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Lectin-Like Oxidized LDL Receptor 1 Mediates the Uptake of the C-Terminal Domain of Hsp70 (A Promising Immune Adjuvant Molecule) and Antigen Peptide Complexes

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed the experiments, analyzed the data, wrote the protocol and wrote the manuscript. Author MKK performed the experiments, wrote the protocol and wrote the first draft of the manuscript. Author SK performed the experiments. Author TM analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Extracellular heat shock protein 70 (Hsp70) is an adjuvant molecule that stimulates the immune system. The C-terminal domain of Hsp70 (C70), without the ATPase domain, is sufficient for antigen cross-presentation. However, the mechanism by which the receptor mediates the uptake of C70–peptide complex remains unclear. We therefore aimed to determine the process by which the receptor mediates the uptake of antigenic peptide-bound C70.
Methodology: Hsp70 and C70 individually cloned into pET28a were expressed in *Escherichia coli* BL21 (DE3) and were purified on Ni-NTA agarose and MonoQ HR5/5. Hsp70 and C70 were labeled with Alexa 555 and Alexa 633, respectively, to detect cellular binding. HEK293 cells stably expressing lectin-like oxidized LDL receptor-1 (LOX-

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1) and KG-1 human dendritic-like cells were incubated with Alexa-labeled Hsp70 and C70 individually or with C70 and antigenic complexes and were observed using fluorescence microscopy. The affinity of LOX-1 toward Hsp70 and C70 was analyzed by chip assay using surface plasmon resonance, which immobilized LOX-1 ligand recognition domain.
Results: HEK293 cells stably expressing LOX-1 and KG-1 cells accepted the C70–peptide and Hsp70–peptide complexes. Anti-LOX-1-neutralizing antibody inhibited the uptake of the C70–peptide complexes by KG-1 cells. The dissociation constant (K_D) of C70 toward the LOX-1 extracellular domain, measured by surface plasmon resonance, was 4.02×10^{-7} M and that of the C70–peptide complex was 6.6×10^{-8} M. C70 increased the LOX-1 affinity by forming a complex with the antigen peptide.
Conclusion: Our findings suggest that LOX-1 is the primary receptor for the C70–peptide and the Hsp70–peptide complexes. C70 is a promising adjuvant molecule that is internalized via LOX-1. In addition, it is convenient to prepare C70 using an *E. coli* expression system and C70 is more stable than full-length Hsp70.

Keywords: C-terminal domain of Hsp70 (C70); lectin-like oxidized LDL receptor-1 (LOX-1); antigen peptides; immune adjuvant.

ABBREVIATIONS

C70; Hsp70 C-terminal domain; DCs, dendritic cells; Hsp, heat-shock protein; LDL, low-density lipoprotein; LOX-1; lectin-like oxidized LDL receptor-1; LPS, lipopolysaccharide; PMA, Phorbol 12-myristate 13-acetate ; RU; response units.

1. INTRODUCTION

Heat shock proteins (Hsps) are highly conserved proteins found in all prokaryotes and eukaryotes [1]. In addition to their intercellular functions, Hsps initiate the host immune response mediated by the antigen-presenting cells [2]. The Hsp70 family is the most conserved class of Hsps. Hsp70 is composed of two functional domains: a highly conserved N-terminal 44-kDa ATPase domain and a 28-kDa C-terminal domain (C70), which contains an 18-kDa substrate-binding region. Recently, efficient cross-presentation of antigenic peptides bonded to Hsp70 or C70 was demonstrated with the B3Z read-out system [3]. C70 has been reported to be sufficient for adjuvant activity. Moreover, C70 is easy to purify and can interact with antigen peptides in the absence of ATP, making it a more useful immune adjuvant molecule than the full-length Hsp70. However, the process of a receptor mediating the uptake of C70–peptide complex is unclear. For the efficient use of C70, it is necessary to identify the receptor for peptide/C70 and confirm that the receptor mediates the uptake of antigenic peptide-bonded C70.

Various surface proteins of immune cells are believed to play a role in the cellular responses induced by Hsp70. Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) was originally identified as a lectin-like receptor that binds and internalizes oxidized LDL on endothelial cells, causing endothelial dysfunction and atherosclerosis [4]. LOX-1 has been identified on smooth muscle cells and macrophages as well as on dendritic cells (DCs). Recently, it was reported that LOX-1 is involved in the binding of Hsp70 and the antigen–peptide complex (peptide/Hsp70) to DCs, and in antigen cross-presentation [5,6].

