Kinetic Study of the Binding of Ca^{2+} to S-100 Proteins

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(Received February 20, 1995)

The effects of trifluoperazine (TFP) and mastoparan (MP) on the rate of Ca^{2+}-release from bovine brain S-100 proteins (s-100a.a′, S-100b) were determined using a stopped-flow method. No remarkable difference was observed in the rate constants for the case of S-100a.a′ and S-100b at pH=7.14. The addition of MP decreased the rate constants to one fifth, while TFP had little influence.

S-100 proteins (molar mass = 21 kg mol^{-1}) are Ca^{2+}-binding proteins which exist mainly in bovine brain as a mixture of the three isoforms S-100a, S-100a′, and S-100b with subunits of $\alpha\beta$, $\alpha'\beta$, and $\beta\beta$, respectively.[1—3] S-100 proteins belong to the same EF-hand protein family as calmodulin, and each subunit has two EF-hand domains. The first EF-hand is exceptional with two extra amino acids in the Ca^{2+}-binding loop, and binds Ca^{2+} more loosely than the second EF-hand. S-100 proteins maximally bind two Ca^{2+} atoms per subunit, with pK_d's of 4.2 and 3.7.[4—6] Although the characteristic chemical properties of S-100a and S-100a′ are quite similar except for a subtle difference in the net charge, those of S-100b are different from S-100a and S-100a′.[3,4]

The physiological function of S-100 proteins has still not been disclosed, so the target proteins of S-100 proteins remain unknown. Trifuoperazine (TFP) and mastoparan (MP) have been used as target models of calmodulin.[7—9] It is also known that S-100 proteins interact with TFP and MP.[10—12] Although studies concerning conformation changes in Ca^{2+}-bound S-100 proteins induced by TFP or MP have been reported,[10—12] the effects of TFP or MP on Ca^{2+}-binding to S-100 proteins have not been examined.

In this study, the effects of TFP and MP on the rate constants of Ca^{2+}-release from S-100 proteins were examined using a fluorescence stopped-flow technique to clarify the characteristic differences between S-100a.a′ and S-100b. Furthermore, by means of dialysis, these kinetic results are shown to be consistent with the static Ca^{2+}-binding ability of S-100 proteins.

Experimental

Materials. S-100a.a′ and S-100b were prepared from bovine brain as previously reported.[13] Quin II, TFP, and MP were purchased from Dojin, Sigma, and the Peptide Institute, respectively. All other chemicals were of analytical grade, and were used without further purification.

Methods. Static fluorescence measurements were carried out with a Hitachi MPF-4 spectrofluorometer at room temperature.

Kinetic measurements were made by a stopped-flow method with a Unisoku USP-501 apparatus. For the excitation of Quin II, 334-nm wavelength light from a Xenon lamp was used. One syringe contained 20 μM S-100a.a′ or S-100b and 100 μM $CaCl_2$, 20 mM Tris(hydroxymethyl)-methylamine (Tris-HCl) (pH=7.14), and 2 mM 2-mercaptoethanol, and the other contained 200 μM Quin II in the Tris-HCl buffer (1 M=1 mol dm^{-3}). In order to study the effects of TFP and MP, 40 μM TFP or MP solution was added to the Ca^{2+}-bound S-100 protein solution. The reaction rate was obtained in the same way as that for Ca^{2+}-release from calmodulin.[14,15]

The ratio of TFP bound to S-100 proteins has been reported to be unity in the presence of $CaCl_2$.[10] So, under the present conditions, the TFP binding sites of the S-100 proteins seemed to be saturated (pK_d=5.4).[10] The concentration of MP in this study was chosen to be 40 μM for the following reasons. Although the stoichiometry and the affinity of MP to S-100 proteins have not been reported, it is known that S-100 proteins form a complex with mellitin or annexin II in a molar ratio of 1 : 2.[16,17] The affinity of annexin II to S-100 proteins (pK_d=6.4) is much higher than that of TFP.[17] Because MP is a peptide similar to mellitin or annexin II, a similar stoichiometry and affinity are predicted.

