

Oligomeric state of human p50 NF- κ B and its complexes with DNA duplexes at reducing conditions studied by SAXS

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Introduction

The human transcription factor NF- κ B plays the important role in the maintenance of immune homeostasis. Besides it regulates the expression of the genes responsible for occurrence of some oncologic and infectious diseases, including AIDS [1]. At various pathologies the determining moment is hyper-activation of factor NF- κ B and the formation of a specific complex of this protein with its recognition sequence in DNA. It is known, that the DNA binding of NF- κ B in a cell is redox-sensitive. To present time many problems concerning its function are already solved [2]. The problem of the regulation of NF- κ B activity to prevent pathology is intensively studied now. To check the efficiency of protein-DNA interaction usually p50 subunit of NF- κ B, responsible for DNA binding, is used. Here we present the study of solution structure of recombinant p50 subunit NF- κ B conjugated with 19 N-terminal amino acids, including 6 His (p50-His6) and its complexes with synthetic DNA duplexes.

Experimental

Human p50 subunit of NF- κ B 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 MgCl₂, 0.5mM DTT, 0.05mM EDTA. Protein concentration was 3.7 mg/ml. 20-mer synthetic specific and nonspecific DNA duplexes were added to protein solution in protein/DNA molar ratio 1:1 and 4:1, respectively. Synchrotron X-ray measurements were done on the small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors $Q=0.008-0.2\text{ nm}^{-1}$. P(R) distance distribution function was calculated by program GNOM [3].

Results

Here we used p50-His6 to diminish maximally the influence of additional part in protein on its function. Guinier plot of all samples had a curvature at the very small scattering angles reflecting some association of protein. Kratky plot exhibited bell shape indicating compact structure of protein. More information can be extracted

from the analysis of P(R) function presented in Fig.1. One can see that the addition of reducing agent (DTT) sharply decreases the amplitude of P(R) function witnessing the disruption of oligomers preferentially to dimers. This fact sheds light on the known importance of reducing agents for protein-DNA binding. Addition of specific DNA-duplexes causes the further disruption of oligomers with the appearance of monomers (peak near 5nm). At the same time the nonspecific DNA-duplex practically does not influence on the P(R) function in agreement with the independent data on the high dissociation constant of such complex. The above results may contribute the valuable information about NF- κ B-DNA interactions. Further model calculations may present stoichiometry of associates in details. This work was supported in part by the RFBR projects no.04-04-97255.

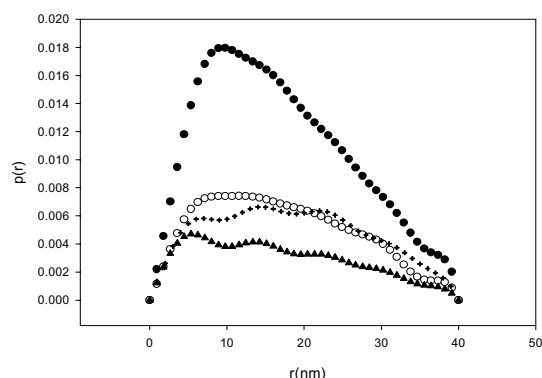


Fig.1 P(R) function for free p50-His6 without DTT (●), with DTT (○) and its complex with specific DNA-duplex (molar ratio 2:1) (□) and the nonspecific one (molar ratio 4:1) (△).

References

- 1) Perkins, TRENDS in Cell Biology, **14**, 64, 2004.
- 2) Ghosh, Nature, **373**, 303, 1995
- 3) Semenyuk, J. Appl. Crystallog., **24**, 537, 1991.

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