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Comparative analysis of stability of two isoforms of rabbit elongation factor eEF1-A against urea studied by SAXS technique

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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 comparison of stability of rabbit eEF1A1 from liver and eEF1A2 from muscle against urea.

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. Urea concentrations were in the range 0-5 M. 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 $Å^{-1}$.

Results

In our previous publication [1], 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 [2] and supposed some disorder in this protein. Such disorder may influence on its stability. Therefore, here we compared the stability of two isoforms against urea monitored by SAXS technique. In Fig.1 the Kratky plot for eEF1A1 is presented at different concentration of urea. One can see that an unfolding of protein is observed in the region of 2-3 M of urea where evaluated radius of gyration (Rg) from Guinier plot increases from 35A to 52A. In Fig.2 the analogous plot is given for eEF1A2. One can see that in this case an unfolding of protein is observed in the region of 3-4 M of urea where Rg increases from 27A to 52A. Thus, eEF1A1 is less stable isoform of factor as we supposed.

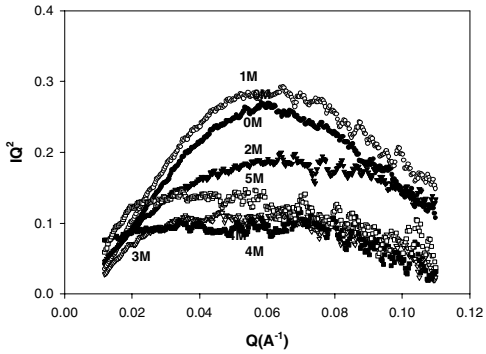

Fig.1 Kratky plot for eEF1A1 at different concentrations of urea (shown in figure).

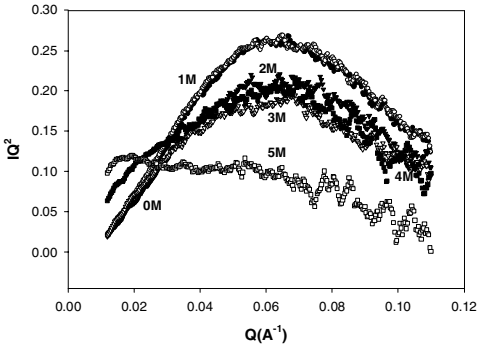

Fig.2 Kratky plot for eEF1A2 at different concentrations of urea (shown in figure).

References

1) Timchenko *et al*., PF reports, **25**, 246, 2008
2) Budkevich *et al.*, Biochemistry, **41**, 15342, 2002

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