Effect of Mastoparan on the Binding of Ca^{2+} to S-100 Proteins in the Presence of Zn^{2+}

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S-100 proteins, which are acidic Ca^{2+}-binding proteins, exist in the brain and modulate enzymes and proteins as does calmodulin. In order to clarify some unique characteristics of S-100 proteins, the effects of mastoparan (MP) on the Ca^{2+} affinity of two S-100 protein isoforms (S-100a.a′, S-100b) in the presence of Zn^{2+} were examined using dialysis and stopped-flow methods. It was clearly demonstrated that the enhancement of S-100a.a′ was distinct from that of S-100b. Although the former isoform was affected only by the addition of MP, the latter was affected by MP or Zn^{2+}.

S-100 proteins are acidic Ca^{2+}-binding proteins, and mainly exist in the brain as a mixture of three dimeric isoforms (S-100a, S-100a′, and S-100b) with subunits of $\alpha\beta$, $\alpha'\beta$, and $\beta\beta$, respectively (Fig. 1).[1—3] The molecular weights of the dimeric form are all about 21000. S-100 proteins are classified as the so-called EF-hand protein, such as calmodulin; in each subunit of S-100 proteins two EF-hand-like Ca^{2+}-binding domains exist. The Ca^{2+} affinity of the C-terminal domain (K_{dca} = 10—63 μM) is greater than that of the N-terminal domain (K_{dca} = 40—200 μM).[4—7] The chemical properties of S-100a and S-100a′ are very similar, while those of S-100b substantially differ from the former two.[3,4]

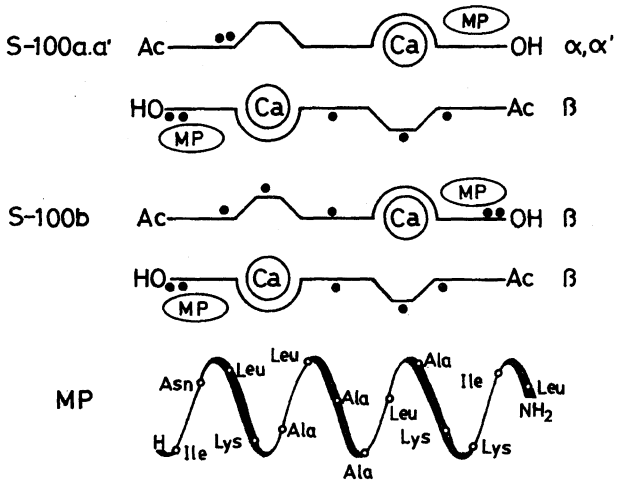

Fig. 1. Schematic models of S-100 proteins and mastoparan (MP).[16,17] The solid circle indicates the position of histidine residue. It has not been reported to assign histidine residues to Zn^{2+}-coordinating residues. The configuration of MP is in hydrophobic medium and cell membrane. Because the configuration of MP bound to calmodulin is considered to be α-helix form,[18] this structure may be valid in the case of MP bound to S-100 proteins. The half circle and the trapezoid show the strong Ca^{2+}-binding sites and the weak Ca^{2+}-binding sites, respectively. The C-terminals of S-100 proteins are estimated to be the MP-binding site.[19]

It has been reported that S-100 proteins have no enzymatic activity, but have roles in protein phosphorylation, cell differentiation, and microtuble protein assembly[8—10] through specific hydrophobic binding with other proteins.[11,12] Some of the roles were substituted by calmodulin.[12,13] The S-100 proteins also bind to mastoparan (MP, Fig. 1), which is a wasp toxin and is used as a target model peptide of calmodulin.[14,15] In a previous report[15] we reported that the addition of MP to S-100 proteins equally enhanced the Ca^{2+}-binding ability of S-100a.a′ (a mixture of S-100a and S-100a′) and S-100b. Baudier et al. also reported that the addition of bee toxin (melittin) to S-100b enhanced the Ca^{2+}-binding ability.[13]

On the other hand, S-100 proteins have a higher affinity to Zn^{2+} (K_{dzn} = 2 μM for S-100a.a′ and 0.16 μM for S-100b) than to Ca^{2+}.[7,20] The Zn^{2+}-binding sites are different from the Ca^{2+}-binding sites.[7,20,23] This Zn^{2+}-binding property is a characteristic of S-100 proteins, and has not been found in other EF-hand proteins. Taking into account the Zn^{2+} concentration (0.3 μM) in the brain, the S-100–Zn^{2+} complex should not be ignored.[21,22]

Although, Zn^{2+}-binding to S-100 proteins is known to enhance their Ca^{2+}-binding ability,[7] the effect of the coexistence of Zn^{2+} and MP on the Ca^{2+} affinity of S-100 proteins has not been examined. It is unclear whether Zn^{2+} and MP compete for each other's binding sites. In this study, the effect of MP and Zn^{2+}-binding on the Ca^{2+} affinity of S-100a.a′ or S-100b isoform was investigated using dialysis and stopped-flow methods.

