The 90-kDa heat shock protein, HSP90, is a major stress protein which is conserved from Escherichia coli to human (1-4). HSP90 of mammals is encoded by two separate genes whose products are designated as HSP90α and HSP90β (5). Both HSP90α and HSP90β exist as homodimers, but a minor part of HSP90 is present as a monomeric form (6). The synthetic peptides corresponding to these putative calmodulin-binding sequences were found to be cross-linked with Ca2+-calmodulin and to prevent the cross-linking of HSP90 and Ca2+-calmodulin. Both HSP90α and HSP90β bind Ca2+. The HSP90 peptides bind HSP90 and thereby inhibit the binding of Ca2+. In addition, the HSP90 peptides augment the self-oligomerization of HSP90 induced at elevated temperatures. These results suggest that the calmodulin-binding domain of HSP90 might interact with another part of the same molecule and that Ca2+-calmodulin might modulate the structure and function of HSP90 through abolishing the intramolecular interaction.

The mouse 90-kDa heat shock protein (HSP90) and Ca2+-calmodulin were cross-linked at an equimolar ratio using a carbodiimide zero-length cross-linker. To identify the calmodulin-binding domain(s) of HSP90, CNBr-cleaved peptide fragments of HSP90 were mixed with Ca2+-calmodulin and cross-linked. Amino acid sequence determination revealed that an HSP90-derived peptide starting at the 488th amino acid residue was contained in the cross-linked products, which contains a calmodulin-binding motif (from Lys565 to Ile628). A similar motif is present also in HSP90β (from Lys611 to Val653). The synthetic peptides corresponding to these putative calmodulin-binding sequences were found to be cross-linked with Ca2+-calmodulin and to prevent the cross-linking of HSP90 and Ca2+-calmodulin. Both HSP90α and HSP90β bind Ca2+. The HSP90 peptides bind HSP90 and thereby inhibit the binding of Ca2+. In addition, the HSP90 peptides augment the self-oligomerization of HSP90 induced at elevated temperatures. These results suggest that the calmodulin-binding domain of HSP90 might interact with another part of the same molecule and that Ca2+-calmodulin might modulate the structure and function of HSP90 through abolishing the intramolecular interaction.

**EXPERIMENTAL PROCEDURES**

Materials—CNBr, lysylendopeptidase, and the Wakosi 5C18 column were obtained from Wako Pure Chemical Industries, Ltd. (Osaka). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxy sulfo succinimide were purchased from Pierce Chemical Co. Gradient gels for SDS-PAGE were from Daiichi Pure Chemical Co., Ltd. (Tokyo). Immobilin F membranes were from Millipore Corp. HSP90 was purified from mouse lymphoma L5178Y cells as described (17), and its concentration was determined following Bradford (22) using a protein assay kit (Bio-Rad). Calmodulin from bovine brains and melittin were obtained from Sigma. Calmodulin was dissolved in a buffer solution (20 mM Tris-HCl, pH 7.5, 1 mM imidazole, 1 mM MgCl2, 10 mM D-mercaptoethanol) and then dialyzed against the same buffer solution. The concentration of calmodulin was determined by UV absorption measurement, assuming a405=1.67. Sheep anti-calmodulin IgG was purchased from Biomedical Technologies, Inc. (Stoughton, MA). Rabbit anti-goat IgG antibody conjugated with peroxidase was from Cappel Laboratories. All other chemicals and reagents used were of the highest grade obtainable.

Synthesis of Peptides—Peptides used in this work, Lys565-Ile628 and Thr265-Tyr331 of HSP90α and Lys611-Val653 of HSP90β, were synthesized with an Applied Biosystems model 430A peptide synthesizer (Applied Biosystems, Inc.).

Radiodination—Two HSP90 peptides or melittin (50 μg) was
Calmodulin-binding Domain of Mouse HSP90

**FIG. 1. Cross-linking of HSP90 and calmodulin.** A mixture of HSP90 (0.99 mg/ml) and calmodulin (0.59 mg/ml) was treated with a zero-length cross-linker, EDC, in the presence of Ca\(^{2+}\) (0.5 mM), followed by SDS-PAGE using a 4–20% gradient gel. For control experiments, either one or two components were omitted as indicated. Positions of monomeric and dimeric forms of HSP90 and calmodulin are indicated by arrows. An arrowhead indicates a major cross-linked product of HSP90 and calmodulin (120 kDa). CaM, calmodulin.