In the current study, we showed that LOX-1 is the primary receptor for peptide/C70 and for peptide/Hsp70. C70 increases the LOX-1 affinity by forming a complex with the antigen peptide. Moreover, we confirmed that C70 can be conveniently prepared using an *Escherichia coli* expression system, and that C70 is more stable than the full-length Hsp70.

2. MATERIALS AND METHODS

2.1 Cell Culture

HEK293 cells were purchased from Riken cell bank (Tsukuba, Japan) and transfected with plasmid carrying CFP-LOX-1 [7] using Lipofectamine Plus (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and stable cell lines were selected using G418 (1.2 mg/mL) in MEM with 10% fetal calf serum (FCS).

KG-1 human dendritic like cells were purchased from Riken cell bank (Tsukuba, Japan). KG-1 cells were differentiated into dendritic like cells by stimulating 0.2×10^6 cells/mL for 5–7 days with PMA (100 ng/mL, Sigma, St.Louis, MO) plus TNF-α (10 ng/mL, R and D Systems, Minneapolis, MN) in RPMI 1640 with 10% heat-inactivated FCS. Treatment with PMA and TNF-α caused some cells to become adherent and the typical morphological changes observed *in vivo* were noted [8].

2.2 Purification of Hsp70and C70

The Hsp70-coding clone was purchased from GeneCopoeia (Rockville, MD), and full-length Hsp70 (Hsp70, residues 1-641) and C region-coding peptide-binding domain (C70, residues 384–641) were cloned into pET 28a (Novagen, Madison, WI), which contains a thrombin-recognition site before the His-tag. *Escherichia coli* BL21 (DE3) were transformed with each plasmid and His-tagged proteins were expressed during 4-h induction with 1 mM IPTG, according to the protocol supplied by Novagen. Harvested bacterial cells were re-suspended in TBS (20 mM Tris, 150 mM NaCl, pH7.5) containing protease inhibitor cocktail (Nakalai, Kyoto, Japan), sonicated, and centrifuged at 10,000 × *g* for 30 min. Clarified supernatants were used as soluble lysates, and each soluble lysate was bound to a column containing Ni-NTA agarose (Qiagen, Hilden, Germany), washed with TBS containing 20 mM imidazole, and eluted with 20–250 mM imidazole gradient using FPLC system (Pharmacia, Uppsala, Sweden). Peak fractions at OD_{280} were pooled and dialyzed against TBS. Although high purity of C70 was observed at this step, an additional purification step was performed as a precaution. Each dialyzed fraction of Hsp70 or C70 was applied to MonoQ HR5/5 (Pharmacia) equilibrated with buffer A (20 mM Tris-acetate, pH 7.6, 1 mM EDTA, 10 μM 2-mercaptoethanol) and eluted with 0–350 mM NaCl gradient. Peak fractions were collected and dialyzed against PBS. The His-tag was removed by incubation with thrombin for 4 h at 25ºC and thrombin was removed by benzamidine sepharose (GE Healthcare, Tokyo, Japan). *E. coli* bacterial cell lysates and purified proteins were examined by SDS-PAGE and proteins were visualized by Coomassie staining. In order to completely deplete the contaminating lipopolysaccharide (LPS) from bacterial cells, a subsequent purification step using polymixin B columns (Pierce, Rockford, IL) was performed, according to the manufacturer protocol, immediately before addition of each protein to DCs.

2.3 Peptides and Antibodies

The antigen peptide OvaBip (SIINFEKLGSGHWDFAWPW) was synthesized by Operon (Tokyo, Japan) and ovalbumin-derived antigen peptide, Ova (SIINFEKL), was purchased from MBL (Nagoya, Japan). When indicated, Hsp70, C70 and OvaBip were labeled with Alexa555 or 633 (Invitrogen, Carlsbad, CA) according to the protocol supplied by manufacturer. Anti-LOX-1 antibody (ab60178) and anti-LOX-1 neutralizing antibody (23C11) were purchased from Abcam (Cambridge, MS).

2.4 Peptide and Hsp70 Complex Formation

Antigen peptide and Hsp70 complex formation was carried out as described previously [3] Briefly, Hsp70 was incubated in binding buffer (PBS containing 1 mM KCl, 2mM $MgCl_2$) for 15 min at 37ºC, with a 20-fold molar excess of OvaBip or Ova in the presence of 1 μM ATP. Subsequently, Hsp70 and peptides complex (OvaBip/Hsp70 or Ova/Hsp70) was incubated for another 25 min at 37ºC in the presence of 100 μM ADP and free peptides were removed by gel filtration using Bio-Gel P-6 Extra Fine (Bio Rad, Foster City, CA). Alexa633-labeled Hsp70 and OvaBip peptide complexes (OvaBip/Alexa633-Hsp70) or Alexa633-labeled Hsp70 and Alexa555-labeled OvaBip complexes (Alexa555-Ovabip/Alexa633-Hsp70) or Alexa633-labeled Hsp70 and Ova peptide (Ova/Alexa633-Hsp70) complex were prepared in the same way. C70 and OvaBip or Ova complex (OvaBip/C70 or Ova/C70) formation was carried out without ATP for 14 h and free peptides were removed by gel filtration. Alexa555-labeled C70 and Ova complex (Ova/Alexa555-C70) was prepared in the same way.

