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Circular Dichromism Study of S-100 Protein Complexes

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S－１００蛋白錯体の円偏光二色性の研究

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Abstract

Effect of Zn^{2+}, Ca^{2+} and mastoparan (MP) on the secondary structure of bovine brain S-100 a.a'($\alpha\ \beta$) and S-100b($\beta\ \beta$) was examined by circular dichromism (CD) spectrum measurements. It was confirmed that the ellipticity ($-[\theta]_{222}$) of S-100a.a' decreased by addition of Zn^{2+} by ca. 12 %, and on the contrary, that of S-100b increased by ca. 6 %. In the case of MP-addition, the value $-[\theta]_{222}$ little changed for S-100a.a' and slightly increased for S-100b. Thus addition of Zn^{2+} and MP has an opposing effect on the ellipticity of respective isoform S-100a.a' and S-100b.

Introduction

S-100 proteins are known to be acidic Ca^{2+}-binding proteins (molar mass ≒ 21 kg/mol), consisting of S-100a($\alpha\ \beta$), S-100a'($\alpha'\beta$) and S-100b($\beta\ \beta$).[1] Since the chemical properties of S-100a and S-100a' are very similar, the mixture of them, denoted as S-100a.a', is regarded as practically homogeneous. The chemical properties of S-100b substantially differ from those of S-100a.a'.[2] In each subunit of S-100 proteins there exist two Ca^{2+}-binding domains which have so-called EF-hand structure.[1, 2] On their affinity to metal cations it is known that S-100 proteins have a higher affinity to Zn^{2+} than to Ca^{2+},[3] and the Zn^{2+}-binding sites differ from the Ca^{2+}-binding sites,[3] and only for S-100b the Zn^{2+}-binding increased its Ca^{2+}-binding affinity.[4]

On the other hand, S-100 proteins also bind a target model compound mastoparan (MP), a wasp toxin peptide, irrespective of the existence of Zn^{2+}- or Ca^{2+}-binding.[4]

For further understanding of the foregoing results, it is important to examine the structural characteristics of the Ca^{2+}-bound S-100 protein complexes, ie the secondary structure.

The secondary structure of those complexes have not yet been examined. In the present report, the effects of Ca^{2+}, Zn^{2+}, and MP on the ellipticity ($-[\theta]_{222}$) of S-100 proteins were

examined using circular dichromism (CD) spectrum method.

Materials and Methods

S-100a.a' and S-100b were prepared from bovine brain as previously reported.[5] MP was purchased from Peptide Institute. CD spectrum was measured using a JASCO J500A spectropolarimeter equipped with a data processor DP500N. The measurements were performed with 29 μM solution of S-100a.a' and 42 μM solution of S-100b, in 20 mM MOPS-NaOH (pH = 7.0) buffer at room temperature using a rectangular quartz cell of 1 mm light path. The concentration of added Zn^{2+} and Ca^{2+} were 0.2 mM and 1 mM, respectively. MP was added to the S-100a.a' solution and to the S-100b solution, with mole ratio of 1:1.

Results and Discussion

The CD spectra of S-100a.a' in the presence and absence of Zn^{2+} are shown in Fig. 1. The Zn^{2+}-binding to S-100a.a' decreased $-[\theta]_{222}$ by ca. 12 %, which shows the corresponding decrease of α-helix structure. All the results for Zn^{2+}, Ca^{2+} and MP on the ellipticity ($-[\theta]_{222}$) of S-100