**FIG. 2. CNBr cleavage of HSP90 and the 120-kDa product.**

A. positions of methionine residues of HSP90a and HSP90β are represented by vertical lines. a.a., amino acids. B, a mixture of HSP90 and calmodulin was treated with EDC in the presence of Ca\(^{2+}\) and resolved by SDS-PAGE (4–20%) as described in the legend to Fig. 1. From gels stained with CBB, bands corresponding to HSP90 and the 120-kDa product were excised and treated with CNBr, followed by SDS-PAGE (10–20%). Open and closed triangles represent the largest CNBr-cleaved peptide fragments of HSP90a and HSP90β, respectively.

**FIG. 3. Cross-linking of CNBr-cleaved peptide fragments of HSP90 and calmodulin.** A, a mixture of CNBr-cleaved peptide fragments of HSP90 and Ca\(^{2+}\)-calmodulin was treated with EDC. The cross-linked products were subjected to amino acid sequence determination either directly or after cleavage with CNBr. Arrowheads indicate sites of CNBr cleavage. The calmodulin-binding site of HSP90 (●) and the HSP90-binding site of calmodulin (△) are symbolically depicted. The NH\(_2\) terminus of calmodulin is acetylated (Ο——Ο). B, CNBr-cleaved peptide fragments of HSP90 were mixed with calmodulin in the presence (lane 2) or absence (lane 3) of Ca\(^{2+}\), treated with EDC, and analyzed by SDS-PAGE (10–20%) followed by staining with CBB. The untreated CNBr-cleaved peptide fragments were also electrophoresed (lane 1). The CNBr-cleaved HSP90 peptides and calmodulin were treated with EDC in the presence of Ca\(^{2+}\) and electrophoresed on a 10–20% gradient gel without (lane 4) or with (lane 5) EDTA. Bands of calmodulin and a 24-kDa product are indicated by arrows and arrowheads, respectively.

Calmodulin-binding Domain of Mouse HSP90

9605
The 24-kDa cross-linked product was subjected to amino acid sequence determination directly (i) or after digestion with CNBr (ii). Eleven cycles of Edman degradation were performed in these determinations. The determined sequences are shown from left to right. Multiple acid residues identical with those of HSPSO or calmodulin are underlined. 9606 residues were detected for some of the cycles and all of them are shown.

The relative intensity of the stained protein bands was estimated using a densitometer of TIAS-2000s (ACI, Japan).

TABLE I

<table>
<thead>
<tr>
<th>Experimental procedure</th>
<th>Determined sequence</th>
<th>Corresponding sequence</th>
</tr>
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<tbody>
<tr>
<td>(i) Direct blot</td>
<td>KENQKH1FY</td>
<td>HSP90α</td>
</tr>
<tr>
<td></td>
<td>L K V X</td>
<td>(486) KENQKH1FYIT</td>
</tr>
<tr>
<td>(ii) CNBr digestion</td>
<td>KQ E V</td>
<td>Calmodulin</td>
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<tr>
<td></td>
<td>L SNLX E</td>
<td>HSP90α</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

HSP90α #500-520: KEOVANSAFVERLKRGLEVI
HSP90β #491-511: KEOVANSAFVERVRKGFEVV

The 24-kDa cross-linked product was subjected to amino acid sequence determination directly (i) or after digestion with CNBr (ii). Eleven cycles of Edman degradation were performed in these determinations. The determined sequences are shown from left to right. Multiple acid residues identical with those of HSPSO or calmodulin are underlined.

The relative intensity of the stained protein bands was estimated using a densitometer of TIAS-2000s (ACI, Japan).

Cross-linking—Either one or two of the following substances, i.e. HSPSO, CNBr-cleaved peptide fragments of HSPSO, the synthetic peptides of HSPSO, and melittin, were mixed with calmodulin in F buffer (30 mM PIPES, pH 6.9, 2 mM MgCl₂, 100 mM KCl, 0.6 mM EGTA) with or without Ca²⁺ (the final concentration was 0.5 mM) and incubated at room temperature for 10 min. Then cross-linking was carried out with EDC and N-hydroxysuccinimide according to the method of Kawasaki et al. (26) with the modification in that the reaction was stopped by boiling in SDS-PAGE sample buffer (23) instead of adding glycine. The 120-kDa product was digested with lysylendopeptidase for the peptide analysis following the method of Kawasaki et al. (26). For the experiment of Fig. 2, after cross-linking, HSP90 and the 120-kDa product were separated by 4-20% SDS-PAGE. The protein bands corresponding to HSP90 and the 120-kDa product were excised separately from CBB-stained gels and subjected to treatment with CNBr, followed by SDS-PAGE (10-20%) (6).

