International Journal of Biological Macromolecules 68 (2014) 225–232

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Dual effect of arginine on aggregation of phosphorylase kinase

Tatiana B. Eronina[a,*,1], Natalia A. Chebotareva[a,1], Nikolai N. Sluchanko[a], Valeriya V. Mikhaylova[a], Valentina F. Makeeva[a], Svetlana G. Roman[a], Sergey Yu. Kleymenov[b], Boris I. Kurganov[a,*]

[a] Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, Moscow 119071, Russia
[b] Kolsov's Institute of Developmental Biology, Russian Academy of Sciences, Vavilova 26, Moscow 119991, Russia

ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form 24 April 2014
Accepted 29 April 2014
Available online 9 May 2014

Keywords:
Phosphorylase kinase
Arginine
Aggregation
HspB6
HspB5
Ca^{2+}, Mg^{2+} ions

ABSTRACT

Arginine is widely used in biotechnology as a folding enhancer and aggregation suppressor. However, its action on the stability of complexly organized oligomeric proteins, on the one hand, and its role in the formation of supramolecular structures, on the other hand, are poorly known. The investigation is concerned with the effects of arginine on protein–protein interactions using phosphorylase kinase (PhK) as an example. PhK, a 1.3 MDa $(\alpha\beta\gamma\delta)_4$ hexadecameric complex, is a Ca^{2+}-dependent regulatory enzyme that catalyzes phosphorylation and activation of glycogen phosphorylase *b*. On the basis of light scattering measurements it was shown that arginine induced aggregation of Ca^{2+}-free PhK. On the contrary, when studying Ca^{2+}, Mg^{2+}-induced aggregation of PhK at 37 °C, the protective effect of arginine was demonstrated. The data on analytical ultracentrifugation are indicative of disruption of PhK hexadecameric structure under the action of arginine. Though HspB6 and HspB5 suppress aggregation of PhK they do not block the disruption effect of arginine with respect to both forms of PhK (Ca^{2+}-free and Ca^{2+}, Mg^{2+}-bound conformers). The dual effect of arginine has been interpreted from view-point of dual behaviour of arginine, functioning both like an osmolyte and a protein denaturant.

1. Introduction

Phosphorylase kinase (PhK; EC 2.7.1.38) catalyzing phosphorylation and activation of glycogen phosphorylase *b* (Ph*b*) plays a key role in the cascade system of regulation of glycogen metabolism in skeletal muscle [1–4]. The PhK with molecular mass of 1320 kDa has a complex molecular organization and consists of four subunits forming hexadecamer $(\alpha\beta\gamma\delta)_4$ [5], where the γ-subunit possesses the catalytic activity and α-, β- and δ-subunits regulate its activity [5–7]. Ca^{2+} and Mg^{2+} ions stimulate PhK activity by inducing changes in the tertiary and quaternary structure of the molecule [7–9] and also stimulate association of PhK hexadecamer molecules [10–13]. Ca^{2+}-free PhK and PhK molecules in the presence of Ca^{2+} and Mg^{2+} ions have different conformations and physico-chemical properties [7,9]. The fact that Ca^{2+}, Mg^{2+}-PhK has dramatically less negative zeta potential in comparison with Ca^{2+}-free PhK can explain self-association of PhK in the presence of Ca^{2+} and Mg^{2+} ions.

Chebotareva et al. [14] proposed that PhK could participate in regulation of the chaperone capacity of the cell. This hypothesis was put forward on the basis of the data pointing on the interaction between native PhK and several members of family of small heat shock proteins (sHsps), namely HspB1 (Hsp27) or α-crystallin, under crowding conditions at 20 °C [12,14]. The addition of sHsps to the PhK solution resulted in the disappearance of large-sized species of PhK. It was suggested that native PhK modulates oligomeric state and chaperone-like activity of HspB1 [14].