2.5 Surface Plasmon Resonance Assay

The surface plasmon resonance assays were carried out with a Biacore 3000 (Biacore, Uppsala, Sweden). We prepared a version of CTLD14, the extracellular ligand-binding domain of LOX-1, which was biotinylated near the N-terminal, as described previously [9]. Biotinylated CTLD14 (1 μg/mL PBS(-) pH7.4) was immobilized on the sensor chip SA (GE Healthcare, Buckinghamshire, UK) via the N-terminal biotin at 25ºC with a flow rate of 20 μL/min to a density of approximately 200 response units (RU) for Hsp70 or 140 response units for C70. The interaction between immobilized CTLD14 and Hsp70, C70, or OvaBip complex was examined at 25ºC with a flow rate of 20 μL/min with PBS (-), pH 7.4, as a running buffer. Proteins or protein-peptide complexes were injected over the flow cell for 2 min, followed by 3 min for dissociation. The response obtained from the control flow cell (without immobilized CTLD14) was subtracted from the CTLD14-immobilized cell to correct for nonspecific binding. BIA evaluation version 4.1 (GE Healthcare) was used to carry out the analysis of the kinetics.

2.6 Binding and Uptake Assay

HEK293 cells or HEK293 cells stably expressing CFP_LOX-1were grown on cover slips for 2 days prior to the experiments, and incubated with 1 μM of Alexa633-Hsp70, Alexa555-OvaBip or Alexa555-OvaBip/Alexa633-Hsp70, for 15 min at 37ºC in a humidified atmosphere containing 5% CO_2. The cells were then fixed with formaldehyde (2% in PBS), mounted, and imaged using a Leica DM IRE2 microscope (Leica Microsystems, Wetzler, Germany) equipped with a 100× NA 1.4 objective and a Cool SNAP-HG digitalized cooled CCD camera (Roper Scientific, Trenton, NJ) driven by MetaMorph software (Universal Imaging, Downing,

PA). To determine the expression level of LOX-1, the CFP fluorescence intensity was examined using an E4 filter with excitation at 436-nm and a 470-nm emission filter. The fluorescence intensity Alexa555 was examined using an Y3 filter with excitation at 535-nm and a 610-nm emission filter and the fluorescence intensity of Alexa633 was examined using an Y5 filter with excitation at 620 nm and a 700-nm emission filter.

KG-1 cells were differentiated into DCs on lysine-coated coverslips for 5–7 days, and the uptake assay was performed as described above. Images of over 100 DCs from 3 independent experiments were acquired. After subtracting the background emission level, the fluorescence intensity of each cell was determined from the mean pixel value of the whole cell by using MetaMorph software. The binding of protein or peptide was calculated from the fluorescence intensity derived from Alexa555 or 633 as described previously [9]. LOX-1 was detected using anti-LOX-1 antibody followed by incubation with Alexa488 labeled 2nd antibody.

2.7 Statistical Analysis

Student's *t*-test was used for comparison of 2 data sets. P values <0.05 were considered statistically significant.

3. RESULTS

3.1 LOX-1 is the Receptor for Hsp70-OvaBip Complex Uptake

To investigate the contribution of LOX-1 in the uptake of Hsp70 and peptide complex, we prepared recombinant Hsp70 labeled with Alexa633 and OvaBip (SIINFEKLGSGHWDFAWPW) labeled with Alexa555. HEK293 cells stably expressing CFP_LOX-1 (expression of CFP_LOX-1 was confirmed by western blotting with anti-LOX-1 antibody as shown in a supplemental figure) were incubated with Alexa633-Hsp70, Alexa555-OvaBip, or Alexa555-OvaBip/Alexa633-Hsp70. We were able to detect little uptake of Hsp70 (Fig. 1A) and OvaBip (Fig. 1B). In contrast to Hsp70 or OvaBip alone, the uptake of both Hsp70 and OvaBip were remarkably increased when it was in a complex (Fig. 1C). Since we evaluated the OvaBip/Hsp70 uptake ability of wild HEK293 cells, no OvaBip/Hsp70 signal were detected (Fig. 1D), we concluded that the OvaBip/Hsp70 uptake were dependent on LOX-1.