HSP90 and ¹²⁵I-iodinated peptides were mixed in PN solution (30 mM PIPES, pH 6.7, 100 mM NaCl) in the presence or absence of nonradioactive peptides and cross-linked as described above.

Immunoblotting—After SDS-PAGE, cross-linked products were electrotransferred onto Immobilon P membranes and fixed with 0.2% glutaraldehyde in order to enhance the retention of calmodulin on the membrane as described (27). The blots were incubated with sheep anti-calmodulin antibody, followed by peroxidase-conjugated rabbit anti-goat IgG antibody.

Amino Acid Sequencing—Cross-linked products of calmodulin and CNBr-cleaved peptide fragments of HSP90 were separated and sequenced with an Applied Biosystems model 470A protein sequenator, either directly or after digesting with CNBr, as follows. The products were resolved by SDS-PAGE on a 10-20% gradient gel and electroblotted onto an Immobilon P membrane as described by Matsudaïra (28). Bands corresponding to the cross-linked product were cut out from the membrane and subjected to amino acid sequencing.

Protein bands of the cross-linked product were excised from stained gels (10-20%) and then subjected to cleavage with CNBr as described (6). The cleaved products were separated on a WakoSil 5C18 reversed phase column with a linear gradient of acetonitrile in the presence of 0.1% trifluoroacetic acid using a Hitachi 655-15 HPLC system. The respective peak fractions were subjected to amino acid sequencing.

Calcium-binding Assay—HSP90 was resolved into two isoforms by SDS-PAGE (6) and electroblotted onto Immobilon P membranes. The blots were incubated in a [³⁵Ca]CaCl₂ (37 kBq/µl, Amersham) solution and processed as described (29). A 100-fold excess of nonradioactive calcium or the HSP90 peptides were added to the incubation mixtures when indicated. After autoradiography, the membranes were stained with CBB (28).

In Vitro Oligomerization of HSP90—HSP90 was incubated at various temperatures in PN solution. When indicated, Ca²⁺ or the HSP90 peptides were added. After 1-h incubations, samples were analyzed by nondenaturing PAGE.
The 120-kDa band was cleaved with CNBr, yielding only when Ca²⁺ was present. The 120-kDa band was confirmed to contain both HSPSO and calmodulin was incubated in the presence of Ca²⁺ with a zero-length cross-linker, EDC, that cross-links close contact sites (21). The blots were subjected to [⁴⁵Ca]calcium-binding assay (28). Lane 1, CBB staining; lane 2, autoradiograph. B, purified HSP90 (5 μg for lanes 1, 3, 5, and 7; 10 μg for lanes 2, 4, 6, and 8) was electrophoresed and blotted as described in A. The calcium-binding assay was performed in the presence of a 100-fold excess of the HSP90β peptide (lanes 1 and 2), HSP90α peptide (lanes 3 and 4), nonradioactive Ca²⁺ (lanes 5 and 6), or none (control) (lanes 7 and 8). Arrowheads indicate the positions of HSP90. The lower lanes are autoradiographs, and the upper ones are the same blots stained with CBB.

RESULTS

Fig. 6. Inhibition of cross-linking of HSP90 and calmodulin by the HSP90 peptides. A, a mixture of HSP90 (0.99 mg/ml) and calmodulin (0.59 mg/ml) was treated with EDC in the absence (lane 1) or presence (lanes 2-5) of the HSP90 peptides. Molar ratios of the HSP90α peptide to HSP90 were 3 (lane 2) and 6 (lane 3). Molar ratios of the HSP90β peptide to HSP90 were 6 (lane 4) and 15 (lane 5). Positions of HSP90 and calmodulin are indicated by respective arrows. Cross-linked products of HSP90 and calmodulin are indicated by an arrowhead. B, the amount of the cross-linked products indicated by an arrowhead in CBB-stained gels (A) was estimated by densitometry. Inhibitions of cross-linking by the HSP90 peptides are expressed as a function (%). Molar ratio of the HSP90α peptide (○) or the HSP90β peptide (●) to HSP90. The result obtained using a control peptide was also shown (△).