The family of sHsps is characterized by a common α-crystallin domain located in the C-terminal part of the molecule and by their ability to prevent irreversible protein aggregation by binding non-native target proteins [15]. HspB5 and HspB6 proteins are typical representatives of sHsps having molecular masses of subunits equal to 20.2 and 17.1 kDa, respectively. Both sHsps are ubiquitously expressed in human tissues and play an important role in maintaining cellular proteostasis [16], but demonstrate almost opposite oligomeric states—HspB5 forms large assemblies of highly dynamic quaternary structure [17], while HspB6 predominantly forms dimers with pronounced tendency to self-association [18]. The detailed mechanism of anti-aggregating action of sHsps remains

* Corresponding authors. Tel.: +7 495 9525641; fax: +7 495 9542732.
E-mail addresses: eronina@inbi.ras.ru (T.B. Eronina), kurganov@inbi.ras.ru (B.I. Kurganov).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijbiomac.2014.04.056
0141-8130/

largely unknown. One of the possibilities is that the large assemblies of sHsps undergo reversible dissociation into suboligomeric species followed by interaction with denatured proteins and subsequent reassociation into large chaperone–substrate complexes [19–22]. However, it is not clear, why some sHsps are inherently present in the form of large oligomers (e.g., HspB1, HspB5) and others—predominantly in dissociated forms (e.g., HspB6, HspB8), while all of them reveal chaperone-like activity. The study of interaction of PhK with HspB5 and HspB6 is of interest from view-point of the deciphering the PhK role in regulation of chaperone potential of the cell.

Arginine (Arg) is widely used in the biotechnology and pharmaceutical industries for increasing protein solubility [23,24], improving protein refolding yield [25,26], suppressing protein aggregation [24,27–30]. The extensive literature on the role of Arg in specific and non-specific protein–protein interactions is reviewed in refs. [23,31–35].

Analysis of the literature data shows that Arg can exhibit the dual effect on protein aggregation, suppressing or facilitating the aggregation process. Suppression of protein aggregation by Arg can be attributed to the interaction of Arg and its clusters with hydrophobic regions of the protein molecule [31,33,36–38]. Binding of Arg prevents intermolecular hydrophobic interactions by the shielding of hydrophobic regions on the protein surface. Facilitation of protein aggregation is presumably due to the conformational destabilization of protein by interaction between the guanidinium group of Arg and some acidic residues of protein [38–40].

According to Sharma et al. [35,41] Arg may contain two properties which explain its dual behaviour: first, it should contract the unfolded state and second, it should also behave like a denaturant. These authors have shown that Arg interacts both with the protein backbone and also with the side chains, while traditional osmolytes do no interact with the side chains.

It should be noted that the majority of the investigations of the effects produced by Arg was carried out with monomeric proteins. One can expect that the testing of the action of Arg on complexly organized proteins will result in the discovery of hitherto unknown properties of Arg. PhK is a suitable model for such investigations because of its complex quaternary structure and ability to form supramolecular assemblies in the presence of Ca^{2+} and Mg^{2+} ions.

The goal of the present work was to study the effect of Arg, HspB5 and HspB6 on Ca^{2+}, Mg^{2+}-induced association of PhK at 37 °C. The fact that self-association of PhK is suppressed by HspB5 and HspB6 is indicative of interaction between PhK and these sHsps. It is known that Arg can modulate the chaperone-like activity of α-crystallin [42] and HspB5 [43]. Therefore the experiments on combined action of Arg and sHsps under study were carried out. The ability of Arg to inhibit PhK self-association can be defined as a value of semi-saturation concentration of Arg. It was surprising that in the case of Ca^{2+}-free PhK Arg provoked protein aggregation. Arg-induced aggregation suppressed by HspB5 and HspB6. The stoichiometry of PhK–HspB6 complex was determined from the dependence of the initial rate of PhK aggregation on HspB6 concentration. The data on analytical ultracentrifugation indicate that Arg induces disrupture of the hexadecameric structure of PhK molecule. This effect was observed both for Ca^{2+}-free and Ca^{2+}, Mg^{2+}-bound conformers of PhK.