3.2 Substrate-binding Domain of Hsp70 is More Stable than Full Length Hsp70

Next, we prepared the 28-kDa C-terminal domain of Hsp70 (C70), which contains the 18-kDa substrate-binding region [1], using an *E. coli* expression system in the same way as that for Hsp70. C70 was expressed as a completely soluble protein and was easy to purify (Fig. 2B). After the first purification step using Ni-NTA agarose, C70 was detected as an almost pure protein (Fig. 2C). In contrast, nearly 40% of Hsp70 was expressed as insoluble protein (Fig. 2A) and the purity after Ni-NTA agarose column purification was less than 90% (Fig. 2C). The yield of purified proteins from a 300 mL culture was 15 mg of C70 and only 8 mg of Hsp70 before cleavage of the His-tag. The final yield after treatment to remove the His-tag and LPS was 3 mg of C70 and 0.8 mg of Hsp70. Although these purification steps caused loss of protein, we treated each protein with thrombin and depleted LPS in order to exclude any effects from the His-tag or contaminating LPS. C70 was stable at 4ºC over a few months, whereas full-length Hsp70 degraded during storage (Fig. 2D).

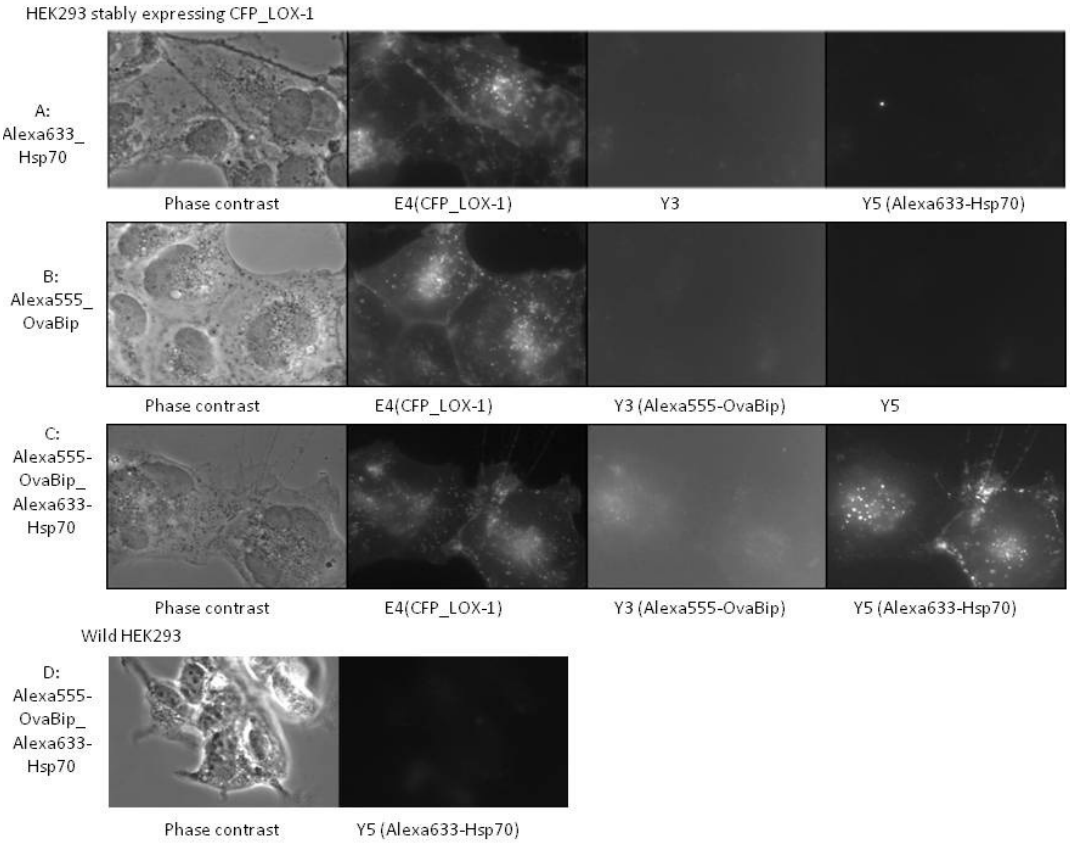

Fig. 1. LOX-1 is the receptor for uptake of Hsp70 and OvaBip complex

HEK293 cells stably expressing CFP_LOX-1 incubated with Alexa633-Hsp70, Alexa555-OvaBip, or Alexa555- OvaBip/Alexa633- Hsp70 and then examined by fluorescence microscopy. (A) Cells incubated with Alexa633-Hsp70. (B) Cells incubated with Alexa555-OvaBip. (C) Cells incubated with Alexa555-OvaBip/Alexa633- Hsp70. (D) Wild HEK293cells incubated with Alexa555- OvaBip/ Alexa633-Hsp70

When HEK293 cells stably expressing LOX-1were pre-treated with OvaBip/C70, the uptake rate of Alexa555-OvaBip/Alexa633-Hsp70 was decreased (Fig. 2E). The result indicated that C70 peptide complex binds to LOX-1 as well as Hsp70.