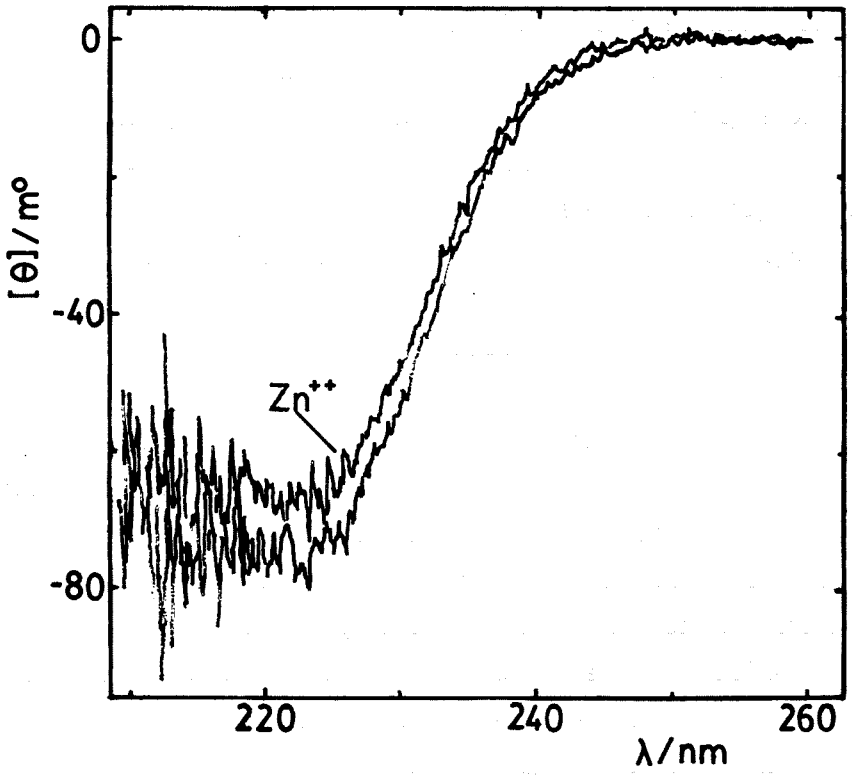

Fig.1. CD spectrum of S-100a.a' in the presence and absence of Zn^{2+}

proteins are summarized in Table 1. The Ca^{2+}-binding to S-100a.a' and S-100b slightly decreased their $-[\theta]_{222}$, similarly as reported by Mani et al.[6, 7] The Zn^{2+}-binding to S-100b increased $-[\theta]_{222}$ by ca. 6 %. The effect of Zn^{2+}-binding to S-100b quite opposite to the one to S-100a.a' (Fig. 1). The $-[\theta]_{222}$ of S-100a.a' was little affected by MP-binding, whereas that of S-100b

Table 1. Effect of Ca^{2+}, Zn^{2+} and MP on $-[\theta]_{222}$ of bovine brain S-100a.a' and S-100b.

	$-[\theta]_{222}$/m°	
Condition	S-100a.a'	S-100b
No additions	77 ± 1	73 ± 1
Ca^{2+}	73 ± 1	71 ± 1
Zn^{2+}	68 ± 1	76 ± 1
MP	75 ± 1	78 ± 1
Ca^{2+}/Zn^{2+}	69 ± 1	73 ± 1
Ca^{2+}/MP	74 ± 1	75 ± 1
Ca^{2+}/Zn^{2+}/MP	71 ± 1	75 ± 1

*) The concentration of S-100a.a' and S-100b were 29 μM and 42 μM, respectively.

was increased by ca. 7 %. The difference in those effects of Zn^{2+} and MP for the cases of S-100a.a' and S-100b would be attributable to their difference in the fractions of α-helix, β-sheet, β-turn, and random structure.

The effect of Zn^{2+} and/or MP on $-[\theta]_{222}$ of Ca^{2+}-bound S-100a.a' was slight. The addition of Zn^{2+} had little effect on $-[\theta]_{222}$ of Ca^{2+}/S-100b, while that of MP caused slight increase of $-[\theta]_{222}$ irrespective of the coexistence of Zn^{2+}.

It has been known that the Ca^{2+}-binding affinity of each isoform are enhanced by MP-binding, and that of only S-100b is enhanced by Zn^{2+}-binding.[4] These enhancement effects have no parallelism to $-[\theta]_{222}$ as shown in Table 1, the cases of Ca^{2+}/S-100a.a', Ca^{2+}/S-00a.a'/MP, and Ca^{2+}/S-100a.a'/Zn^{2+} being representative.

In summary, the effect of Zn^{2+}-binding to S-100a.a' and S-100b on their $-[\theta]_{222}$ were apparently opposite. The effect of Zn^{2+} and/or MP-binding to Ca^{2+}/S-100a.a' and Ca^{2+}/S-100b was rather complicated.

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