International Journal of Biological Macromolecules 50 (2012) 1341–1345

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Concentration dependence of chaperone-like activities of α-crystallin, αB-crystallin and proline

Svetlana G. Roman[a,b,*], Natalia A. Chebotareva[a], Boris I. Kurganov[a]

[a] Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, Moscow 119071, Russia
[b] Department of Physics, Moscow State University, Leninskie Gory, Moscow 119992, Russia

ARTICLE INFO

Article history:
Received 5 December 2011
Received in revised form 16 March 2012
Accepted 22 March 2012
Available online 29 March 2012

Keywords:
α-Crystallin
αB-Crystallin
Glycogen phosphorylase *b*
Proline
Aggregation
UV irradiation

ABSTRACT

Chaperone-like activities of α-crystallin, αB-crystallin and proline were studied using a test system based on aggregation of UV-irradiated glycogen phosphorylase *b* (Ph*b*) from rabbit skeletal muscle. The biphasic character of the dependence of the initial rate of aggregation (v_{agg}) of UV-irradiated Ph*b* on the concentration of α-crystallin or αB-crystallin is indicative of the existence of two types of chaperone–protein substrate complexes differing significantly in affinity between the components of the complex. The dependence of v_{agg} on the proline concentration is sigmoid (Hill coefficient is equal to 1.6) suggesting that the positive cooperative interactions between the proline molecules bound on the surface of the protein particles occur. When studying the combined suppressive action of α-crystallin and proline on aggregation of UV-irradiated Ph*b*, a slight antagonism between proline used at a fixed concentration (0.15 M) and α-crystallin was observed. At higher concentration of proline (0.5 M) each chaperone acts independent of one another.

1. Introduction

The chaperone-like activities of α-crystallin, one of the representatives of the family of small heat shock proteins [1–6], and chemical chaperone proline [7–13] were studied by numerous investigators using different aggregation test systems (thermal aggregation of proteins, aggregation accompanying protein refolding, aggregation induced by relatively low concentrations of guanidine hydrochloride or urea and others). The main drawback of all these test systems is that a chaperone being tested can affect the stages preceding the aggregation stage. For example, when using a test system for which heat-induced unfolding of a protein substrate, glycogen phosphorylase *b* (Ph*b*) or glyceraldehyde-3-phoshate dehydrogenase from rabbit skeletal muscle, precedes the aggregation stage, we observed that α-crystallin [14–17] and proline at relatively low concentrations [12] could reveal accelerating effect on the stage of unfolding of the protein molecule. This circumstance hinders the quantitative estimation of the chaperone-like activity.

To overcome the above-mentioned drawback of aggregation test systems, we proposed a new test system based on aggregation of UV-irradiated protein substrate (see [18]). In this work we showed that UV irradiation of Ph*b* resulted in denaturation and inactivation of the enzyme molecules. Denatured Ph*b* molecules are assembled in clusters with the hydrodynamic radius of 10.4 nm which are relatively stable at 20 °C, however reveal a tendency to further sticking at higher temperatures (for example, at 37 °C). The test system of such a type allows detecting the effect of agents possessing chaperone-like activity exclusively on the aggregation stages. It is significant that in this case we obtain the true characteristics of the chaperone-like activity of the agents being tested without distortions caused by the effect of the agents under study on the stage preceding the aggregation stage (for example, on the stage of protein denaturation).

Analysis of data on titration of a protein by a ligand gives information on the stoichiometry of the protein–ligand complex and the affinity of the ligand to the protein. When molar concentrations of the protein and ligand exceed significantly the value of the dissociation constant for the protein–ligand complex, the titration data allow the stoichiometry of the complex to be determined. When molar concentration of the ligand (inhibitor) exceeds significantly molar concentration of the protein (enzyme), the titration data (or the dependences of the enzyme activity on the inhibitor concentration) allow the affinity of the ligand to the protein to be determined.

In the present work we compared the antiaggregation activities of α-crystallin, αB-crystallin and proline with UV-irradiated Ph*b* as a protein substrate. Analysis of the concentration dependences of

* Corresponding author at: Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, Moscow 119071, Russia. Tel.: +7 495 9525641; fax: +7 495 9542732.
E-mail address: svetabaj@gmail.com (S.G. Roman).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.03.015

the chaperone-like activity allowed us to determine the stoichiometry of α-crystallin–protein substrate and αB-crystallin–protein substrate complexes and to characterize the affinity of proline to the protein substrate.

2. Experimental

2.1. Materials

Hepes and proline were purchased from "Sigma" (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 Phb, α- and αB-crystallin

Ph*b* was isolated from rabbit skeletal muscle by the method of Fisher and Krebs [19] using dithiothreitol instead of cysteine; the enzyme was crystallized four times. The preparations of Ph*b* were electrophoretically homogeneous. Ph*b* concentration was determined spectrophotometrically at 280 nm using the absorbance coefficient $A^{1\%}_{cm}$ of 13.2 [20]. Molar concentration of Ph*b* was calculated per subunit (M_r = 97.4 kDa).

Isolation of α-crystallin was performed from freshly excised lenses of 2-year-old steers according to the procedure described previously [21]. Concentration of α-crystallin was determined spectrophotometrically at 280 nm using the absorption coefficient $A^{1\%}_{cm}$ of 8.5 [22]. Molar concentration of α-crystallin was calculated per subunit (M_r = 20 kDa).

Isolation of αB-crystallin was performed as described previously [23]. Concentration of αB-crystallin was determined spectrophotometrically at 280 nm using the absorption coefficient $A^{1\%}_{cm}$ of 6.93 (Swiss-Prot P02489). Molar concentration of αB-crystallin was calculated per subunit (M_r = 20 kDa).