3.3 Surface Plasmon Resonance Assay

To evaluate the affinity of Hsp70, C70 alone, or protein and OvaBip complexes to LOX-1, we performed a surface plasmon resonance assay. We prepared soluble CTLD14, which is a domain of LOX-1 that is responsible for ligand recognition [7]. The recombinant CTLD14 was purified as a dimer, which is the native form of LOX-1 on the cell surface [7]. Because the CTLD14 had been almost 100% biotinylated near the N-terminus in the bacterial expression system, it could be directly immobilized onto an SA sensor chip without any further treatment.

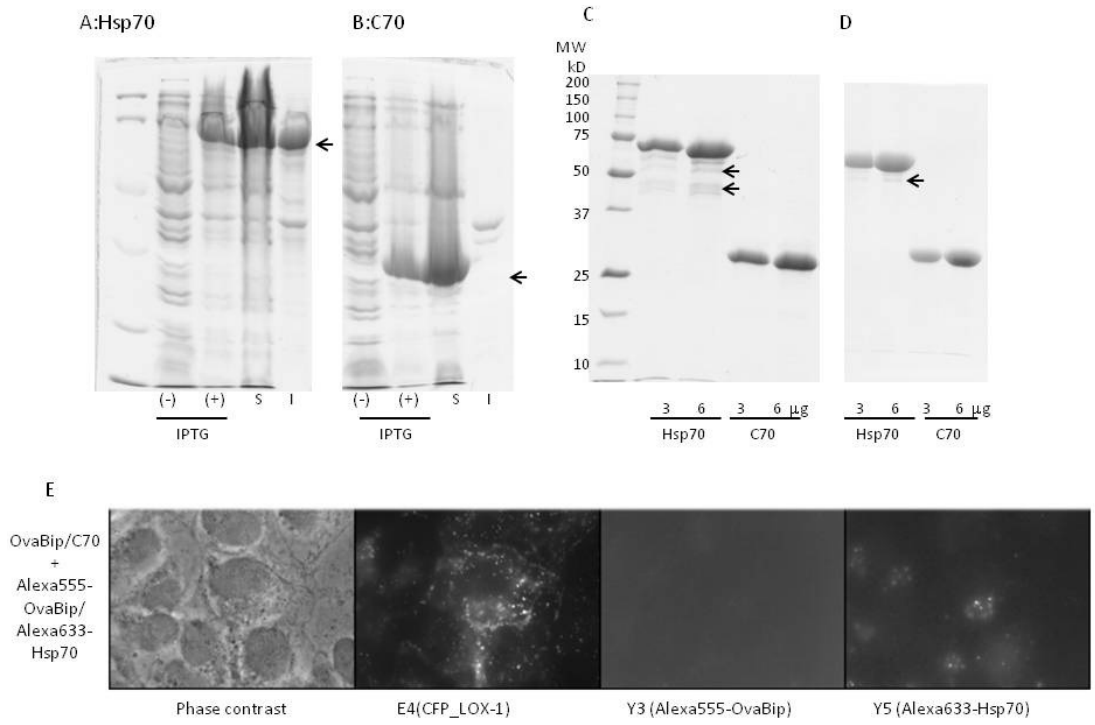

Fig. 2. Expression and stability of recombinant Hsp70 and C70

Recombinant Hsp70 and C70 were purified as described in material and methods. E. coli bacterial cell lysates and purified proteins were examined by SDS-PAGE and proteins were visualized by Coomassie staining. (A) Hsp70, (B) C70. (-): bacterial culture without IPTG, (+) with IPTG for 4 h; S: soluble fraction, I: insoluble fraction. Arrows indicate the expected molecular mass of Hsp70 or C70. (C) Hsp70 and C70 eluted from Ni-NTA Agarose column. Each lane contained 3 µg or 6 µg total proteins. Arrows indicate contaminating protein. (D) Stability of Hsp70 and C70. Purified proteins were stored at 4ºC for 3 months and 3 µg or 6 µg total proteins were run in each lane. Arrow indicates the degraded Hsp70. (E) HEK293 cells stably expressing CFP_LOX-1 incubated with OvaBip/C70 followed by incubation with Alexa555-OvaBip/Alexa 633-Hsp70

