Thermochimica Acta 516 (2011) 88–90

Contents lists available at ScienceDirect

Thermochimica Acta

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

Short communication

Thermodynamics of the interaction between bovine binder of sperm BSP1 and low-density lipoprotein from hen's egg yolk

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ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form
23 December 2010
Accepted 7 January 2011
Available online 18 January 2011

Keywords:
Isothermal titration calorimetry
Low-density lipoproteins
Binder of sperm protein
Sperm preservation
Egg yolk extender

ABSTRACT

Egg yolk is used in extender to protect sperm from cold shock and freezing during preservation. It is the low-density lipoprotein (LDL) fraction of egg yolk that protects sperm. Even though essential for sperm capacitation, the major proteins from bull seminal plasma, the Binder of SPerm (BSP) proteins, are detrimental for sperm preservation because they induce a continual phospholipids and cholesterol efflux from sperm membranes. The BSP proteins were proposed to bind to egg yolk LDL, preventing the sperm membrane damage. We characterized the binding between the BSP proteins and the LDL by isothermal titration calorimetry, providing the thermodynamics and quantitative description of this putative association. The association between BSP1 (major BSP proteins) and LDL is characterized by an affinity constant (K_a) of $3.4 \pm 0.4\,\mu M^{-1}$. A protein/LDL ratio of 104 ± 5 was determined indicating that 104 molecules of BSP1 would bind to one LDL particle. This stoichiometry leads to proposing that the association involves 1.6 ± 0.1 phosphatidylcholines (PC) per BSP protein. This finding is satisfactorily consistent with the fact that each BSP1 protein has 2 binding sites for choline group. In conclusion, the formation of a high affinity complex between BSP1 and LDL is proposed to be important for the protection of sperm by egg yolk extender.

1. Introduction

Mammalian sperm can be preserved following dilution in extenders. Media, such as hen egg yolk, are routinely used. Egg yolk can protect bovine sperm during liquid storage [1–3] and cryopreservation [4–7]. A concentration of 20% egg yolk is generally added to the extender. The constituent of egg yolk that provides the protection of sperm against cold shock and freezing damages is the low-density lipoprotein (LDL) [6,8,9].

The mechanism of sperm protection by egg yolk is not well known. We proposed a novel mechanism suggesting that the LDL could protect sperm by sequestering a family of lipid-binding proteins from bovine seminal plasma, known as Binder of SPerm or BSP proteins (reviewed in [10,11]). BSP proteins are secreted by the bull seminal vesicles and are constituted of BSP1, BSP3 and BSP5 [12,13]. BSP proteins are beneficial in vivo because they bind to sperm membranes via the choline-containing phospholipids at ejaculation and induce a cholesterol and phospholipid efflux from sperm membranes, increasing the fertility of sperm (reviewed in [14]). However, in vitro, sperm is in continuous contact with an excess of BSP proteins that induce lipid efflux from the sperm membrane, resulting in decreased sperm resistance to cold shock and freezing [15]. Egg yolk LDL can bind BSP proteins in a rapid and saturable manner. The resulting lipoprotein: protein complex appears to be stable even after freeze thawing of the semen [16]. When semen is diluted in an extender containing egg yolk, the LDL present in egg yolk would sequester most of the BSP present in seminal plasma. This would prevent the lipid efflux induced by the BSP proteins (reviewed in [10]).

In order to gain a better understanding of the mechanism of sperm protection by egg yolk, we characterized the thermodynamics of the interaction between BSP proteins and LDL by using isothermal titration calorimetry (ITC). This approach presents a quantitative description of the affinity of BSP for LDL and provides significant insights into the nature of the interaction between these two species.

2. Experimental

2.1. Chemicals

BSP1 was isolated as described previously [13,17]. Egg yolk LDL was prepared as previously [16]. Tris(hydroxymethyl)-aminomethane (Tris) was from Sigma (St. Louis, MO). All other

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doi:10.1016/j.tca.2011.01.003

chemicals used were of analytical grade and obtained from commercial suppliers.