Binding of Calmodulin to HSP90—A mixture of HSP90 and calmodulin was incubated in the presence of Ca²⁺ with a zero-length cross-linker, EDC, that cross-links close contact sites between two molecules. The mixture was resolved by SDS-PAGE as shown in Fig. 1. A major cross-linked product of 120 kDa, indicated by an arrowhead (Fig. 1, lane 4), was yielded only when Ca²⁺ was present. The 120-kDa band was not detected when either HSP90 or calmodulin was omitted. The 120-kDa band was confirmed to contain both HSP90 and calmodulin by the peptide analysis with lysylendopeptidase (data not shown). On the basis of its molecular mass, the 120-kDa product was supposed to consist of three HSP90 and one calmodulin polypeptide. In addition to the 120-kDa product, dimeric forms (215 kDa) of HSP90 were observed as indicated by an arrow (Fig. 1).

The 120-kDa product was cleaved with CNBr, yielding peptide fragments derived from both HSP90α and HSP90β (Fig. 2). As we have previously shown (6), the band indicated by an open triangle was derived from HSP90α and that indicated by a closed triangle was derived from HSP90β. These results clearly indicate that both HSP90α and HSP90β contain respective calmodulin-binding domains.

Cross-linking of CNBr-cleaved Peptide Fragments of HSP90-and Calmodulin—Short peptide fragments corresponding to calmodulin-binding domains are known to bind calmodulin as the intact proteins do (21). Given this advantage, CNBr-cleaved peptide fragments of HSP90 were used instead of the native HSP90 in cross-linking with Ca²⁺-calmodulin. An outline of the experiments to identify the calmodulin-binding domain(s) of HSP90 is shown in Fig. 3A. This approach depends upon an important assumption that CNBr does not destroy the calmodulin-binding domain(s).

Purified mouse HSP90 was completely cleaved with CNBr. The peptide fragments were mixed with calmodulin in the presence of Ca²⁺, followed by treatment with EDC. The mixtures were resolved by SDS-PAGE (Fig. 3B, lane 2). Several bands stained with CBB were observed above the calmodulin band only when the peptide fragments were cross-linked with calmodulin in the presence of Ca²⁺ (Fig. 3B, lanes 1-3). One (24 kDa) of these bands was identified as a complex of an HSP90-derived peptide fragment and calmodulin based upon the following results. Firstly, the 24-kDa band contained the amino acid sequences of both HSP90 and calmodulin (see below). Secondly, the band was stained with anti-calmodulin antibody (data not shown). Thirdly, the band exhibited, like calmodulin itself, a Ca²⁺-dependent mobility shift in SDS-PAGE (Fig. 3B, lanes 4 and 5).

Amino Acid Sequence Determination of the HSP90-derived Peptide Contained in the Cross-linked Product—The 24-kDa product was subjected to amino acid sequence determination from the NH₂ termini. The results are shown in Table I. It should be noted that the NH₂ terminus of calmodulin is acetylated (30). The sequence obtained corresponds to that of a fragment derived from mouse HSP90α beginning at the 486th amino acid residue. If HSP90α is assumed to be completely cleaved with CNBr, this peptide fragment is expected to consist of 35 amino acid residues. A similar sequence is also present in HSP90β (5).

The 24-kDa product was further treated with CNBr, after which a peak fraction recovered by reverse phase chromatography (data not shown) was subjected to amino acid sequencing. Two sequences were expected; one is the same as that directly determined on the 24-kDa product and the other is that of a CNBr-cleaved peptide fragment of calmodulin. In fact, 2 or 3 amino acid residues sequentially detected can be
aligned into two sequences. One is the expected HSP90α-derived peptide sequence, and the other corresponds to the sequence of calmodulin beginning at the 52nd amino acid residue (30).

The above results clearly show that the 24-kDa product contains both a peptide fragment of HSP90α and calmodulin. Therefore, the calmodulin-binding domain of HSP90α resides within this fragment. In fact, a putative calmodulin-binding domain of HSP90α, i.e. the 21 amino acid residues from Lys⁹⁰⁰ to Ile⁵₂⁰, is present. A positively charged, amphiphilic α-helix (Fig. 4) was predicted for this sequence according to the method of Chou and Fasman (31). The corresponding sequence (Lys⁴⁹⁸ to Val⁵¹¹) is also present in HSP90β.