First, we examined the binding of Hsp70. Sensorgrams (Fig. 3A) showed an increase in RU reflective of Hsp70 binding (association) and a very slow decrease in response consistent with a loss of mass from washout (dissociation) after each injection (arrows). We analyzed the sensorgrams by fitting them with a simple 1:1 Langmuir model. Parameters were summarized in Table 1. The K_D value was calculated to be 2.44×10^{-9} M. A χ^2 value of 3.43 indicates an adequate fit of the model to the data. Furthermore, SE was less than 10%. Next, we analyzed the interaction between OvaBip/Hsp70 and CTLD14 (Fig. 3B). The K_D value was calculated to be 1.25×10^{-10} M. Although the k_d was very low and it was difficult to calculate the accurate K_D value, this very slow dissociation rate shows the stability of OvaBip/Hsp70 and CTLD14 in PBS at neutral pH.

We then examined the binding of C70 to CTLD14 (Fig. 3C) and OvaBip/C70 to CTLD14 (Fig. 3D). The K_D value between C70 and CTLD14 was calculated to be 4.02×10^{-7} M. The χ^2 value was 0. 2. The K_D value between OvaBip/C70 and CTLD14 was calculated to be 6.6 $\times 10^{-8}$ M. The □^{2} value was 0. 6. Although the RU for C70 was low compared to that of full-

length Hsp70, C70 increased its affinity for LOX-1 by forming a complex with the peptide. The low k_d indicated that the interaction between the OvaBip/C70 and CTLD14 was stable.

Table 1. Summary of parameters analyzed by BIA evaluation

	k_a(1/Ms)	SE(k_a)	k_d(1/s)	SE(k_d)	K_D(M)
Hsp 70	4.83×10^4	734	1.18×10^{-4}	8.88×10^{-5}	2.44×10^{-9}
OvaBip/Hsp70	2.35×10^5	3.82×10^3	2.95×10^{-5}	6.5×10^{-5}	1.25×10^{-10}
C70	1.60×10^4	979	6.41×10^{-3}	1.78×10^{-4}	4.02×10^{-7}
OvaBip/C70	1.48×10^4	300	9.80×10^{-4}	7.66×10^{-5}	6.6×10^{-8}

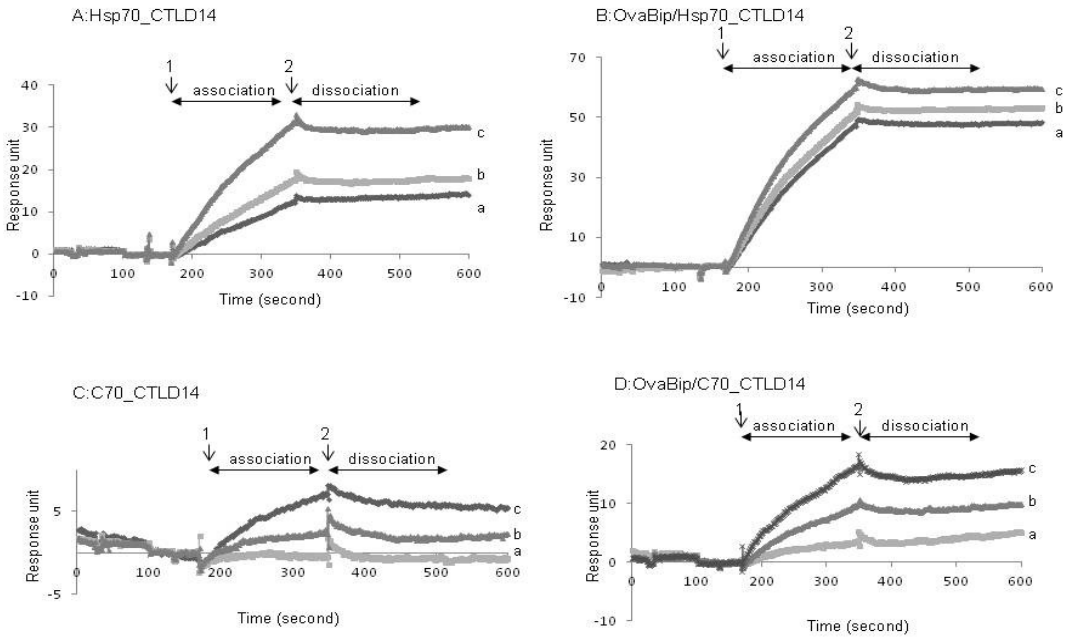

Fig. 3. Sensorgrams of specific binding of serially diluted ligands to immobilized CTLD14 on SA sensor chip

