BBA - Biomembranes 1860 (2018) 611–616

Contents lists available at ScienceDirect

BBA - Biomembranes

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

Interaction of Hsp90 with phospholipid model membranes

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ARTICLE INFO

Keywords:
Hsp90
Lipid binding
Membrane
SPR

ABSTRACT

Heat shock protein 90 (Hsp90) is an essential molecular chaperone with versatile functions in cell homeostatic control under both normal and stress conditions. Hsp90 has been found to be expressed on the cell surface, but the mechanism of Hsp90 association to the membrane remains obscure. In this study, the direct interaction of Hsp90 and phospholipid vesicles was characterized, and the role of Hsp90 on membrane physical state was explored. Using surface plasmon resonance (SPR), we observed a strong interaction between Hsp90 and different compositions of lipid. Hsp90 had a preference to bind with more unsaturated phospholipid species and the affinity was higher with negatively charged lipids than zwitterionic lipids. Increasing the mole fraction of cholesterol in the phospholipid led to a decrease of binding affinity to Hsp90. Circular dichroism (CD) spectroscopy of Hsp90 in PC membranes showed more α-helix structure than in aqueous buffer. The differential scanning calorimeter (DSC) and fluorescence polarization results showed Hsp90 could affect the transition temperature and fluidity of the bilayer. We postulate from these results that the association between Hsp90 and membranes may involve both electrostatic and hydrophobic force, and constitute a possible mechanism that modulates membrane lipid order during thermal fluctuations.

1. Introduction

Hsp90 is an essential molecular chaperone assisting in the folding and conformational regulation of client proteins and play a crucial role in regulating cell homeostasis and promoting cell survival. It is one of the most abundant cellular proteins, which accounts for 1–2% of total proteins in unstressed cells and is upregulated to 4–6% in many cells in response to stress [1]. Numerous studies have focused on the intracellular location and function of Hsp90, and the evidence revealing the presence of Hsp90 on the cell membrane is growing in recent years [2–8], but the biological significance of surface localization of Hsp90 remains elusive. It was reported that Hsp90 is an iron-binding protein associated with the plasma membrane of Hela cells and regulate the redox state of cells [4]. Hsp90 is also present in membrane microdomains following lipopolysaccharide induced cell activation and involved in signal transduction [5,6]. The extracellular and membrane bound Hsp90 plays an important role in cell motility, migration, invasion, and metastasis of tumor cells [7,8].

Plasma membrane is the barrier to the external environment and alterations of the lipid phase of membranes caused by stress or pathophysiological conditions could generate membrane initiated signaling processes related to heat shock protein expression [9]. Cell stressors such as heat shock, oxidative stress and hypoxia could stimulate Hsp90 translocation to the membrane or secretion [10]. The association of membrane phospholipid with Hsp70 and small heat shock protein (sHsp) has been discovered, it showed the interaction strongly depends on the lipid composition and the extent of lipid unsaturation, and Hsps could regulate membrane fluidity and preserve structural and functional integrity of membranes [11–13]. The regulation of Hsps expression and their feedback effect in modulations of membrane physical state and function are essential for cell survival and potentially of high therapeutic value.

Although Hsp90 has been characterized as membrane bound protein, neither the details of its interaction with lipid nor the physiological implications have been studied. Thus this research was conducted to characterize the interaction of Hsp90 with membrane and enrich our understanding of the physiological function of this protein on plasma membrane.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC), 1-

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https://doi.org/10.1016/j.bbamem.2017.11.011
Received 9 August 2017; Received in revised form 7 November 2017; Accepted 17 November 2017
Available online 21 November 2017

palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC), 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphoglycerol (POPG), 1,2-dipalmitoyl-*sn-glycero*-3-phospho-(1′-rac-*glycerol*) (DPPG) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol was obtained from Sigma-Aldrich (St. Louis, MO, USA). *N*, *N*, *N*-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl) phenylammonium *p*-toluenesulfonate (TMA-DPH) was purchased from AAT Bioquest (CA, USA). All of the other chemicals were of the highest analytical grade and were purchased from commercial suppliers.

2.2. Purification of Hsp90

Hsp90 was extracted and purified from duck muscles according to our previous study with slight modifications [14]. 30 g of muscle sample was homogenized with 100 mL Tris-HCl buffer (100 mM, pH 8.0) at 12000 rpm using an Ultra Turrax (T25, IKA, Germany). The homogenate was then centrifuged for 20 min at 12000*g* (Allegra 64R, Beckman, USA), supernatant was collected and filtered through four-layer gauze. Afterwards, the filtrate was precipitated by ammonium sulfate at a saturation of 70% at 4 °C for 12 h under gentle stirring, and centrifuged for 20 min at 12000*g*, the precipitate was dissolved in Tris-HCl buffer (100 mM, pH 8.0) and dialyzed for 24 h in Tris-HCl buffer (100 mM, pH 8.0).

The crude protein was loaded in DEAE-cellulose 52 column (2.5 × 60 cm) with Tris-HCl buffer (50 mM, pH 8.0), and the target protein peak was eluted with Tris-HCl buffer (50 mM, pH 8.0) containing 1 M NaCl. Then the fraction containing Hsp90 was applied to Source 15Q column (1.0 × 5.0 cm), which has been previously equilibrated with Tris-HCl buffer (50 mM, pH 8.0) and eluted with the same buffer, until unbound protein had passed through the column. The bound proteins were eluted with a linear gradient of 0–1.0 M NaCl in equilibration buffer. Hsp90 was pulled and concentrated by ultrafiltration using a 10 kDa cut-off membrane. The protein concentration was determined with the Bradford Protein Assay Kit (Jiancheng, Nanjing, China), and the purity of protein was analyzed by SDS-PAGE.

