Conformation of domains of elongation factor eEF-1A *Dictyostelium discoideum* studied by synchrotron x-ray scattering

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Introduction

The eukaryotic translation elongation factor eEF-1A (formerly EF-1α) is a functional analogue of bacterial factor EF-1A (formerly EF-Tu). The function of EF-1A in prokaryotic cells is well studied. EF-1A*GTP delivers the elongator aminoacyl-tRNA to the ribosome and promotes the accurate interaction of the tRNA anticodon with the codon of mRNA located at the ribosomal A-site. Following the codon-anticodon recognition, hydrolysis of GTP in the complex with EF-1A takes place. As a result, the factor affinity for aminoacyl-tRNA and ribosome is lost. EF1A*GDP leaves the ribosome and, after the GDP/GTP exchange, the protein can participate in the next elongation cycle.

The most studied structure of EF-1A is procaryotic one. Protein molecule consists of three distinct domains joining by flexible loops. GDP/GTP exchange dramatically changes mutual positions of the domains toward more compact state demonstrating the high plasticity of EF-1A protein domains [1]. Procaryotic EF1A has globular structure in solution with minor changes in the structure upon aa-t-RNA binding [2]. At the same time we have found that eucaryotic eEF-1A has no fixed rigid structures in solution detected by small-angle neutron scattering and scanning microcalorimetry [3].Here we present the conformation of eEF-1A *Dictyostelium discoideum* domains with and without GDP.

Experimental

Domains of eEF-1A *Dictyostelium discoideum* were isolated from the HB-101 strain of *E.coli* carried the pGEX-dm D plasmid kindly provided by G.Liu (USA). The buffer conditions are: 30mM Tris-HCl (pH7.4), 25mM KCl, 5mM $MgCl_2$, 2mM DTT. Protein concentrations were 1-5 mg/ml. 20mkM and 200mkM concentrations of GDP were used. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q=0.008-0.2 nm^{-1}.

Results

Domain I and II showed about linear shape of Guinier plot with some increase at the very small scattering angles reflecting some association of protein. Evaluated radii of gyration (Rg) from Guinier plot were (8.0+/-0.1)nm and (3.0+/-0.1)nm for domain I and II, respectively. Kratky plots for both domains exhibit bell shape, particularly, domain II as seen from Fig.1. Evaluated molecular masses from I(0) and the above results showed that domain I creates globular associates starting from trimer. Domain II preferably exists as a globular dimer. At the same time, dimer III exhibits essentially nonlinear shape of Guinier plot reflecting noticeable association of particles. Kratky plot for domain III shows two maxima one for associates and another for monomer showing compact conformation of monomer. It occured that GDP influences only on the conformation of domain III stimulating the further association of macromolecules. From the above results one can conclude that domains are globular, and the observed not rigid structure of the whole eEF-1A [3] may be due to flexible link between domains of hinge type. Further model calculations can present stoichiometry of associates in details.

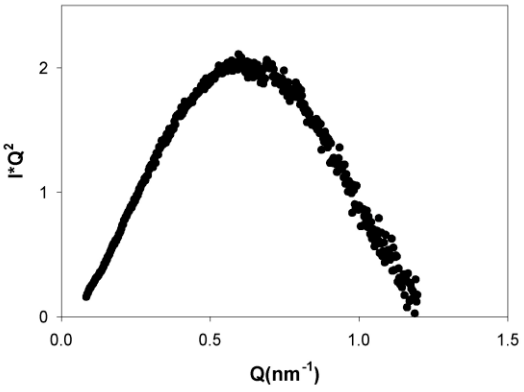

Fig.1 Kratky plot for domain II of eEF-1A.

References

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