Experimental

Materials. S-100a.a′ and S-100b isoforms were prepared from bovine brain, as previously reported.[24] These isoforms were then treated with trichloroacetic acid to remove any bound Ca^{2+}. 8-Amino-2-[(2-amino-5-methylphenoxy)methyl]-6-methoxyquinoline-*N*,*N*,*N*′,*N*′-tetraacetic acid (Quin II, a metal cation chelator)

and MP were purchased from Dojin (Kumamoto, Japan) and the Peptide Institute (Osaka, Japan), respectively. All other reagents were of analytical grade and used without further purification.

The quantities of metal ion bound to the S-100 proteins was determined by static dialysis, as previously reported.[15,25] The metal-ion complex samples were prepared as follows: 100 μM $ZnSO_4$ and 100 μM $CaCl_2$ were added to a 20 μM S-100a.a′ solution, and 200 μM $ZnSO_4$ and 100 μM $CaCl_2$ were added to a 20 μM S-100b solution, respectively, under the presence or absence of 40 μM MP.#) All of the concentrations were the final value in a dialysis tube. The buffer component was 20 mM Tris-HCl (pH = 7.14), including 2 mM 2-mercaptoethanol. The amount of metal ions was measured by an atomic-absorption spectrometer (AA-630-11, Shimadzu). The amounts of the Zn^{2+} or Ca^{2+} bound to the S-100 proteins were determined by subtracting the amount of the metal-ion in a dialyzed outer buffer from the total.

A kinetic study of the release of Ca^{2+} from the complex was performed by a stopped-flow method with a Unisoku-501 apparatus (Hirakata, Japan), as previously reported.[15] In brief, one syringe contained 20 μM S-100a.a′, 100 μM $CaCl_2$, and 100 μM $ZnSO_4$ in a 20 mM Tris-HCl (pH = 7.14) buffer including 2 mM 2-mercaptoethanol, or 20 μM S-100b, 100 μM $CaCl_2$, and 200 μM $ZnSO_4$ in the same buffer. Another syringe contained 400 μM (for S-100a.a′) or 600 μM (for S-100b) Quin II in the same Tris-HCl buffer. The fluorescence intensity was monitored at wavelengths greater than 470 nm with excitation at 334 nm. To test the effects of MP, a 40 μM solution of MP was additionally added to the mixture of S-100 proteins.

Results and Discussion

The effects of MP or the Zn^{2+} affinity of S-100 proteins obtained by the static-dialysis method are listed in Table 1. The addition of MP did not affect the Zn^{2+}-binding strengths of S-100a.a′ and S-100b. Since the affinity of MP to S-100 proteins (K_d = ca. 1 μM) is almost the same as that of Zn^{2+},#,[13,26] the present results suggest that the Zn^{2+}-binding sites in S-100 proteins are different from the MP-binding sites.

The effects of MP on the Ca^{2+}-binding of the two isoforms of the S-100 protein, with or without the presence of Zn^{2+}, are summarized in Table 2. Under the coexistence of Zn^{2+}, the Ca^{2+}-binding ability of S-100a.a′ was drastically enhanced by the addition of MP, while that of S-100b was little affected.

Table 1. Amounts of Zn^{2+} Bound to S-100 Proteins Dialyzed against 20 mM Tris-HCl and 2 mL 2-Mercaptoethanol at 1 °C

Condition	Zn^{2+}/protein (mol/mol)	
	S-100a.a′	S-100b
No additions	2.8±0.3	5.9±0.3
MP	2.6±0.3	5.8±0.3

Zn^{2+} content was measured by atomic absorption spectrophotometer. The five runs were made and the data are the mean ±S. D.