2.2. ITC experiments

BSP1 was dissolved in Tris–HCl buffer (10 mM), pH 7.4. LDLs were diluted in the same buffer. The pH of the solutions was measured and re-adjusted if necessary with 0.1 N NaOH solution. BSP1 solution was filtered through a porous membrane of 0.2 μm. LDL solution was not filtered, in order not to affect their fragile structure. ITC experiments were performed at 37 °C on a MicroCal VP-ITC calorimeter (MicroCal Inc., Northampton, MA). Samples were degassed before ITC experiment, except for LDL. The injector-stirrer syringe was loaded with BSP1 solution (3.2 mg/ml). The reaction cell (V = 1.4527 ml) was filled with LDL solution (0.1 mg/ml). The run consisted of 18 aliquots of 10 μl injected in 12 s with a delay of 500 s between successive injections, under constant stirring (300 RPM). The dilution of BSP1 (occurring upon injection in the cell) and of LDL (by the injected buffer volume) led to small contributions (endothermic and exothermic, respectively) that were subtracted from the enthalpies measured during the titration. For each run, the initial injection was 0.5 μl injected in 1.5 s and the associated data point was discarded from the titration as no peak was typically observed [18]. Data were analyzed using the Origin software (MicroCal, version 5.0). The reported values and the standard deviation are obtained from triplicates.

3. Results and discussion

The isothermal titration of LDL (0.1 mg/ml) with BSP1 (3.2 mg/ml) was performed at 37 °C (Fig. 1). BSP1 was used in this study because it is the major protein (~80%) from the BSP protein family [19]. The interaction between BSP1 and LDL is exothermic (Fig. 1, top panel) as indicated by the negative peaks of the titration. The area of the peaks gradually decreases during the titration, indicating that the binding of BSP1 progressively saturates the LDL. At the end of the titration, the peaks display a constant area that essentially corresponds to the dilution enthalpy of the injected BSP1 solution in the cell filled with BSP1-saturated LDL suspension. The areas of the peaks associated with the BSP1 injections were determined and, for each titration, the enthalpy variations (ΔH) as a function of the evolution of the BSP1 to LDL ratio in the cell were obtained (Fig. 1, bottom panel).

Despite the complexity of the investigated system, the titration curves could be analyzed with a standard Scatchard binding model (Table 1). This model assumes that the LDL particles provide n independent and equivalent binding sites to BSP1 and these sites are characterized by an affinity constant, K_a. The molar enthalpy of association of BSP to LDL particles (ΔH_a) is considered to be constant during the titration. This model was previously used to successfully describe the association of BSP1 with lipid model membranes [20,21]. The data were analyzed considering LDL as binding units with an average molecular weight estimated to 760 kDa [16]. The calorimetric titrations were fitted using ΔH_a, n, and K_a as adjustable parameters. The results indicate a strong association between BSP1 and LDL particles, characterized by a K_a of $3.4 \pm 0.4\ \mu M^{-1}$. This finding is consistent with previous studies of the BSP–LDL complex characterized by gel filtration, electrophoresis and ultracentrifugation suggesting qualitatively a high affinity binding between BSP proteins and LDL [16]. The ΔH_a was -106 ± 7 kJ/mol. The molar free energy of binding (ΔG_a) and the molar change in entropy (ΔS_a) was calculated using:

$$\Delta G_a = -RT \ln K_a \quad (1)$$

$$\Delta G_a = \Delta H_a - T\Delta S_a \quad (2)$$

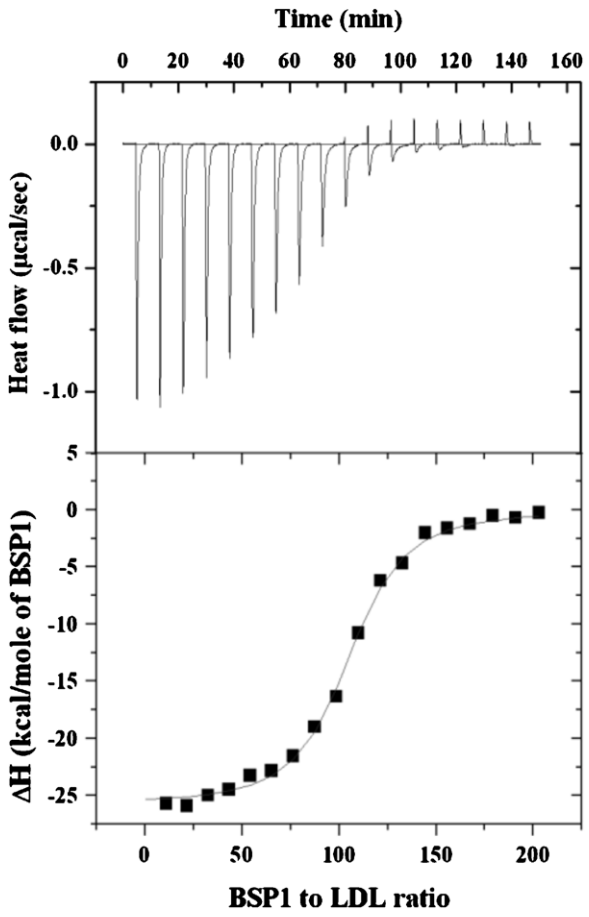

Fig. 1. Isothermal titration calorimetry of 0.1 mg/ml LDL suspensions with 3.2 mg/ml BSP1 at 37 °C. Top panel: heat flow changes resulting from the injections. Bottom panel: isothermal titration. The squares represent the data points derived from the injections whereas the curve represents the best fit obtained with Scatchard model, using ΔH_a, n, and K_a as adjustable parameters.