Ligands diluted with running buffer (PBS) were injected (at the points indicated by arrows 1) over flow cells at a flow rate of 20 µL/min for 2 min, followed by 3 min of dissociation (started at the points indicated arrows 2). Curves from the reference cell without immobilized CTLD14 were subtracted from the binding curve. (a) 0.045 µM, (b) 0.09 µM and (c) 0.18 µM of ligand was injected. The dissociation constant of ligand and CTLD14 was calculated based on the 1:1 (Langmuir) binding model. (A) Hsp70, (B) OvaBip/Hsp70, (C) C70 and (D) OvaBip/C70

3.4 Substrate-binding Domain of Hsp70 (C70) Uptake Through LOX-1

Finally, we evaluated the uptake of well-known antigen peptide, Ova (SIINFEKL) and Hsp70 or C70 complexes. Each Ova/Hsp70 and Ova/C70 was taken up into LOX-1 expressing HEK293 (Fig. 4A) as well as OvaBip/Hsp70 complex. Next, we examined each complex uptake through LOX-1 in DCs. To stimulate their differentiation into DCs, KG-1 cells were treated with a combination of PMA and TNF-α; the typical morphological changes were apparent (Fig. 4B-1). Once differentiated, KG-1 DCs, which express LOX-1 (Fig. 4B-1), were incubated with Hsp70, Ova, or Ova/Hsp70. We were able to detect the uptake of Hsp70 and

Ova/Hsp70 (Fig.4B-2, 3). In contrast to peptide alone, the uptake of Ova was increased when it was in a complex with Hsp70 (Fig. 4C-1). Moreover, when cells were pre-treated with anti-LOX-1-neutralizing antibody, the uptake rate was clearly decreased (to less than 15%) (Fig. 4C-1). Next, we examined whether C70 was also taken up into DCs through LOX-1. Although the amount of Alexa555-C70 alone that was taken up by DCs was not very high compared to that of full-length Hsp70 (Fig. 4B-4), Ova/Alexa555-C70 showed increased uptake efficiency (Fig. 4B-5) and 23C11 inhibited the uptake of Ova/Alexa555-C70 (Fig. 4C-2). These results indicate that only C70 is sufficient to form the antigen peptide complex and be internalized into DCs via LOX-1.