2.3. UV irradiation of Phb

UV irradiation of Ph*b* (15 μM) was conducted in 1 cm path quartz cell at 10 °C. A high-pressure Hg lamp (DRK-120, 120 W, Russia) was used in the irradiation experiments. A filter that passes UV light (270–390 nm) while blocking other wavelengths in the light spectrum was applied. The power of incident light was 5 mW/cm^2. The summary UV radiation dose obtained by Ph*b* was equal to 13.5 J/cm^2. Previously we showed that, judging from the measurements of the enzymatic activity and the data on differential scanning calorimetry, Ph*b* completely lost enzymatic activity and native structure under these conditions of irradiation [18]. The samples of UV-irradiated Ph*b* were centrifuged at 6800 × *g* for 5 min (10 °C) using a 5804R Eppendorf centrifuge.

2.4. Aggregation kinetics studies

Aggregation of UV-irradiated Ph*b* in the absence and presence of proline, α- and αB-crystallin was detected by changes in light scattering intensity at 90° upon incubation at 37 °C. The light scattering intensity (I) was monitored by 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. The initial parts of the aggregation curves were analyzed by the following equation proposed by us earlier [18]:

$$I = I_0 \exp\left\{(\ln 2)\left(\frac{t}{t_{2I}}\right)^n\right\}, \quad (1)$$

where I_0 is the initial value of the light scattering intensity at time zero, t_{2I} is the time value at which $I = 2I_0$ and n is a constant. The reciprocal value of t_{2I} may be considered as a measure of the initial rate of aggregation. To characterize the antiaggregation activity of proline, α- and αB-crystallin, we used $(1/t_{2I})/(1/t_{2I})_0 = (t_{2I})_0/t_{2I}$ ratio, where $(t_{2I})_0$ is the t_{2I} value obtained for aggregation of UV-irradiated Ph*b* in the absence of any additives.

2.5. Combined action of α-crystallin and proline on aggregation of UV-irradiated Phb

To characterize the mutual influence of proline (inhibitor 1) and α-crystallin (inhibitor 2) on each other, when testing suppression of aggregation of UV-irradiated Ph*b*, we used parameter j proposed by Silonova et al. [24]:

$$j = \frac{i_{1,2}}{1-(1-i_1)(1-i_2)}. \quad (2)$$

In this equation i is a degree of inhibition: $i_1 = 1-(t_{2I})_0/(t_{2I})_1$ for inhibitor 1, $i_2 = 1-(t_{2I})_0/(t_{2I})_2$ for inhibitor 2 and $i_{1,2} = 1-(t_{2I})_0/(t_{2I})_{1,2}$ for the inhibitor 1/inhibitor 2 mixture. When the action of one inhibitor is not dependent on the presence of the other, parameter j is equal to unity. The case $j > 1$ corresponds to synergism and the case $j < 1$ corresponds to antagonism in the combined action of two inhibitors.

2.6. Calculations

Origin 7.0 (OriginLab Corp., USA) software was used for the calculations. To characterize the degree of agreement between the experimental data and calculated values, we used the coefficient of determination R^2 (without considering the statistical weight of the measurement results) [25].

3. Results

3.1. Effect of α-crystallin and αB-crystallin on kinetics of aggregation of UV-irradiated Phb

Previously we demonstrated that α-crystallin suppressed aggregation of UV-irradiated Ph*b* [18]. To have a quantitative estimation of the antiaggregation activity of α-crystallin, we analyzed the initial parts of the kinetic curves of aggregation obtained at various concentrations of α-crystallin. As an example, Fig. 1 shows the dependences of light scattering intensity (I) on time obtained in the absence of α-crystallin (A) and in the presence of α-crystallin at the concentrations of 0.75 (B), 11.5 (C) and 76 μM (D). When α-crystallin concentration is varied in the range of 0–30 μM, the initial parts of the dependencies of I on time are satisfactorily described by Eq. (1). For the dependencies of I on time obtained in the absence of α-crystallin (Fig. 1A) and in the presence of α-crystallin at the concentrations of 0.75 and 11.5 μM (Fig. 1B and C) the following values of parameters t_{2I} and n were obtained: $t_{2I} = 1.62 \pm 0.06$, $n = 1.4 \pm 0.1$ ($R^2 = 0.951$), $t_{2I} = 2.87 \pm 0.07$, $n = 1.08 \pm 0.03$ ($R^2 = 0.986$) and $t_{2I} = 5.32 \pm 0.11$, $n = 1.07 \pm 0.06$ ($R^2 = 0.932$), respectively. At the concentrations of α-crystallin exceeding 30 μM Eq. (1) becomes invalid. A typical kinetic curve obtained at high concentration of α-crystallin (namely, 76 μM) is represented in Fig. 1D.

The ratio $(t_{2I})_0/t_{2I}$ characterizes the relative initial rate of aggregation of UV-irradiated Ph*b*. Fig. 2A shows the dependences of $(t_{2I})_0/t_{2I}$ on α-crystallin concentration obtained at two concentrations of UV-irradiated Ph*b* (1.5 and 3.0 μM). These dependences demonstrate the inhibiting ability of α-crystallin. As one can see in Fig. 2A, the titration curve consists of two linear parts. The linear character of the initial part of the dependences of $(t_{2I})_0/t_{2I}$ on α-crystallin concentration testifies that tight α-crystallin–UV-irradiated Ph*b* complex is formed under these conditions. In other words, all added α-crystallin becomes bound