Two peptides corresponding to the sequences of Lys⁸⁰⁰-Ile⁵₂⁰ of HSP90α and Lys⁹⁵¹-Val¹¹¹ of HSP90β were synthesized and examined in their ability to bind to calmodulin. The HSP90 peptides were mixed separately with calmodulin in the presence or absence of Ca²⁺ and treated with EDC. The results shown in Fig. 5 revealed that both of the HSP90 peptides bind to calmodulin in a Ca²⁺-dependent manner (lanes 2 and 3 versus lanes 5 and 6) although the binding of the HSP90α peptide was less strictly dependent on Ca²⁺ (lane 5). Furthermore, we have observed that melittin, a potent calmodulin-binding peptide, inhibits the binding of the HSP90 peptides to calmodulin (data not shown).

When either one of the above HSP90 peptides was added to the mixture of HSP90 and calmodulin, the formation of the cross-linked products between HSP90 and calmodulin was significantly reduced (Fig. 6). Inhibition of the cross-linking was not observed with a control peptide, which corresponds to an unrelated sequence, Thr⁹⁷⁸-Tyr⁹⁸⁰, of HSP90α (Fig. 6B).

Taken together, we concluded that the peptide Lys⁸⁰⁰-Ile⁵₂⁰ of HSP90α and Lys⁹⁵¹-Val¹¹¹ of HSP90β are calmodulin-binding domains of these corresponding HSP90 isoforms, respectively.

The Calmodulin-binding HSP90 Peptides Bind HSP90—Kang and Welch (32) have previously shown by using the [⁴⁵Ca]CaCl₂ overlay technique that Ca²⁺ binds HSP90. In accord with this, we have observed that [⁴⁵Ca]Ca²⁺ binds both HSP90α and HSP90β (Fig. 7A, lanes 2 and Fig. 7B, lanes 7 and 8). The binding was not detected in the presence of an excessive amount of nonradioactive Ca²⁺ (Fig. 7B, lanes 5 and 6). Unexpectedly, we found that the synthetic peptides corresponding to the calmodulin-binding domains of two HSP90 isoforms inhibited the binding of [⁴⁵Ca]Ca²⁺ to HSP90 (Fig. 7B, lanes 1–4). The control peptide did not prevent the binding of [⁴⁵Ca]Ca²⁺ to HSP90 (data not shown). It would be possible that the calmodulin-binding HSP90 peptide blocked or preoccupied the Ca²⁺-binding site(s) on HSP90, because Ca²⁺ did not bind to the calmodulin-binding HSP90 peptides (data not shown). In fact, we observed that the ¹²⁵I-labeled calmodulin-binding HSP90 peptides bind HSP90 (Fig. 8). Cross-linked products of the ¹²⁵I-labeled HSP90α peptide and HSP90 were detected when a mixture of the peptide and HSP90 was treated with EDC (Fig. 8, lanes 1 and 4). When cross-linking was performed in the presence of an excess amount of the unlabeled HSP90α peptide, the cross-linked products of the ¹²⁵I-labeled HSP90α peptide and HSP90 detected by autoradiography disappeared, and those of the unlabeled HSP90α peptide and HSP90 detected by CBB staining appeared instead (Fig. 8, lanes 2 and 5). The unlabeled HSP90β peptide weakly but significantly reduced (50% inhibition) the cross-linking between the radioiodinated HSP90α peptide and HSP90 (Fig. 8, lane 3). These results clearly indicate that the HSP90α peptide binds to HSP90. In addition, we observed that melittin inhibits the binding of the HSP90α peptide to HSP90 (Fig. 8, lane 6). Using the same cross-linking method, we found that melittin binds HSP90 (Fig. 8, lanes 7 and 8) and that the binding is inhibited by the HSP90α peptide (Fig. 8, lane 9).

The above observations prompted us to examine the effects of Ca²⁺ and the calmodulin-binding HSP90 peptides on properties of HSP90. As previously shown by Lanks (9), purified HSP90 forms self-oligomers upon exposure to elevated temperatures (Fig. 9A). We found that an addition of Ca²⁺ to purified HSP90 at 37 °C does not induce its self-oligomerization (data not shown) but enhances the self-oligomerization induced at 43 °C (Fig. 9B). Furthermore, we found that both of the synthetic peptides corresponding to the calmodulin-binding domains of HSP90α and HSP90β augmented the temperature-induced oligomerization of HSP90 although the HSP90α peptide was more effective than the HSP90β peptide (Fig. 9C).
Calmodulin-binding Domain of Mouse HSP90

**Fig. 9. Self-oligomerization of HSP90.** A, purified HSP90 (0.50 mg/ml) was incubated separately at 25, 31, 37, or 43 °C for 1 h, followed by analysis on nondenaturing PAGE with 7% gels (inset). Monomer, dimer, and oligomer bands are indicated. Densitometry of CBB-stained gels was performed to determine relative amounts of the oligomers to the total HSP90. B, purified HSP90 (0.50 mg/ml) was incubated at 43 °C for 1 h in the presence of Ca²⁺ at various concentrations. C, purified HSP90 (0.47 mg/ml) was incubated at 43 °C for 1 h with increasing amounts of the HSP90α peptide or HSP90β peptide. Only the densitometric results are shown in B and C.

