STRUCTURE AND FUNCTIONS OF BIOPOLYMERS

Eukaryotic elongation factor 1a disintegrates aggregates of phenylalanyl-trna synthetase

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Translation elongation factor 1A (eEF1A) provides binding and transporting of the appropriate codon-specified aminoacyl-tRNA to the aminoacyl site of the ribosome. Two active tissue-specific isoforms of eEF1A have been identified in mammals. Herewith we report that two isoforms of eEF1A disintegrate the aggregates of phenylalanyl-tRNA synthetase like molecular chaperones which are involved in protein folding and renaturation after stress.

Key words: translation elongation factor 1A, phenylalanyl-tRNA synthetase, chaperone, aggregation, thermodenaturation.

Introduction. Eukaryotic translational elongation factor (eEF1A) plays an important role in the process of protein synthesis, catalyzing GTP-dependent binding of aminoacyl-tRNAs to the ribosomal A site. eEF1A is a part of the eEF1H complex which also contains eEF1вгд-subunits catalyzing GTP/GDP exchange in eEF1A [1]. eEF1A exists in two isoforms – eEF1A1 and eEF1A2. The first form is expressed in the majority of tissues but in the process of development it is replaced by the other form – eEF1A2 – in neural, cardiac, and muscular tissues [2].

eEF1A is one of the most abundant proteins in the cell, after actin and tubulin. This fact gave grounds for searching its additional functions. In fact, the results of recent researches indicate a great number of so called non-canonic functions of the factor. There are functions among them, connected with the chaperon-like capacity of eEF1A. For example, eEF1A binds actin filaments and microtubes *in vitro* and *in vivo* [3] and influences assembly and stability of cytoskeleton components [3, 4]. It reminds molecular chaperons properties and their ability to control multimolecular aggregation. Several works have shown that prokaryotic translational elongation factor 1A is capable of binding and renaturating denatured and incorrectly folded proteins into their functionally active conformation [6, 7], which indicates possible participation of EF1A in protein folding.

Molecular chaperons are a family of polypeptide-binding proteins, which participate in numerous processes in the cell, among which are protein folding *de novo*, formation of multi-molecular complexes, degradation of irreversibly damaged proteins [8].

Possible chaperon-like capacity of eEF1A from rabbit liver was studied in our laboratory, namely its ability to sta-

bilize and restore functionally active conformations of ARSs [9, 10].

In this study, using the method of dynamic light scattering we have shown that both isoforms of the eEF1A similar to molecular chaperons disintegrate the aggregates of PheRS formed in the process of thermal denaturation.

Materials and Methods. The following reagents were used in the work: ATP, GTP, phenylalanyl (Sigma, USA); tris HEPES (Calbiochem, USA); GDP, DTT (Boehringer Mannheim, Germany); в-mercaptoethanol, glycerin, $MgCl_2$(Merck, Germany); PMSF (Serva, Germany); SP-Sepharose, Sephacryl S-400, heparin-Sepharose (Pharmacia, Sweden); filters GF/C, DEAE-cellulose (Whatman, Great Britain); nitrocellulose filters (pore diameter 0.45мм) (Sartorius GmbH, Germany); hydroxyapatite (Bio-Rad, USA); [^{14}C] phenylalanyl (50Ci/mol, Amersham, Great Britain). Other reagents were of analytical and special grade.

Phenylalanyl tRNA-synthetase (PRS) was isolated from rabbit liver as described in [11], with the exception for using heparin-Sepharose instead of tRNA-Sepharose on the last step of purification.

The initial rate of tRNA aminoacylation was measured as earlier described [11, 12]. The specific activity of the aminoacyle-tRNA synthetase was calculated on the basis of the dependence of initial aminoacylation rate on the enzyme concentration.

eEF1A1 was isolated from rabbit liver according to the method, developed in our laboratory [13]. eEF1A2 was isolated from rabbit skeletal muscles. The scheme of eEF1A2 isolation included all the steps of purification, described in [13], except gel filtration on Sephacryl S-400. The activity of two isoforms of eEF1A was determined in the reaction of GDP/[^{3}H]GDP exchange, as described in [13].

The distribution function of PheRS molecules according to the size was studied using laser correlation spectrophotometer ZetaSizer-3 "Malvern Instrument", which was equipped with multi computing correlator type 7032ce, helium-neon laser LH-111, 25mW, wave-length 633nm and constant temperature holder. The measurements were performed in glass cuvette, diameter 10mm. The registration and statistics were performed during 60-180sec, the obtained autocorrelation function (ACF) was processed using standard computer programme PCS – Size mode v.1.61.

Results and Discussion. As it was shown in the previous works [9, 10] the PheRS activity in the reaction of tRNA aminoacylation reduced substantially after 10min incubation at 42°C. The recovery of the activity was achieved by the addition of eEF1A to the inactivated enzyme. Moreover, complete preservation of the enzyme activity during thermal inactivation at 42°C was observed in the presence of eEF1A. Thus, it was shown that eEF1A is capable of restoring the activity of partly inactivated PheRS and keeping it in functionally active conformation at conditions of thermal denaturation.