The dialysis was performed as follows.[18] An S-100 protein solution (3 ml) was dialyzed once overnight against 20 mM Tris-HCl and 2 mM 2-mercaptoethanol (100 ml) at 1 °C. The component of the S-100 protein solution was the same as that of the syringe solution used in the stopped-flow technique. The amount of Ca^{2+} contained in the sample solution was measured by a Shimadzu 630-11 atomic absorption spectrophotometer. The amount of Ca^{2+} bound to the S-100 proteins was calculated by subtracting the amount of Ca^{2+} in the solvent from the total amount. This showed the relative measure of the Ca^{2+}-binding strength of the S-100 protein molecule under three conditions: no additions, in the presence of TFP, and in the presence of MP.

Results and Discussion

It is still difficult to quantitatively separate S-100a and S-100a′, so a mixture of S-100a and S-100a′ was

used. No difference has been observed between them with respect to Ca^{2+}-binding.

Figure 1 shows the fluorescence spectrum of Quin II (a calcium chelator) in S-100a.a′ solution. It is reported that Quin II has a strong affinity for Ca^{2+} at neutral pH ($pK_d = 7.1$).[15] The intensity was enhanced five times by the addition of $CaCl_2$. This was also found in the case of S-100b. The addition of TFP or MP to the solution had no effect on the spectrum. Since no appreciable interaction between S-100 proteins and Quin II was observed, the above fluorescence change must correspond to the Ca^{2+} uptake of Quin II.

Figure 2 shows the change in the intensity, i.e. the reaction rate, induced by mixing the Quin II solution and the Ca^{2+}-bound S-100b solution at 3 °C. It has already been confirmed[14,15] that the association of Quin II and Ca^{2+} is much faster than the Ca^{2+}-release reaction of a Ca^{2+}-binding protein. Therefore, the curves in Fig. 2 can be regarded as the rate of Ca^{2+} release from S-100b (Fig. 2a) and the MP-S-100b complex (Fig. 2b). A significant difference was observed between the rates of Ca^{2+} release from Ca^{2+}-bound S-100b and from MP-S-100b. The addition of MP caused a decrease in the rate. Although the reaction may proceed through two steps, the data were analyzed based on a single first-order exponential equation. The rate constants are $k = 31 \pm 3$ s^{-1} for S-100b and $k = 6.5 \pm 0.2$ s^{-1} for MP-S-100b. The effect of MP on the Ca^{2+}-release rate was also observed for S-100a.a′. The rate constant of S-100a.a′ decreased from 31 ± 2 s^{-1} to 6.3 ± 0.3 s^{-1}. No difference in the rate constants was observed between S-100a.a′ and S-100b in both the presence and absence of MP (Table 1).

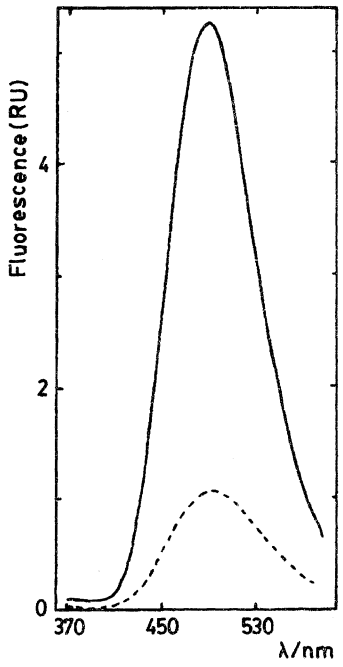

Fig. 1. Fluorescence spectra of 100 μM Quin II in 10 μM S-100a.a′, 20 mM Tris-HCl, and 2 mM 2-mercaptoethanol. The solid line and dashed line denote the spectrum in the presence and absence of 50 μM $CaCl_2$, respectively. Quin II was excited at 334 nm. Fluorescence intensity is given in arbitary units.