2.3. Preparation of liposomes from phospholipids

Liposome vesicles were prepared by dissolving a defined amount of dried lipid in chloroform in a round bottom flask, followed by drying under a rotary evaporator and the lipid film was left under vacuum overnight to remove all traces of solvent. The cholesterol concentration was 25 or 50% on a molar basis of the total lipids. PBS buffer (pH 7.0) was added to the lipid and the dispersion was vortexed. Subsequently the liposome suspension was sonicated in a bath sonicator at 4 °C until a clear suspension of unilamellar vesicles was obtained. The vesicles were then extruded through 100 nm polycarbonate membranes mounted on an extruder for 20 times (Morgec Inc.)

2.4. SPR measurements

SPR experiments were conducted on a Biacore X100 Plus (GE Healthcare, Uppsala, Sweden) equipped with L1 chip (GE Healthcare). Before each liposome injection, the chip surface was cleaned by injecting 91 μL of 40 mM octylglucoside, and washed thoroughly with PBS buffer. Liposomes (0.5 mg/mL) were deposited on sensor chips at a low flow rate (10 μL/min) until a stable resonance unit (RU) level was obtained, then it was washed with 2 × 50 mM NaOH to remove unstably bound liposome. To block the liposome-unpacked surface, the chip surface was treated with 0.1 mg/mL BSA for 1 min at 10 μL/min. Hsp90 solutions in PBS buffer were injected at various concentrations from 0.14 to 2.22 μM, and the lipid-coated surface was regenerated with 25 mM NaOH as long as a consistent baseline was maintained. All analyses were performed at 25 °C.

2.5. Circular dichroism measurements

Circular dichroism (CD) measurements were performed to estimate the secondary structure of Hsp90 (0.1 mg/mL) in the presence and absence of POPC liposome (0.5 mg/mL). The data were collected from 200 to 260 nm at 1.0 nm intervals on an Chriascan circular dichroism spectrometer (Applied Photophysics, Cambridge, England) with a path length of 1 mm using a quartz cell at room temperature.

2.6. Differential scanning calorimetry

DSC studies were carried out on a TA Q2000 DSC (TA Instruments, New Castle, USA) according to Welker et al. [11] and Zou et al. [15] with slight modifications. The scanning rate was 10 °C/h, and the sample concentrations were 50 mg/mL DPPC, DPPG or DPPC/25% cholesterol liposomes and 10 mg/mL Hsp90 in PBS. Data analysis was performed with the Origin software package (OriginLab).

2.7. Fluorescence polarization measurement

Fluorescence polarization was measured using the incorporation of the fluorescent probe TMA-DPH, using a FM4P-TCSPC spectrometer (Horiba Jobin Yvon Inc., NJ, USA) provided with a thermostated holder. 5 μL of TMA-DPH in DMSO was added to 500 μL 1 mM DPPC, DPPG or DPPC/25% cholesterol liposomes prepared as described above to reach a final concentration of 0.5 μM TMA-DPH, and mixture were incubated in the dark at 55 °C for 60 min. The polarization measurements were performed within the temperature range from 30 to 50 °C by gradually increasing the temperature 5 °C for every measurement. Excitation and emission wavelengths were 355 and 430 nm. The degree of fluorescence polarization was calculated applying the eq.

$$P = (I_{VV} - GI_{VH})/(I_{VV} + GI_{VH}),$$

where I_{VV} and I_{VH} are the intensities measured with its polarization plane parallel (I_{VV}) and perpendicular (I_{VH}) to that of the exciting beam. G is a factor used to correct polarization of the instrument and is given by the ratio of vertically to horizontally polarized emission components when the excitation light is polarized in the horizontal direction.

3. Results and discussion

3.1. Effect of phospholipid composition on the interaction with Hsp90

The sensorgrams for the interaction of Hsp90 with lipids of different composition deposited on sensor chip are shown in Fig. 1, and the kinetic parameters are presented in Table 1. With all lipids a strong association is observed followed by weak dissociation. The interaction with DPPC, POPC and PLPC showed the extent of lipid unsaturation could influence the association constant (Ka) that association is faster to the phospholipid with higher degree of unsaturation. The equilibrium constants for dissociation (KD) was in a decreasing order of PLPC > POPC > DPPC, and there was around 3-fold difference between PLPC and DPPC. At 25 °C the POPC and PLPC phospholipid is in liquid-crystalline phase and DPPC in gel phase. The increased fatty acids unsaturation is accompanied by increased membrane fluidity, and Hsp90 was more prone to bind to more unsaturated lipid, changes in membrane fluidity may influence the Hsp90-lipid interaction. Several studies have shown the physical state of phospholipid membranes plays important roles in protein association, and the mechanisms of the interactions are complex and involve both electrostatic and hydrophobic forces [16].

The binding level and binding affinity increased with POPS and POPG vesicles as compared with that of the neutral lipids, indicating the binding was more pronounced to the negatively charged liposomes than zwitterionic liposomes. The results demonstrate a strong ionic interaction between charged residues on the protein surface and