**DISCUSSION**

Calmodulin-binding Domains of HSP90 Isoforms—In this study, we have identified the sequences of Lys⁶⁰⁰-Ile⁶⁰⁹ of HSP90α and Lys⁶¹¹-Val⁶¹¹ of HSP90β as calmodulin-binding domains of the corresponding proteins. Amphiphilic α-helical structures, which are common in a number of calmodulin-binding proteins (21), are predicted for their amino acid sequences according to the method of Chou and Fasman (31). We could not strictly rule out the possibility that there is another calmodulin-binding site within the HSP90 molecule which might have been destroyed by treatment of HSP90 with CNBr. The stoichiometry of the binding of calmodulin to HSP90 (Fig. 1) suggested, however, that they are unique calmodulin-binding sites of two HSP90 isoforms.

According to the amino acid sequences previously reported (5), there should be a CNBr-cleaved peptide fragment of HSP90β having the sequence similar to that of HSP90α which we have identified in this work as a calmodulin-binding domain. The cross-linked product(s) subjected to amino acid sequencing did not contain any HSP90β-derived peptide fragment, however (Table I). This may be compatible with the result shown in Fig. 5 that the cross-linked product(s) between the HSP90α peptide and calmodulin were yielded to a higher degree than that between the HSP90β peptide and calmodulin. Moreover, the HSP90β peptide is less potent than the HSP90α peptide in inhibiting the cross-linking between HSP90 and calmodulin (Fig. 6). All of these results could imply that the binding affinity of the HSP90β peptide to calmodulin is significantly lower than that of the HSP90α peptide.

On the other hand, it was shown in Fig. 2, however, that the yield of an HSP90β-derived peptide fragment (indicated by a closed triangle) from the complex of HSP90 and calmodulin was much higher than that of an HSP90α-derived peptide fragment (indicated by an open triangle) even though the cleavage was incomplete. Furthermore, both HSP90α and HSP90β equally bind actin filaments (6), and the binding was inhibited by Ca²⁺-calmodulin by 80% or more (18), suggesting that Ca²⁺-calmodulin equally binds both of the HSP90 isoforms. It is possible that the affinity of the HSP90β peptide to calmodulin is significantly weaker than that of the intact HSP90β, whereas the HSP90α peptide has an affinity comparable to that of the intact HSP90α. Neighboring amino acid sequences around the calmodulin-binding HSP90β peptide in the intact HSP90β may be important for acquiring the full affinity to calmodulin.

Binding Sites on HSP90 of Ca²⁺ and the Self Peptides—For several enzymes and proteins (33–36), calmodulin-binding domains have been proposed to interact with functionally key domains such as catalytic domains of the same polypeptides, thereby inhibiting the functions of the proteins. In these cases, the binding of calmodulin to the calmodulin-binding domains results in releasing the inhibition and, therefore, activates the proteins (33–36). We found that the calmodulin-binding HSP90 peptides also bind HSP90 polypeptides (Fig. 8). On the analogy of the calmodulin-binding proteins mentioned above, it is possible that the calmodulin-binding domains of HSP90 interact with another domain(s) within the same polypeptide. We have also shown in the present study that melittin and Ca²⁺ bind HSP90. The binding of the HSP90 peptides to HSP90 was competitively inhibited by melittin and vice versa (Fig. 8). The calmodulin-binding HSP90 peptides inhibit the binding of Ca²⁺ to HSP90 (Fig. 7). These results are consistent with the possibility that all of the calmodulin-binding HSP90 peptides, melittin, and Ca²⁺ might bind to a unique site on HSP90. If this is the case, the hypothetical site is functionally analogous to calmodulin. This site obviously differs, however, from calmodulin, because the binding of Ca²⁺ to the site was inhibited by the binding of the self peptides, whereas calmodulin binds the HSP90 peptides and HSP90 in a Ca²⁺-dependent manner. Further structural and functional analysis is necessary to clarify this apparent inconsistency.

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Calmodulin-binding Domain of Mouse HSP90