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NF-κB p50 subunit forms a tetramer in solution at presence of specific DNA duplex as observed by SAXS

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Introduction

The eukaryotic transcription factor NF-κB is a key protein of immune and anti-inflammatory response of organism. X-ray analysis showed that NF-κB interacts with specific DNA duplex, containing κB recognition site, in a form of homodimer (p50-p50) or heterodimer (p50-p65) [1]. However, it is known that for some immediately activated genes there are three κB sites in their promoters, so, one can suppose that NF-κB interacts with DNA in a higher oligomeric form. Here we present the study of conformation of p50 subunit and its R59E mutant form in a free state and in a complex with specific DNA duplex in solution observed by SAXS.

Experimental

Human p50 subunit of NF-κB in the form p50-His6 (p50WT, M=44.7kDa) and its mutant form R59E 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 1-3 mg/ml. 20-mer synthetic specific DNA duplex with κB-33 site was added to protein solution in protein/DNA molar ratio 1:1 and 2:1. 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=0.008-0.2Å^{-1}.

Results

Guinier plots of all samples are shown in Fig.1 in absolute scale. One can see that there is a curvature of all plots at the very small scattering angles reflecting some association of protein. From linear part of Guinier plots we evaluated the molecular mass (M_w) of particles from I(0) and found that in free state p50 has essential presence of tetramer (M_w ~150kD) whereas M_w ~90kD of R59E is close to the value of dimer. The presence of specific DNA duplex increases M_w up to 170kD for both proteins corresponding to M_w of tetramer (see Fig.1). Kratky plot (Fig.2) demonstrates that there is a shift of maximum to larger values of Q for p50 indicating a decrease of particle size what follows also from Guinier plot (Fig.1). It reflects the essential compactization of p50 molecule upon DNA binding. For R59E there is some shift of maximum to smaller values of Q indirectly pointing out the formation of tetramer. Recently we have shown by gel-shift procedure that R59E has low affinity to specific DNA duplexes, and SAXS results may be interpreted as DNA duplexes stimulate the formation of tetramers not causing the essential change of protein conformation in contrary to the p50 case.

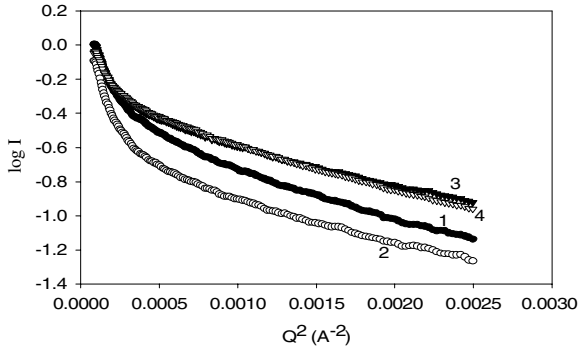

Fig.1 Guinier plot for p50WT (1), R59E (2), p50:DNA (1:1) (3), R59E:DNA(1:1) (4).

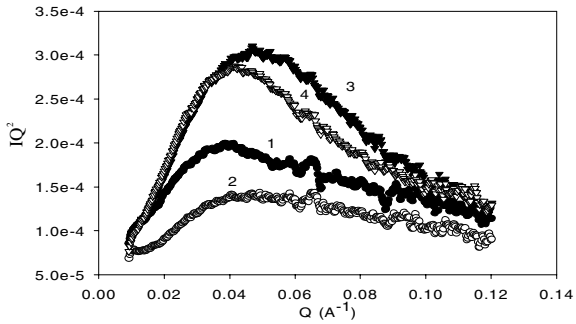

Fig.2 Kratky plot for p50WT (1), R59E (2), p50:DNA (1:1) (3), R59E:DNA (1:1) (4).

References

1) Ghosh, Nature, **373**, 303, 1995.

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