding R59E) are monomers. R59E has noticeably higher stability and dissociate on monomers only at 5M of urea (see Table 1). By gel-shift it was found that mutant proteins R57A, C62W and K147W can form a DNA-protein complex with a greatly low affinity as against the wild type, whereas R59A and R59E don't interact with the DNA duplex containing the κB-site. On the basis of our results we can conclude that in perspective the best inhibitors for the regulation of NF-κB-dependent transcription in cells may be obtained on the basis of mutant proteins R59E and R59A.

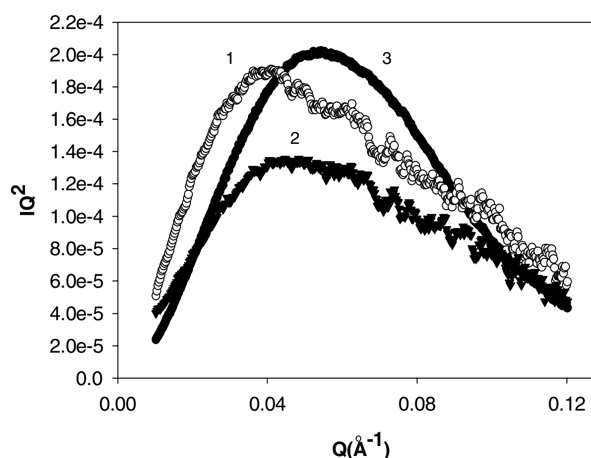

Fig.1 Kratky plot for p50WT (1), R59E (2). BSA (3).

Table 1. Molecular masses of proteins at different concentrations of urea.

C(urea),M	M (p50WT), kDa	M (R59E), kDa
0	114	123
3	43	86
4	52	87
5	-	51

References

1) Perkins N.D. and Gilmore T.D., Cell Death and Differentiation, **13**, 759, 2006.

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