New Physiological Function of Chaperones, Facilitating Reconstitution of Apoenzymes

Boris I Kurganov* and Natalia A Chebotareva
Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia

One of the important branches of modern biochemistry and molecular biology is the investigation of structure and function of molecular chaperones. The main function of the family of molecular chaperones named as small heat shock proteins (sHsps) is suppression of aggregation of non-native protein species formed under stress conditions or during folding of the newly synthesized polypeptide chains [1-3]. The low molecular mass of monomers (from 12 to 43 kDa) and tendency to form large oligomers with high molecular masses up to 1000 kDa are typical of this protein family [4-14]. The presence of a conservative a-crystallin domain in the structure of sHsp seems to be important for the formation of stable dimers, whereas variable N- and C-terminal ends seem to participate in the formation of large oligomers [2-4,7]. It is supposed that the polydispersity and quaternary structure dynamics play an important role in cellular sHsp chaperones function [2,12]. sHsp cannot provide folding of the polypeptide chain; however, they form complexes with non-native proteins and can transfer the latter either to ATP-dependent chaperones that provide assistance to protein folding or proteasomes, where proteolytic degradation of the unfolded proteins occurs [15-19].

The properties of sHSPs are being studied intensively over the past two decades. However the most of these investigations are carried on in the diluted solutions. In the living cell all the processes proceed in the medium with high concentrations of macromolecules (proteins, nucleic acids, polysaccharides) which occupy the significant part of the cellular volume (up to 40%). That is why the part of the cellular volume becomes inaccessible for proceeding biochemical processes. The term “molecular crowding” implies the effect of excluded volume.

One of the biochemical processes, which proceeds in the cell and may be sensitive to crowding environment, is the process of reconstruction of holoenzyme from apoenzyme and cofactor. Reconstitution of holofor glycogen phosphorylase b (Phb; EC 2.4.1.1) is a convenient system for the study of the effect of crowding and chaperones on the reconstruction process. Phb in solution exists as a dimer which consists of two identical monomers with a molecular mass of 97.4 kDa. The catalytic site of the enzyme is located in a deep hydrophobic cavity in the center of the subunit; it contains one molecule of covalently bound cofactor pyridoxal 5'-phosphate (PLP), which is necessary for the catalytic activity. Removal of PLP results in the loss of the enzymatic activity of Phb and dissociation of dimer to monomers [20,21]. Monomeric form of apo Phb reveals a high propensity to self-association [20,22-24]. Reconstruction of Phb from apoenzyme and PLP is accompanied by the recovery of the catalytic activity and quaternary structure of the enzyme [20,25]. Therefore PLP can be considered as a catalytic and conformational cofactor of muscle Phb [26]. Since crowding affects protein conformation and processes of self-association of proteins, one would expect that the reconstruction of holo Phb is under control of crowding.

Chebotareva et al. [27] showed that crowding stimulated high-order association of apo Phb. These data agree with the predictions of the theory of molecular crowding and are supported by numerous experiments with various proteins [27-32]. Crowding-induced association of apo Phb is accompanied by the diminishing of the rate of interaction with cofactor because of steric hindrances. The mobile equilibrium between oligomeric forms of apo Phb was found to be sensitive to chaperones. This was demonstrated for a-crystallin, a representative of the family of sHsps, and for chemical chaperone, proline. Chaperones favour the formation of small oligomeric forms of apo Phb resulting in the acceleration of reconstruction of Phb from apoenzyme and cofactor (Figure1). It is of interest that such a dissociating effect of chaperones was observed with phosphorylase kinase (PhK) from rabbit skeletal muscle. In the presence of Mg2+ and Ca2+ PhK reveals a high tendency to self-association. It has been shown that crowding agent trimethylamine N-oxide greatly favours self-association of PhK, a-crystallin and proline suppressing PhK self-association under crowding conditions [30-32]. Chebotareva et al. [33] showed that interaction of Hsp27 with native PhK under crowding conditions resulted in dissociation of large oligomers of PhK hexadecameric molecules.

Thus, investigations of reconstitution of apo Phb allow putting forward an idea on a new physiological function of chaperones. This function consists in acceleration of reconstruction of holoenzyme from apoenzyme and cofactor by counteracting apoenzyme self-association that becomes especially significant under crowding conditions in the cell.

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*Corresponding author: Boris I Kurganov, Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia, Tel: +7(495)-952-5641; Fax: +7(495)954-2732; E-mail: kurganov@inbi.ras.ru

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