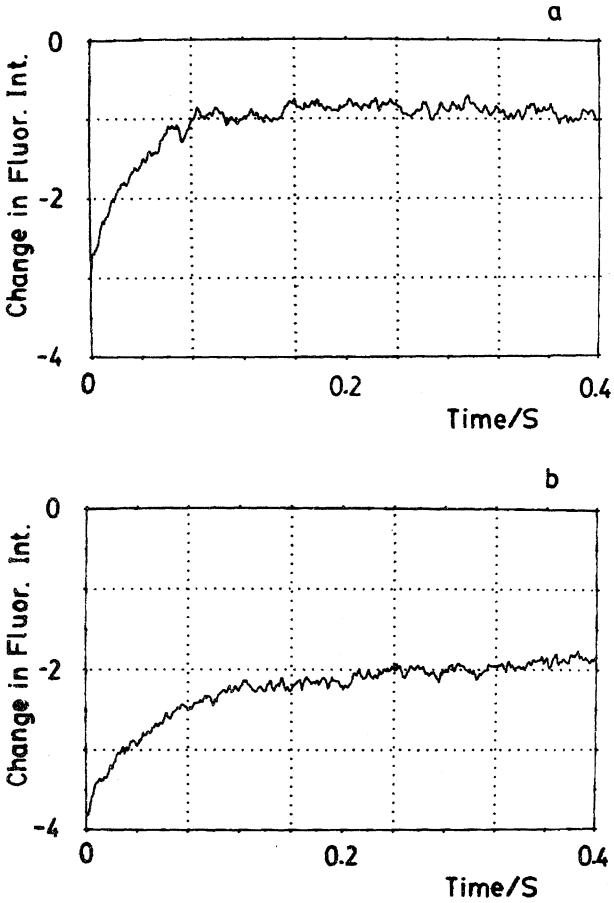

Fig. 2. Ca^{2+}-release reactions from S-100b in the absence (a) and the presence (b) of MP. A. solution of 20 μM S-100b and 100 μM $CaCl_2$ in 20 mM Tris-HCl (pH=7.14), and 2 mM 2-mercaptoethanol was mixed with a solution of 200 μM Quin II in the same solvent. The concentration of added MP was 40 μM. Quin II fluorescence was monitored at wavelengths greater than 470 nm with excitation at 334 nm. The temperature was 3 °C. The amplitude of the ordinate is the relative value. Each trace represents an accumulated signals of 10—12 measurements.

Trifluoperazine was added to the S-100 protein solution at a molar ratio of 1:2. Table 1 shows the effects of TFP on the Ca^{2+} release from S-100a.a′ and S-100b at 3 °C. The rate constants of the reaction of S-100a.a′ and S-100b were 42 ± 5 and 34 ± 2 s^{-1}, respectively. The addition of TFP caused a slight increase in

Table 1. Kinetic Parameters of Ca^{2+}-Release Reactions at 3 °C

	k/s^{-1}		
	Pure solvent	With TFP	MP
S-100a.a′	31±2	42±5	6.3±0.3
S-100b	31±3	34±2	6.5±0.2

the k for S-100a.a′. On the other hand, no remarkable effect was observed on S-100b. This result suggests that the binding of S-100a.a′ with Ca^{2+} was weakened by the binding of TFP. The difference in the affinity to Ca^{2+} is presumably caused by the difference in the subunit structure of S-100a.a′ (α,β) and S-100b (β,β). Namely, the results of static experiments[3,4] are consistent with those of the present kinetic study. In conclusion, there is no significant difference in the rate constant of Ca^{2+}-release from S-100 proteins in the presence and the absence of TFP.

In contrast to TFP, MP caused an obvious decrease in k. Because both TFP and MP are amphiphilic agents, they are liable to produce foam on mixing in the stopped-flow apparatus. This effect becomes remarkable above 10 °C. Therefore, the error in the k of Ca^{2+} release increases with an increase in temperature (Fig. 3). From the temperature dependency of k, the activation energy of the Ca^{2+}-release reaction was estimated to be ca. 15 kcal mol^{-1} for both S-100a.a′ and S-100b (Fig. 3). The activation energy obtained in the presence of TFP or MP was ca. 15 and ca. 14 kcal mol^{-1}, respectively, for both S-100a.a′ and S-100b. A quantitative comparison of the values is difficult, however.