where R is the gas constant and T is the temperature in Kelvin. ΔG_a, and ΔS_a were estimated to -38.8 ± 0.3 kJ/mol and -217 ± 23 J/mol K, respectively. These values indicate that the association is enthalpy-driven and relatively strong interactions between LDL and BSP1 should exist.

The fitted stoichiometric parameter, n, was 104 ± 5, indicating that one LDL particle could bind on average, 104 BSP1. It was previously demonstrated that, at saturation, approximately 243 moles of BSP proteins could bind to a mole of LDL particles (actually LDL-II, which is the most abundant type in egg yolk) [16]; this value is in the same order of magnitude as that reported here, inferred from a thermodynamics method. The interaction between BSP proteins and LDL particles in the previous [16] and the present studies were examined under the same conditions (buffer, pH and salts) except that room temperature was used in the case of the gel filtration experiment whereas the temperature was set to 37 °C in the present study. It was previously estimated, upon its association to lipid membranes, that BSP1 defines an average cross-sectional area of ~6.75 nm^2 [22]. If one assumes that LDL particles have a spherical shape with an average diameter of 37.5 nm [23], their average surface should correspond to 4418 nm^2. Therefore, it is estimated

Table 1
Thermodynamic parameters for association of BSP1 with LDL at 37 °C.

System	K_a (μM^{-1})	ΔH_a (kJ/mol)	ΔG_a (kJ/mol)	ΔS_a (J/mol/K)	nBSP1/LDL
LDL	3.4 ± 0.4	−106 ± 7	−38.8 ± 0.3	−217 ± 23	104 ± 5

that about 16% of the LDL surface is covered with BSP1, assuming a coating with monomeric BSP1. At this point, however, the quaternary structure of LDL-bound BSP1 is not identified. Despite the fact that BSP1 appears to exist under a polydispersed/aggregated form in solution [24], it was proposed that BSP1 oligomers would dissociate upon their binding to membranes and monomers and/or dimers would be formed [21,24]. The LDL coverage estimated above is based on the formation of BSP1 monomeric coating and should, therefore, be considered as a maximum value.

In the present model, the definition of binding sites is indeed not precisely defined because of the complex structure and composition of LDL particles. LDL are formed of approximately 11% (w/w) proteins and 89% (w/w) lipids [25]. They are surrounded by a monolayer of phospholipids and cholesterol and these lipidic species correspond to 26, and 4% (w/w) of total lipids, respectively. The major phospholipid of LDL is phosphatidylcholine (PC) with a weight proportion of ~73% [25]. Considering that the mass of a LDL particle was estimated to 760 kg/mol [16], and that the average molar mass of LDL PCs is estimated to 770 g/mol, it is calculated that 104 BSP1 moles bind 170 moles of PC, assuming that all the BSP molecules bind exclusively to PC moieties. Therefore, it is inferred that there are 1.6 PC per bound BSP1. This result is interesting because previous equilibrium dialysis studies indicated that one BSP1 would bind 1.8 choline molecules [26]. Moreover, the crystal structure of BSP1 and phosphorylcholine complex was obtained and four phosphorylcholine groups were found to bind to a BSP1 dimers [27]. Therefore we could propose that the PC molecules of the LDL monolayer act as binding anchors for the BSP1 proteins. Proteins from the LDL surface must also be a part of a binding site.

4. Conclusion

The use of ITC to study the thermodynamics of the interaction between egg yolk LDL and BSP1 protein demonstrated the high affinity of the BSP proteins for the LDL particles, with a K_a of 3.4 μM^{-1}. It was estimated that 104 molecules of BSP1 could bind one LDL particle. This would correspond to 1.6 PC per bound BSP1. The strong interaction between LDL particles and BSP1, which is now quantified, could be the key to the sperm protection by egg yolk extender.

Acknowledgement

The authors thank the Natural Sciences and Engineering Research Council of Canada for the financial support.

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