#) It is known that mellitin or annxin II, a peptide similar to MP, form a complex with S-100 proteins in a molar ratio of 1 : 2[13,27] and their affinity to S-100 proteins, in terms of K_d, is ca. 1 μM and 0.4 μM, respectively.[13,27] Thus, when a similar stoichiometry and affinity are assumed, the concentration of 40 μM would be sufficient for 20 μM S-100 proteins solution.

Table 2. Amounts of Ca^{2+} Bound to S-100 Proteins under the Coexistence of Zn^{2+} Dialyzed against 20 mM Tris-HCl and 2 mM 2-Mercaptoethanol at 1 °C

Condition	Ca^{2+}/protein (mol/mol)	
	S-100a.a′	S-100b
Zn^{2+}	0.65±0.04	1.2±0.15
Zn^{2+}, MP	1.6±0.1	1.4±0.2
No additions[a)]	0.74±0.10	0.61±0.03
MP[a)]	1.5±0.1	1.4±0.1

Ca^{2+} content was measured by atomic absorption spectrophotometer. The five runs were made and the data are the mean±S. D.
a) Previous work; Ref. 15.

This is apparently different from the case for the previous report;[15] however, the Ca^{2+}-binding ability of S-100b was 2-fold increased in the presence of Zn^{2+} alone, which was in accord with the data of Baudier et al.[7] The difference in the Ca^{2+} affinity between two isoforms, S-100a.a′ and S-100b, was thus clearly demonstrated. The Ca^{2+}-binding ability of one isoform, S-100a.a′, was affected by MP, but not by Zn^{2+}. On the contrary, that of another isoform, S-100b, was enhanced by MP or Zn^{2+}. This enhanced effect of Zn^{2+} was not simply additive to that of MP, suggesting a cooperating structural change of S-100b.

The effects of MP on the Ca^{2+}-binding ability were also investigated using the stopped-flow method. The fluorescence intensity of Quin II increases by about five-times by the binding of Ca^{2+}, while the binding of Zn^{2+} causes a slight decrease in the intensity. The MP, S-100a.a′, and S-100b did not affect the fluorescence intensity. The Ca^{2+}-release from S-100a.a′ or S-100b in the presence of Zn^{2+} is shown in Fig. 2. Since the Ca^{2+}-association of Quin II is much faster than Ca^{2+}-release from the Ca^{2+}-protein complex,[27,28] the increase in the fluorescence intensity shows the rate of Ca^{2+}-release from the S-100 proteins or from the these-MP complexes. The addition of MP caused a decrease in the rate for both isoforms; the effect is particularly remarkable for S-100a.a′. The data in Fig. 2a were well reproduced, and fitted on solid curves obtained by assuming of first-order kinetics. However, the addition of MP to S-100b caused a slight deviation from the exponential curve within a 40 ms range of the dotted line (Fig. 2b). This deviation may be related to Ca^{2+}-release from the low-affinity site(s) in S-100b. The first-order rate constants of Ca^{2+}-release (k_{off}) were obtained by a curve-fitting method, and are summarized in Table 3. Although the addition of MP remarkably decreased k_{off} for all cases, the effect of MP for two isoforms in the presence of Zn^{2+} was not so marked as that in the absence of Zn^{2+}. The k in the presence of Zn^{2+} were slightly smaller than those in the absence of Zn^{2+}, while those in the presence of Zn^{2+} and MP were slightly larger than those in the presence of only MP.[15] These results suggest that the Zn^{2+}-binding of S-100 proteins causes a conformational change which affects k. Irrespective of the presence of MP, slight differences in k between S-100a.a′ and S-100b were observed. This suggests that the Ca^{2+}-binding characteristics of the two isoforms dif-

