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Characterization of physical properties of two isoforms of translation elongation factor 1A

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Two tissue-specific isoforms of mammalian translation elongation factor 1A, eEF1A1 and eEF1A2, are 98% similar. However, some of their functions in the organism are different which in some cases could lead to the induction of carcinogenesis. We supposed that slight difference of primary sequences may cause significant differences of spatial structures of eEF1A isoforms affecting, in its turn, the ability of one or another isoform to interact with protein partners. The differential scanning microcalorimetry and circular dichroism in "near" and "far" UV regions were used to determine that potentially oncogenic eEF1A2 isoform possesses a more compact spatial organization than eEF1A1, and the differences are revealed at the levels of both secondary and tertiary structure of proteins.

Keywords*: translation elongation factor eEF1A1, protein biosynthesis, differential scanning microcalorimetry, circular dichroism*

Introduction. eEF1A is believed to be a multifunctional protein, the canonical functions of which are to promote efficient binding of aminoacyl-tRNA to 80S ribosome and to provide correct codon-anticodon interaction in ribosomal A site [1]. Two tissue-specific isoforms of eEF1A have been identified, namely, eEF1A2, expressed only in neural and muscular tissues, and eEF1A1, discovered in all the other tissues [2, 3]. The reason of two isoforms presence is not yet understood. The appearance of eEF1A2 in non-specific tissue has been found to relate to the induction of carcinogenesis [4-8]. It remains unknown in what way eEF1A2, 98% similar to eEF1A1, which is specific for this kind of tissue, may be related to the formation of tumours in the latter. We supposed that even slight aminoacid substitutions in eEF1A2 are capable of causing some divergence in spatial structures of this protein globule, which may result in the modification of its known functions and appearance of some new ones. To check our assumption we have performed the comparative analysis of molecular dynamics of both isoforms, and found out the possibility of differences in the secondary structure and dynamics of these protein globules [9, 10]. Current work presents experimental data on the comparison of eEF1A1 and eEF1A2 using differential scanning microcalorimetry and circular dichroism (CD) in "near" and "far" UV regions.

Materials and Methods. *Isolation and characterization of eEF1A*. eEF1A1 was isolated from

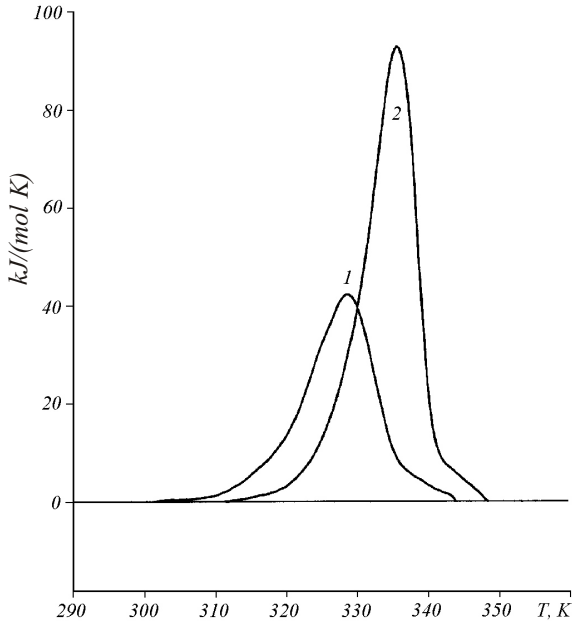

Fig.1 Temperature dependence of excessive heat capacity for eEF1A in 30 mM tris-HCl, pH 7.5, containing 20% glycerol, 6 mM в-mercaptoethanol, 1 mM MgCl, and 150 mM KCl:*1* – melting curve of eEF1A1; *2* – melting curve of eEF1A2

rabbit liver using the combination of ion-exchange chromatography and gel-filtration, as described in [11], with slight modifications.

eEF1A2 was isolated from rabbit muscles using the same scheme, but for the first stage of purification – chromatography on Sephacril S-400, which did not effect the preparation purity.

The activity of eEF1A was determined in the reaction of GDP/[^{3}H]GDP exchange [12].

Differential scanning microcalorimetry. Calorimetric measurements were conducted in precision scanning microcalorimeter SCAL-1 (SCAL Co. Ltd., Pushchino, Russia) in glass cells (0.3 ml) with the rate of 1.0 K per 1 min under excessive pressure of two atmospheres [13]. The dialysis of all protein samples against corresponding buffer was conducted before measurements. Concentrations of proteins used were in the range of 2.3-2.5 mg/ml. Thermodynamic analysis of the profile of the excess of heat capacity was carried out according to [14].

