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Comparative analysis of solution structure of two isoforms of rabbit elongation factor eEF1-A by SAXS technique

Alexander Timchenko[1], Igor Serdyuk[1], Boris Negrutskii[2], Alexandra Novosylna[2], Evgeniya Deryusheva[3] , Evgeny Prituzhalov[3] ,Kazumoto Kimura[4], Hiroshi Kihara[5*]
[1] Institute of Protein Research, Pushchino, Russia, 142290;
[2] Institute of Molecular Biology & Genetics NAS, Kiev, Ukraine, 03143;
[3] Tula State University, Russia, 300600;
[4] Center of Medical Informatics, Dokkyo-University School of Medicine, Mibu, Tochigi 321-0293,
[5]Dept. of Physics ,Kansai Medical University, Hirakata Osaka 573-1136

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 [1]. 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 The eEF1A1 and eEF1A2 isoforms contain 462 and 463 amino acid residues correspondingly with 34 replacements and sole deletion of penultimate residue in eEF1A1. eEF1A1 has seven modified residues as eEF1A2 is characterized by four modified residues. Since the sporadic amino acid substitutions could not be attributed to the functional difference of the isoforms at the current level of knowledge, we reasoned that the background for the functional difference of eEF1A1 and eEF1A2 might lay in the changes of spatial structure of the proteins. .Here we present the comparison of conformations of rabbit eEF1A1 from liver and eEF1A2 from muscle.

Experimental

eEF-1A were purified from rabbit liver and muscle using a combination of gel filtration, ion-exchange, and hydroxyapatite chromatographies in the presence of 20% glycerol and 20mkM GDP. 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=0.008-0.2 $Å^{-1}$.

Results

Guinier plot for eEF1A1 shows the good linear dependence. Evaluated radius of gyration (Rg) from Guinier plot was (48.0+/-1) Å which is considerably higher than that (25-30 Å) expected for globular protein of such molecular mass (50 kD). At the same time the value of molecular mass evaluated from I (0) is (51.0+/-2.0) kD. In our previous neutron scattering experiments [2] we also detected the high value of Rg for eEF1A1 and concluded that the protein has no fixed rigid structure in solution .For eEF1A2 the Guinier plot demonstrates the nonlinear behavior at the very small scattering angles reflecting some association of protein. It makes difficult to evaluate Rg from the plot. Nevertheless Rg value can be evaluated from the position of maximum on the Kratky plot [3]. In Fig.1 the Kratky plot for eEF1A1 and eEF1A2 are presented. One can see the bell-shape of the plot for eEF1A2. The calculated Rg value was 29 Å. For eEF1A1 there is also maximum but the peak is asymmetrical with small decrease on the right side which may reflect the presence of unstructured regions in the protein. This conclusion is in agreement with our data on sedimentation analysis of eEF1A1. Thus, the conformations of two isoforms of EF1A are different in agreement with our model calculations on the basis of amino acid sequence (unpublished results).

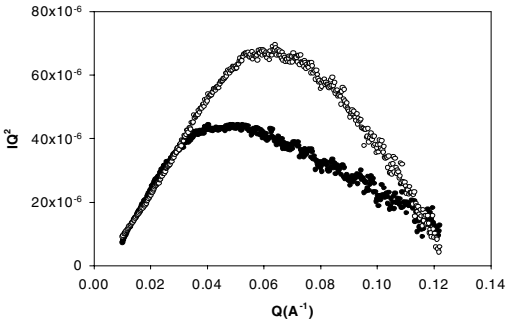

Fig.1 Kratky plot for eEF1A1(•), eEF1A2(o).

References

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* E-mail:kihara@makino.kmu.ac.jp