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Solution structure of two isoforms of rabbit elongation factor eEF1-A recovered from SAXS data

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Introduction

The main function of higher eukaryotic translation elongation factor eEF1A is the delivery of correct aminoacyl-tRNA to the A site of mRNA-programmed ribosome in translation cycles. There are two tissue and development-specific isoforms of eEF1A, which are 97% homologous. Importantly, despite on strong similarity of amino acid sequences, the isoforms appear to differ in some functions. It was found that the appearance of eEF1A2 in non-inherent tissues can be coupled to the cancer development. We reasoned that the background for the functional difference of eEF1A1 and eEF1A2 might lay in the changes of spatial structure and stability of the proteins. Here we present the solution structure of rabbit eEF1A1 from liver and eEF1A2 from muscle recovered from SAXS data.

Experimental

eEF-1A were purified from rabbit liver and muscle as described in [1]. The buffer conditions are: 30mM Tris-HCl (pH7.5), 10mM KCl, 1mM $MgCl_2$, 6mM β-mercaptoethanol, 20mkM GDP, 20%(v/v) glycerol. Protein concentrations were 2-3 mg/ml. 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=0.008-0.12 $Å^{-1}$.

Results

In our previous publication [2], we reported the difference in structure of two isoforms of eEF1A studied by SAXS technique. We noticed essential higher dimensions of eEF1A1 in correspondence with our previous neutron scattering data [1] and supposed some disorder in this protein. To find the solution structure of these isoforms we developed the special package of programs MOLMOD for recovering structure of multidomain proteins in solution from SAXS data. In this algorithm each domain with known crystal structure is approximated by ball with the radius of gyration of the domain. The SAXS pattern from the system of balls (representing the protein domains) with a variation of distance between them is fitted to the experimental one to find the positions of balls. Centers of gravity of domains are set in the centers of balls and the interdomain links are recovered. The SAXS pattern from such structure is compared with the experimental one. In Fig.1 the result of such fit and plausible solution structure of EF1A1 are presented. The same is drawn in Fig.2 for EF1A2. One can see the essential higher dimension of EF1A1 in respect to EF1A2 in agreement with our previous observation [2].

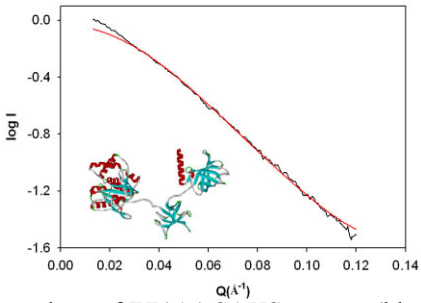

Fig.1 Comparison of EF1A1 SAXS pattern (black) with the calculated one from the model (red).

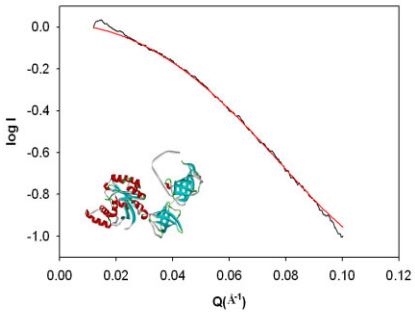

Fig.2 Comparison of EF1A2 SAXS pattern (black) with the calculated one from the model (red).

References

1) Budkevich *et al.*, Biochemistry, **41**, 15342, 2002

2) Timchenko *et al.*, PF reports, **25**, 246, 2008

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