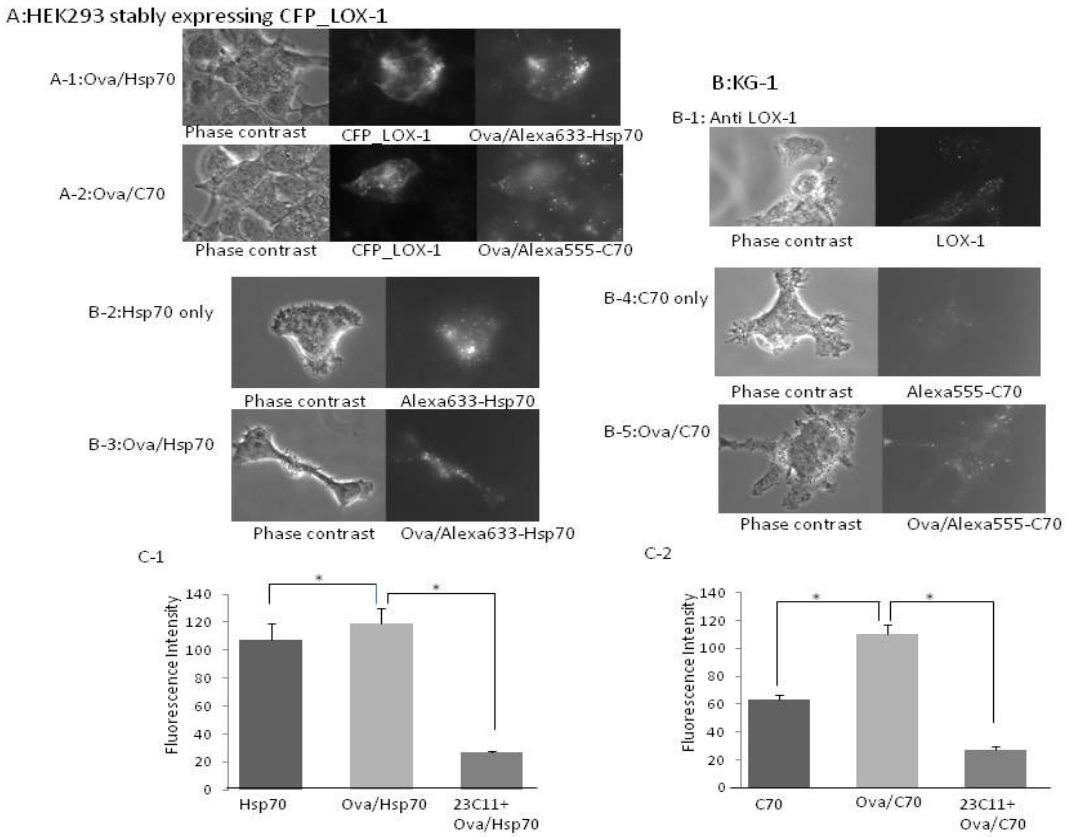

Fig. 4. LOX-1 is the receptor for uptake of Ova/Hsp70 and Ova/C70

*A: HEK293 cells stably expressing CFP_LOX-1 incubated with Ova/Alexa633-Hsp70 (A-1) or Ova/Alexa555-C70 (A-2), and then examined by fluorescence microscopy. B: KG-1 cells were differentiated into DCs, incubated with proteins or peptide/protein complexes, and then examined by fluorescence microscopy. (B-1) DCs stained with anti-LOX-1 antibody. (B-2) DCs incubated with Alexa633-Hsp70. (B-3) DCs incubated with Ova/Alexa633-Hsp70. (B-4) DCs incubated with Alexa555-C70. (B-5) DCs incubated with Ova/Alexa555-C70. (C) DCs incubated with LOX-1-neutralizing antibody (23C11) followed by incubation with Ova/Alexa633-Hsp70 or Ova/Alexa555-C70. Images of over 100 DCs from 3 independent experiments were acquired. Fluorescence intensity was quantitatively evaluated using region-measurement analysis (MetaMorph) as described in Material and Methods. Data are expressed as the fluorescence intensity after subtracting the background emission level ± SD, *P<0.05*

4. DISCUSSION

Hsp70 has been established as an immune adjuvant for cross priming with antigen peptides [10]. Subsequently, it was demonstrated that recombinant Hsp70 expressed in *E. coli* works as an immune adjuvant as well as Hsp70 isolated from tissues. One study also showed that C70, which contains the substrate-binding region but not the ATPase domain of Hsp70, was sufficient for antigen cross-presentation [3]. It was reported that high expression of Hsp70 in *E. coli* led to the formation of inclusion bodies, resulting in defects in solubility and bioactivity [11]. In this study, we showed several advantages of C70 over full-length Hsp70: it is 100% soluble even when expressed at high levels; the purification is simple; the yield is 4 times higher and it is stable when stored for several months.

In the process of cross-priming, DCs internalize Hsp70 with bound peptides through receptor. Binding of Hsp70 to the surface receptor is therefore an important gateway for antigen presentation. In fact, several scavenger receptors are involved in mediating uptake of Hsp70 and peptide complex by DCs [5,6] and recent report has indicated the potential importance of LOX-1 [12]. We evaluated the contribution of LOX-1 in binding antigen peptide by DCs using fluorescent microscopy and LOX-1-neutralizing antibody and demonstrated that LOX-1 works to uptake Ova/Hsp70 and Ova/C70.

Surface plasmon resonance assay revealed that Hsp70 itself showed high affinity for LOX-1. While the affinity between LOX-1 and C70 alone was not very high, the affinity increased when C70 made complex with antigen peptides. These results indicate that C70 acquires a high affinity for LOX-1 by forming a complex with antigen peptide. Moreover, we observed a slow dissociation rate constant between OvaBip/C70 and LOX-1 at neutral pH. The result indicates that once the C70 and peptide complex binds to LOX-1, it is not dissociated during the early endocytosis step when the surrounding environment is at a neutral pH. It has been demonstrated that the binding site for Hsp70 and C70 are located in lipid raft microdomains [3] and a very recent research has reported that LOX-1 is predominantly localized in caveolae/lipid rafts [13]. The results support that LOX-1 is the primary receptor for antigen peptide uptake.

5. CONCLUSION

Our study demonstrates that the C70 is sufficient for antigen uptake on human DCs and that LOX-1 is the primary receptor for C70–peptide complex as well as for Hsp70–peptide complex. C70 increased the affinity for LOX-1 by forming a complex with antigen peptides. In addition, we confirmed that C70 is easy to prepare using an *E. coli* expression system and that C70 is more stable than full-length Hsp70. These findings suggest that C70 is one of the promising adjuvant molecules for therapeutic applications.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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