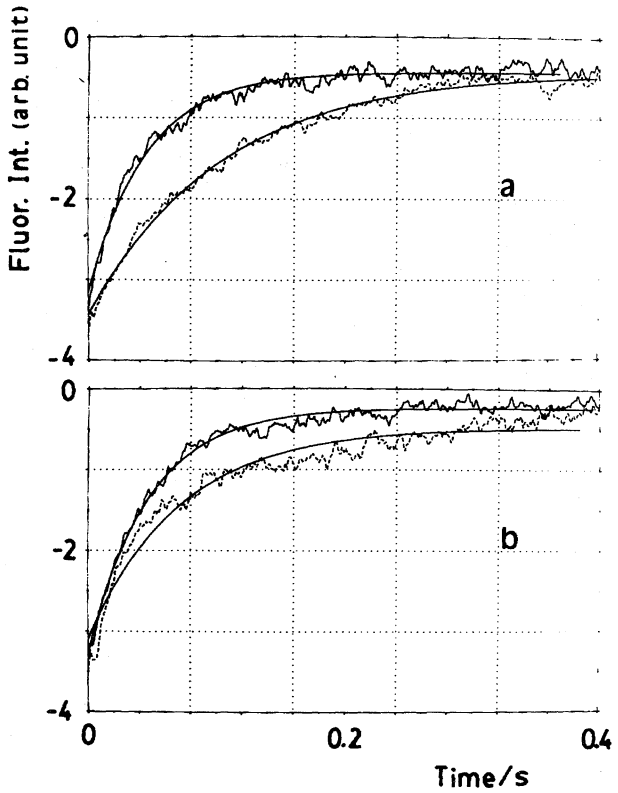

Fig. 2. Ca^{2+}-release rates from S-100a.a′ (a) and S-100b (b). A solution of 20 μM S-100a.a′, 100 μM $CaCl_2$, and 100 μM $ZnSO_4$ in 20 mM Tris-HCl (pH = 7.14) and 2 mM 2-mercaptoethanol was mixed with a solution of 400 μM Quin II (a). The solid line in (a) and (b) shows the Ca^{2+}-release rate in the absence of MP. The dotted line in them denote the addition of 40 μM MP to S-100 protein solution. In the case of S-100b (b), 200 μM $ZnSO_4$ and 600 μM Quin II were used. Change in Quin II fluorescence was monitored at wavelengths greater than 470 nm with excitation at 334 nm. The temperature was 3 °C. The abscissa indicates the time after the mixing of the foregoing two solutions. The amplitude of the ordinate is the relative value. Zero in the ordinate denotes the slightly upper level of the saturation of fluorescence increase. Each trace represents an accumulated signals of 10—11 measurements.

Table 3. Rate Constants of Ca^{2+}-Release Reactions at 3 °C

Condition	k/s^{-1}	
	S-100a.a′	S-100b
Zn^{2+}	26.0±1.5	21.6±0.9
Zn^{2+}, MP	10.1±0.6	14.3±0.7
No additions[a)]	31±2	31±3
MP[a)]	6.3±0.3	6.5±0.2

a) Previous work; Ref. 15

fer from each other in the presence of Zn^{2+}. Since the effect of MP on k for S-100b was confirmed, the slight increase in the amount of Ca^{2+} bound to S-100b caused by MP-binding (1.2→1.4, Table 2) can not be ignored.

In conclusion, the effect of Zn^{2+} and/or MP on the Ca^{2+}-binding ability was found to be distinct for each isoform. In the absence of MP, the Zn^{2+}-binding to S-100b alone showed an enhancement of its Ca^{2+}-binding ability. Since Zn^{2+}-binding to S-100b induced the exposure of hydrophobic amino acid residues,[29] the observed increase in the Ca^{2+}-binding ability of S-100b may be closely related to its hydrophobicity. The Zn^{2+}-binding or the MP-binding to S-100b increased the Ca^{2+}-binding ability, and the effects of MP were not independent of the effects of Zn^{2+} (Table 3). It was reported that although the association of calmodulin with some peptide changed its dumbell-like structure to a more compact sphere shape, the EF-hand domain maintained its structure.[30] The association yielded an increase in the Ca^{2+}-binding affinity by more than ten times, even though the full mechanism for this enhancement has not been clarified.[31] However, such a conformational change may not be applied in the present case, since the S-100 proteins lack a flexible hinge region connecting two EF-hand domains.[2,16] To clarify the mechanism concerning the increase in the Ca^{2+} affinity by Zn^{2+} and MP, it is necessary to compare their tertiary structure of Ca^{2+}/S-100b/MP, Ca^{2+}/S-100b/Zn^{2+}, and Ca^{2+}/S-100b/MP/Zn^{2+} complexes.

The Ca^{2+} affinity of S-100a.a′ was remarkably enhanced by MP-binding, while it was not affected by Zn^{2+}-binding. This was quite different from the case of S-100b. As illustrated in Fig. 1, S-100b is a homo-dimer containing ten histidine residues, and S-100a.a′ is a hetero-dimer containing seven histidine residues. The difference in the primary structure must yield a difference in Ca^{2+}-binding characteristics between them. The tertiary structure of both isoforms with Ca^{2+}, Zn^{2+}, and MP will provide a clear understanding.

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