

Oligomeric state of human p50 NF- κ B-GST and its complexes with DNA duplexes studied by synchrotron small-angle x-ray scattering

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Introduction

The human transcription factor NF- κ B was found in many tissue-specific cells. It regulates the expression of immunoglobulin light chains in B-cells, as well as the expression of the genes responsible for occurrence of some oncological and infectious diseases, including AIDS. At various pathologies the determining moment is hyperactivation of factor NF- κ B and the formation of a specific complex of this protein with its recognition sequence in DNA. To present time many problems concerning its function are already solved [1]. The problem of NF- κ B inhibition to prevent pathology is intensively studied now. Two possibilities of inhibition can be applied: the usage of synthetic DNA having κ B site of DNA and mutant forms of NF- κ B. To check the efficiency of protein-DNA interaction usually p50 subunit of NF- κ B is used [2]. Here we present the study of solution structure of p50-GST and its complexes with synthetic DNA duplexes including modified ones [3].

Experimental

Human p50 subunit of NF- κ B were isolated from the HB-101 strain of *E.coli* carried the pGEX-1 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 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 $Q=0.008$ - 0.2 nm^{-1} .

Results

Free p50-GST showed about linear shape of Guinier plot with small increase at the very small scattering angles reflecting some association of protein (see Fig.1). Evaluated radius of gyration (R_g) from Guinier plot was $(6.8\pm 0.1)\text{nm}$. Kratky plot exhibited bell shape indicating compact structure of protein. Addition of specific DNA-duplexes causes the nonlinear shape of Guinier plot as seen from Fig.1. pointing out the essential association of protein molecules. The nonlinear shape of Guinier plot for complexes with nonspecific DNA-duplexes is expressed in the less extent. Evaluated molecular masses

from $I(0)$ and the above results showed that free p50-GST has globular conformation and exists as dimer. Binding of specific DNA-duplexes forces p50-GST to be tetrameric and globular. At the same time p50-GST is preferably dimeric upon binding of nonspecific DNA-duplexes. The above results may contribute the valuable information about NF- κ B-DNA interactions. Further model calculations can present stoichiometry of associates in detail.

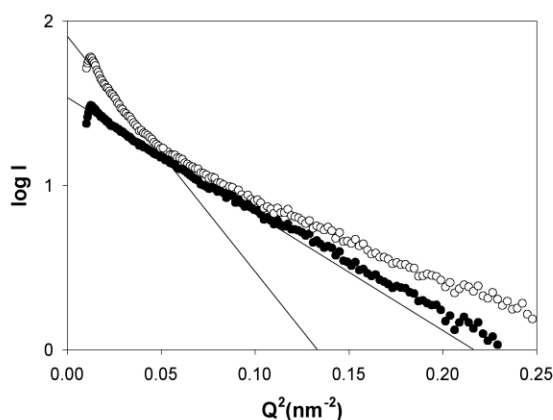


Fig.1 Guinier plot for free p50-GST (●) and its complex with specific DNA-duplex (molar ratio 1:1) (○).

References

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