PheRS is a special case among aminoacyl-tRNA synthetases because of its ability to associate with ribosomes, high value of isoelectric point (pI=8.45), and complex sub-

Table 1.
Size distribution of PheRS molecules during thermal denaturation

PheRS concentration	Rh, nm				
	0 min, 42?C	5 min, 42?C	10 min, 42?C	15 min, 42?C	20 min, 42?C
0.2 μ PheRS	6.9 ± 0.1	3.4 ±0.08	7.1 ±0.08	10.2 ±0.07	10.2 ± 0.1

Table 2.
The influence of eEF1A1 and eEF1A2 on denatured PheRS

Conditions	Rh, nm			
	0 min, 25?C	5 min, 25?C	10 min, 25?C	15 min, 25?C
0.2 μM PheRS(20 min,42?C)+10 μM eEF1A1	12.4 ±0.1	12.5 ±0.06	7.5 ±0.1	7.5 ±0.05
0.2 μM PheRS(20 min,42?C)+10 μM eEF1A2	12 ±0.07	12.2 ±0.05	8 ±0.08	7.9 ±0.1

unit structure $б_2в_2$-type [14]. It is noteworthy that neither individual enzyme subunits nor subunit dimers possess enzymatic activity [15].

To characterize native, denatured, and renatured states of PheRS we chose the method of dynamic light scattering (DLS). This method, which is also called quasielastic light scattering (QELS) or photon-correlation spectroscopy (PCS), is a noninvasive method of detecting molecules in solutions or particles in suspensions, permitting the analysis of different dynamic processes in solutions. The advantage of this method is a possibility of working with relatively low protein concentration (0.05 – 1mg/ml) as well as in the presence of glycerin which is of principal importance for performing experiments with PheRS and eEF1A.

The value of the hydrodynamic radius R_h obtained for native PheRS corresponds to the literature data. R_h for human PheRS was found to be 6.74nm at 20°C [17] while R_h for rabbit PheRS obtained at our experiments is 6.9±0.1nm (Table 1).

To denaturate PheRS the enzyme preparation was diluted to the concentration of 0.05mg/ml (0.2мM) in the 25mM KPO_4 buffer, containing pH7.5, 25mM KCl, 15% glycerol, 6mM 2-mercaptoethanol, and then incubated at 42EC for 0 – 20min.

Thermal denaturation of proteins is often accompanied by the aggregation of denatured molecules. As shown in Table 1 the kinetics of thermal denaturation of PheRS has a complex character. At first the accumulation of inactive monomers (Rh=3.4±0.08, which corresponds to the size of individual enzyme subunit) occurs during denaturation, after 10min the aggregates accumulation is observed. The aggregates consist of 3 protein molecules according to the R_h value. These data correlate with the results presented in the work [15], where a tendency to aggregation was shown for individual subunits of PheRS.

The ability of eEF1A to restore the native conformation of denatured PheRS was checked in the series of experiments, when the enzyme activity was measured after 10 min preincubation at 25°C of thermally denatured PheRS with eEF1A. It was shown that after such preincubation of the inactivated enzyme with either isoform of the elongation factor (molar ratio PheRS/eEF1A1 or eEF1A2=1/50) the value of hydrodynamic radius became close to the value of the native enzyme(Table 2). Some difference in the values of R_h for native and renatured phenylalanyl-tRNA synthetase may be explained by the formation of the PheRS•eEF1A complex in the process of aggregates disruption. This result along with the fact that eEF1A restores the activity of PheRS at the same conditions [10] suggests that the factor, similar to the molecular chaperons, is able to restore native state of PheRS after its thermal denaturation.

Chaperon-like activity of eEF1A acquires special importance in the cells of higher eukaryotes, characterized by a high level of compartmentalization of the translational apparatus. eEF1A seems to support the functionally active conformation of protein synthesizing machine in translational compartments during consecutive elongation cycles.

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Т. А. Лукаш

Эукариотический фактор элонгации трансляции 1А разрушает агрегаты фенилаланил-тРНК синтетазы

Резюме

Фактор элонгации трансляции 1А обеспечивает транспорт и связывание соответствующей кодон-специфичной аминоацил-тРНК синтетазы с аминоацильным сайтом рибосомы. В клетках млекопитающих выявлены две тканеспецифичные изоформы eEF1A. Показано, что eEF1A1 и eEF1A2 подобно молекулярным шаперонам, участвующим в фолдинге и ренатурации белка, разрушают агрегаты фенилаланил-тРНК синтетазы.

Ключевые слова: фактор элонгации трансляции 1А, фенилаланил-тРНК синтетаза, шаперон, агрегация, термоденатурация

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