CD in "far" and "near" UV regions. The secondary structure of proteins was investigated by CD spectroscopy using spectropolarimeter JASCO-600 (Japan) at wavelengths of 190-250 nm ("far" UV range) and 250-310 nm ("near" UV range). Molar ellipticity was calculated using the following equation:

$$[\ ] = [\ ]_{exp}\, M_{res}/(LC),$$

where C – concentration of protein (mg/ml), L – optical path length of cuvette (mm), $[\ ]_{exp}$ – measured ellipticity (degrees) and M_{res} – mean molecular mass of peptide residue (Da), calculated from its aminoacid sequence. Measurement of "far" UV was carried out in 0.1 mm cuvette, and measurement of near CD was conducted in 1 mm cuvette. Concentration of proteins was 1 mg/ml.

Results and Discussion. *Differential scanning microcalorimetry*. Differential scanning microcalorimetry allows obtaining and comparing thermodynamic parameters, characteristic for heat-induced conformational changes of proteins [14, 15].

Computer analysis of molecular dynamics of eEF1A isoforms testified to the possibility of conformational changes in eEF1A1 molecule due to interdomain interactions, while eEF1A2 is specific for a more closed conformation [9, 10]. Therefore, one can expect the comparison of the thermodynamic parameters of the two isoforms of eEF1A to be informative to reveal the difference in thermal stability and enthalpy of denaturation. Since the stability of protein molecule is associated with its structure, the analysis of thermodynamic characteristics of the isoforms may evidence either to structural similarity or difference in their structures, and characterize relative compactness of these proteins.

Temperature dependences of the excessive partial heat capacity are presented in Fig. 1. Almost two-fold difference of denaturation heat of two isoforms is observed. For eEF1A1 H_{total} is 580.0 Kj\mol, while for eEF1A2 H_{total} is 910.0 Kj/mol. Comparison of the melting curves also shows that melting of the eEF1A1 molecule starts earlier than that of eEF1A2. The maxima of transition are 55.5°C for eEF1A1 and 62.7°C for eEF1A2. Half-width temperature of the transition (T) equals 11.0°C for eEF1A1, and 8.1°C for eEF1A2. Thus, eEF1A isoforms possess different spatial structure. Significant increase in enthalpy of denaturation

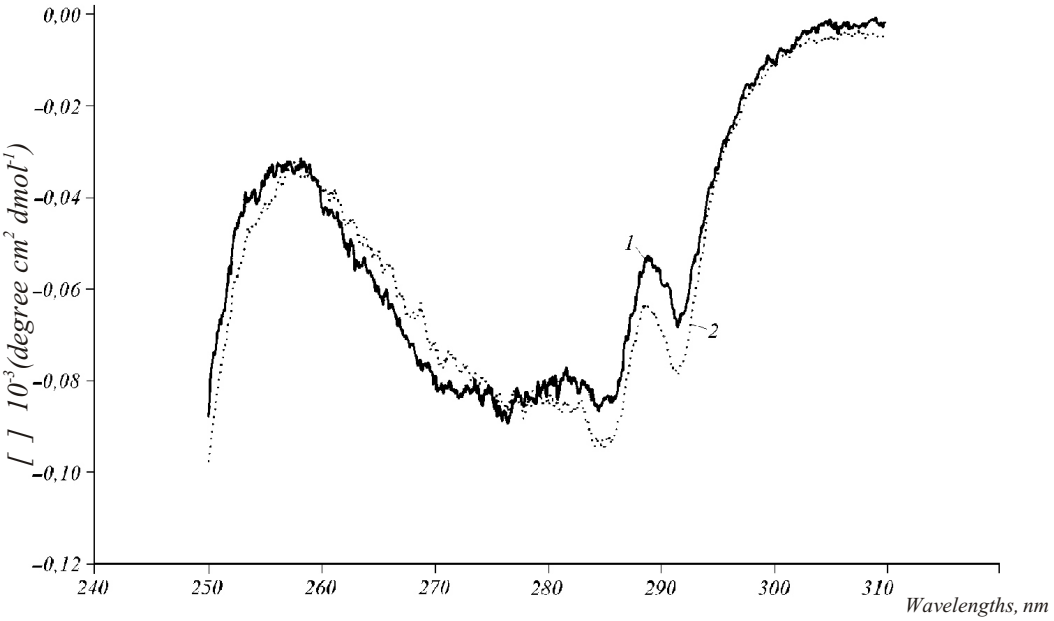

Fig.2 CD spectra of eEF1A isoforms in near UV region: *1* – eEF1A1; *2* – eEF1A2

and decrease in half-width transition at eEF1A2 melting testify to a more compact conformation of eEF1A2, which has been earlier assumed using the data of molecular dynamics [9, 10].

"Near" UV CD. CD spectrum in near UV region (250-350 nm), presenting signals of such chromophores as aromatic amino acids and disulphide bonds, may provide useful information about the tertiary structure of a protein.

Signals in 250-270 nm region are specific for phenylalanine residues, 270-290 nm region – for tyrosine, and the ones in 280-300 nm region are inherent to tryptophan. Disulphide bonds provide weak signals along the entire near UV spectrum [16, 17].