2. Experimental

2.1. Materials

HEPES, Na-glycerol β-phosphate, L-arginine monohydrochloride, tris(hydroxymethyl)aminomethane (Tris), 2-mercaptoethanol (ME), phenylmethyl sulfonyl fluoride (PMSF), ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) were purchased from "Sigma" (USA), dithiothreitol (DTT) was purchased from "Panreac" (Spain), trimethylamine N-oxide dihydrate (TMAO) was purchased from "Fluka" (USA), NaCl was purchased from "Reakhim" (Russia). All solutions for the experiments were prepared using deionized water obtained with the Easy-Pure II RF system (Barnstead, USA).

2.2. Isolation of PhK, HspB5 and HspB6

PhK was purified from rabbit skeletal muscle as described earlier [13]. Preparations of PhK in 25 mM Na-glycerol β-phosphate buffer, pH 7.05, containing 1 mM EDTA, 0.5 mM ME and 50% glycerol were stored at −20 °C. Prior to experiments, PhK was dialyzed against 40 mM HEPES buffer, pH 6.8, containing 100 mM NaCl, 0.2 mM DTT and 0.1 mM EGTA at 4 °C for a night with two changes of dialyzing buffer.

Cloning of the full-length cDNAs of human HspB6 wild type protein (Uniprot ID O14558) and HspB5 (Uniprot ID P02511) was described in refs. [44,45]. Proteins were expressed in *E. coli* using induction by isopropyl-β-thio-galactoside as described earlier [44,45]. Overexpressed proteins were extracted by sonication and then fractionated by ammonium sulfate followed by ion-exchange chromatography and size-exclusion chromatography as previously described [46,47]. Fractions from the size-exclusion chromatography column containing purified proteins were combined, dialyzed against buffer B (20 mM Tris–HCl buffer, pH 7.6, containing 10 mM NaCl, 0.1 mM EDTA, 0.1 mM PMSF and 15 mM ME), concentrated on Amicon concentrators (Millipore) and stored frozen at −20 °C. Protein concentration was determined using a Nanophotometer P330 (Implen) using extinction coefficients $A_{280}^{0.1\%}$ for PhK equal to 1.24 [5] and for HspB6 and HspB5 equal to 0.582 and 0.693, respectively [47]. All proteins were homogeneous according to SDS-gel electrophoresis [48].

2.3. Aggregation kinetics studies

The kinetics of PhK aggregation at 37 °C was studied in 40 mM HEPES buffer, pH 6.8, containing 100 mM NaCl, 0.2 mM DTT and 0.1 mM EGTA by registration of the increment in the light scattering intensity using a commercial Photocor Complex (Photocor Instruments, Inc., USA) with a He–Ne laser (Coherent, USA, Model 31-2082, 632.8 nm, 10 mW) as a light source. Dynamic light scattering (DLS) measurements and calculation of the hydrodynamic radius (R_h) of the protein aggregates were performed as described in our previous works where DLS was used for the study of thermal aggregation of proteins [22,49–53]. Cary Eclipse spectrofluorometer (Varian Inc., Australia) equipped with automatic Peltier multicell holder was also used for the kinetic experiments.

The aggregation process was initiated by the addition of an aliquot of PhK to the final volume of 0.5 mL. To study the effect of Arg, chaperones or crowding agents on PhK aggregation, the latters were preincubated with buffer in the cell for 5 min before the addition of an aliquot of the protein.

To analyze the kinetic curves of PhK aggregation registered by the measurements of the increment in the light scattering intensity (I) and to characterize of the anti-aggregation activity of chaperones of protein nature and chemical chaperones, we used the approaches elaborated by us previously [30,54–57]. To calculate the lag period (t_0) on the dependences of I on time and to characterize the initial rate of aggregation, we used the following empiric equation for description of the initial parts of the kinetic curves:

$$I = I_0 + v(t - t_0)^2, \quad (1)$$