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# Comparative analysis of stability of human NF-κB p50 subunit and its mutant forms C62W and R59E against urea studied by small-angle x-ray scattering

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## Introduction

The human transcription factor NF-κB is an inducible DNA-binding protein which promotes the expression of a lot of genes the majority of which participate in the host immune response [1]. Recently it was shown that the hyperactivation of factor NF-κB plays a crucial role in tumorigenesis resulting in the hyperexpression of oncogenes. Thus 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 comparative analysis of stability of wild type p50 subunit of NF-κB and its mutant forms C62W and R59E with considerably reduced DNA-binding activity at different urea concentrations.

## Experimental

Wild type and mutant forms C62W and R59E of human p50 subunit of NF-κB (replacement of Cys62 for Trp and Arg59 for Glu, respectively) were isolated from the *E.coli* BL21(DE3) carried the plasmids encoding corresponding genes. For the construction of genes 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. Proteins were incubated with the 3, 4 and 5M of urea. 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

To follow the protein conformation it is better to plot scattering patterns in Kratky coordinates ($I*Q^2$ versus Q). One can see in Fig.1 and Fig.2 that Kratky plots exhibit bell shape for all three studied proteins without urea (data for C62W are omitted to avoid crowding), indicating a compact structure of proteins. The position of maximum corresponds to a dimer of proteins. Addition of urea up to 4 M concentration does not change the compact structure of proteins as seen from Figures. At 5 M of urea Kratky plot shows plateau indicating the coil conformation due to unfolding of proteins. At 3 M of urea the position of peak shifts to smaller scattering vector values reflecting the association of proteins. The similar behavior of scattering patterns on urea concentration for all studied proteins means the proximity of stability of mutant forms of p50 subunit to that of wild type. It permits to consider the mutant forms as perspective ones for NF-κB inhibition in a cell.

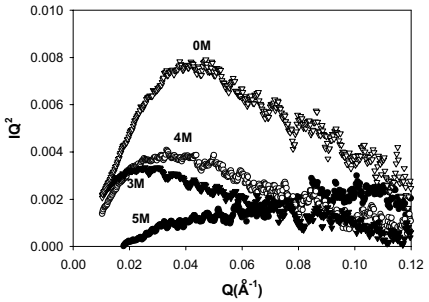


**Fig.1** Kratky plot for wild type of p50 subunit at the indicating urea concentrations.

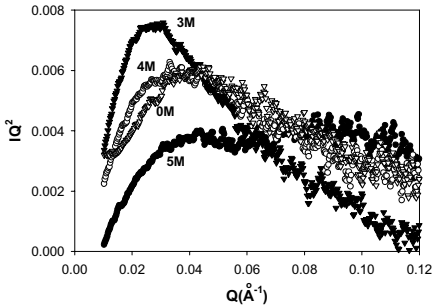


**Fig.2**
Kratky plot for R59E mutant form of p50 subunit at the indicating urea concentrations.

## References

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

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