Taking into account that both eEF1A1 and eEF1A2 are 98% similar, have the same positions in the primary structures, and contain almost equal amount of aromatic amino acids (there is only one substitution of Phe for Ser in eEF1A2; overall amount of amino acids in eEF1A1 (eEF1A2) is Trp – 5(5), Tyr – 12(12), Phe –14(13)), the mentioned method could be useful to test the identity or difference of tertiary structures of the isoforms.

Near UV CD spectra of eEF1A1 and eEF1A2 are shown in Fig.2. The difference in CD signals was observed in the entire spectrum of near UV region. We could not exclude the fact that alterations in the Phe-specific region of CD spectrum might result partially from the difference in 393rd position of primary structures of the isoforms (Phe393 in eEF1A1 or Ser393 substitution in eEF1A2). However, the difference in the rest of the spectrum is the direct consequence of changes in the tertiary structure of these proteins. Therefore, the data of near UV CD testify in favour of evident differences in tertiary structures of the isoforms.

"Far" UV CD. It proved to be important to determine whether local differences of spatial structures of eEF1A isoforms are accompanied by changes in the secondary structure of these proteins. The latter can be investigated using CD spectroscopy in far UV region (190-250 nm). Peptide bonds become chromophores at these wavelengths, and signal appears if the bonds are in regularly folded surrounding. The specific form and size of CD spectrum are mainly conditioned by -helical structures in protein molecule [18].

Far UV CD spectra of eEF1A1 and eEF1A2 are shown in Fig.3. The increase in the molar ellipticity peak at 210 nm was observed for eEF1A2 as compared to eEF1A1. Since this wavelength is specific for -helical structures, the increment of CD signal evidences to partial loss of -helical structures in the secondary structure of eEF1A2. These data correlate with the results of our molecular dynamics simulation on partial unwinding of -helix Lys36-Glu48 of eEF1A2 [9, 10].

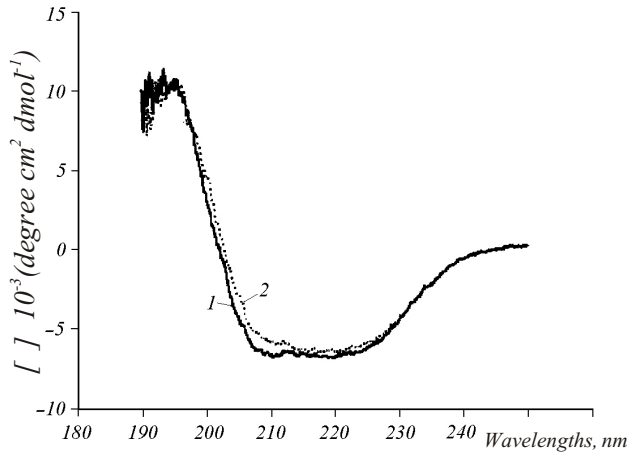

Fig.3 CD spectra of eEF1A isoforms in far UV region: *1* – eEF1A1; *2* – eEF1A2

Therefore, the comparative analysis of spatial structures of two tissue-specific eEF1A isoforms, using biophysical methods, has been carried out for the first time. One of the isoforms is involved in ovarian cancer and cancer of lungs in humans [4, 5, 7, 8]. The structure of eEF1A2 has been shown as more compact and characterized by partial unwinding of -helix Lys36-Glu48. Some differences have been also discovered in the tertiary structures of protein isoforms. These specificities of spatial organization may result in the exposure of functional sites in eEF1A2, capable of binding signal molecules, which may serve as one of the reasons for oncogenicity of this isoform.

Current work is among the first studies, proving that 98% similar isoforms of homologous proteins may have different spatial conformations. Thus, not only the changes in the primary structure, revealed *e.g.* in the appearance of new domains, or in elimination or appearance of new sites of post-translational modifications, but also the changes in their spatial conformation are important for the functional divergence of the protein isoforms. These data will allow extending the direction of searching for multiple families of various isoproteins.

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Характеристика фізичних властивостей двох ізоформ фактора елонгації трансляції eEF1A

Резюме

Дві тканиноспецифічні ізоформи фактора елонгації 1А ссавців (eEF1A1 і eEF1A2) ідентичні на 98 %. Але деякі їхні функції в організмі значно різняться, що в окремих випадках може бути пов'язано з індукцією канцерогенезу. Ми припустили, що незначна різниця у первинних послідовностях може призводити до суттєвих відмінностей у просторовій структурі ізоформ eEF1A. Це, в свою чергу, може впливати на здатність тієї чи іншої ізоформи взаємодіяти з білками-партнерами. В даній роботі методами диференційної сканувальної мікрокалориметрії і кругового дихроїзму на ділянках далекого і ближнього УФ встановлено, що потенційно онкогенна ізоформа eEF1A2 має компактнішу просторову структуру, ніж eEF1A1, причому відмінності проявляються на рівні як вторинної, так і третинної структури білків.

Ключові слова: фактор елонгації трансляції eEF1A, біосинтез білка, диференційна сканувальна мікрокалориметрія, круговий дихроїзм.

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