The above decrease in k on the addition of MP suggests that the binding of MP to S-100 proteins strengthens the Ca^{2+}-binding ability of the S-100 proteins. This hypothesis was briefly confirmed by the static dialysis method (Table 2). The Ca^{2+}-binding strengths of the S-100 proteins were roughly estimated from the content of Ca^{2+} bound to the S-100 proteins. Table 2 shows two conclusions: The addition of MP largely strengthens the Ca^{2+}-binding ability of S-100a.a′ and S-100b to the same extent, and the Ca^{2+}-binding ability of S-100a.a′ and S-100b is not remarkably affected by the addition of TFP.

Table 2. Amounts of Ca^{2+} Bound to S-100 Proteins 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
No additions	0.74±0.10	0.61±0.03
TFP	0.61±0.11	0.65±0.09
MP	1.5±0.1	1.4±0.1

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

The effects of the addition of TFP and MP to S-100 proteins were significantly different as shown by the kinetic and static data. Although these compounds bind to S-100 proteins by hydrophobic interactions, their molecular shapes and molecular weights are clearly different. It is also known that the MP (M.W.=1479) form shows an α-helix structure in the binding with calmodulin,[5] and that TFP (M.W.=480) has a plane of aromatic rings. In addition, the stoichiometries are different as previously described. Therefore, the conformational changes in the S-100 proteins induced by the binding of TFP must be different from those induced

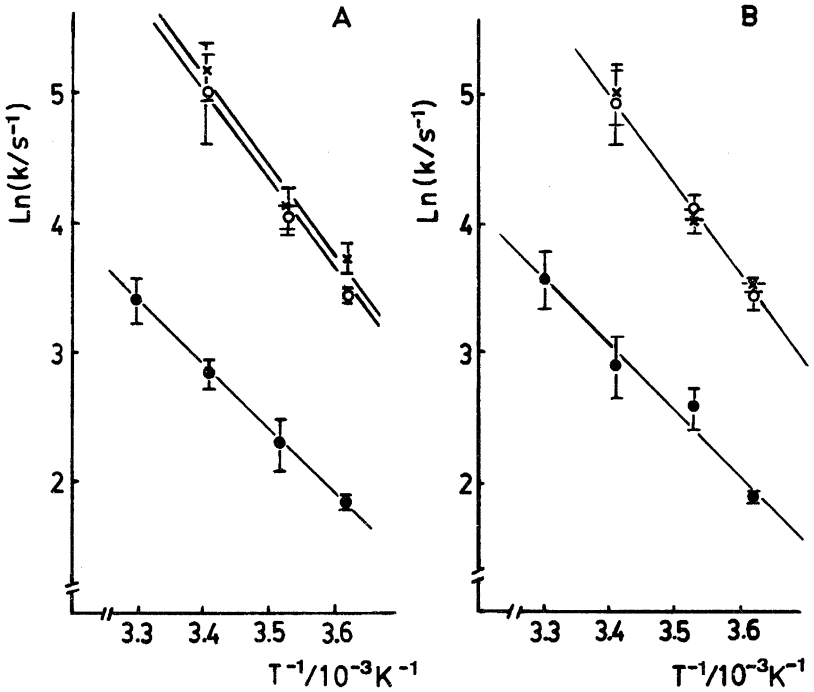

Fig. 3. Temperature dependence of k of Ca^{2+}-release from S-100a.a′ (A) and S-100b (B) in the absence of additions (○) and in the presence of either TFP (×) or MP (●). Each data point represents the average±S.E. of 3 or 4 time course.

by the binding of MP. It is considered that the conformation around the EF-hand structure is influenced by the binding of MP and TFP. The difference in the conformation may result in the difference in the rate of Ca^{2+} release from S-100 proteins induced by Quin II.

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