Interaction of Trifluoperazine with S100 Protein: a ^{19}F NMR Study*

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Abstract

^{19}F NMR spectra were measured to investigate the interaction of trifluoperazine (TFP) with porcine brain S100 protein (S100) under various conditions. It was found that TFP binds to S100 irrespective of Ca^{2+}. However, in the presence of Ca^{2+} the apparent affinity of TFP to protein (K_d = 20 μM) was greater than that in its absence (K_d = 85 μM). Zn^{2+} also enhanced the binding of TFP to S100. The ratio of TFP bound to S100 was estimated to be nearly unity in the presence of Ca^{2+}. It was also found that KCl only markedly affected the interaction of TFP with S100 in the presence of Ca^{2+}. The ^{19}F NMR chemical shift of the TFP–S100 solution changed much depending upon the pH of the solution in the presence of Ca^{2+}, while no remarkable pH dependence of the ^{19}F NMR chemical shift was observed for the TFP–S100 solution in the absence of Ca^{2+}. These pH effects are in contrast wih those observed for the TFP–calmodulin solution.

Introduction

S100 is an acidic Ca^{2+}-binding protein with a molecular weight of 21 000 which is structurally related to calmodulin (CaM) [1–3]. CaM is an ubiquitious and multifunctional Ca^{2+}-dependent regulatory protein [4], whereas S100 seems to be a nervous specific protein [1], but its biological function is not well known. While CaM has high- and low-affinity binding sites for TFP, an antipsychotic drug belonging to phenothiazine [5, 6], from the study of equilibrium dialysis it was suggested that S100 is unable to bind with TFP [7]. Recently, however, it was reported that chlorpromazine (CPZ), another phenothiazine drug, binds to S100 both in the presence and absence of Ca^{2+} [8].

In this paper, we have used ^{19}F NMR to study the interaction of TFP with S100 under various conditions where no aggregation of TFP occurred. It was found that TFP binds to S100 irrespective of Ca^{2+}. However, in the presence of Ca^{2+} the affinity of TFP to protein was greater than that in its absence. In addition, it was found that Zn^{2+} also enhances the TFP binding to S100 and that KCl reduces the Ca^{2+} role in the TFP binding to S100. The binding site of TFP on the S100 molecule was also discussed.

Experimental

S100 was purified from porcine brain by ammonium sulfate fractionation followed by column chromatography on DEAE-Sephadex A-50 and Sephadex G-75 equipment [9]. Protein concentrations in the solutions were determined by the method of Lowry *et al.* [10] using bovine serum albumin as the standard. The purity of the protein was checked by SDS–polyacrylamide gel electrophoresis [11]. TFP was purchased from Sigma. All other reagents used were of the highest guaranteed grade and were used without further purification.

^{19}F NMR spectra were measured on a Brucker CXP-300 FT NMR spectrometer at 282.3 MHz with external D_2O for the frequency lock at 298 ± 0.5 K. Chemical shifts in Hz were referred to signals of ^{19}F nuclei of TFP (0.1 mM) in 0.1 M MES–KOH buffer (pH 7.0). Resonances occurring in the downfield region were taken as positive in Hz [12, 13].

Results and Discussion

^{19}F NMR spectral behavior of TFP by adding S100 in MES–KOH buffer (pH 7.0) was studied in the

*Abbreviations used throughout: TFP, trifluoperazine; S100, S100 protein; CaM, calmodulin; K_d, dissociation constant; CPZ, chlorpromazine; NMR, nuclear magnetic resonance; MES, 2-(*N*-morpholino)ethanesulfonic acid; SDS, sodium dodecyl sulfate.

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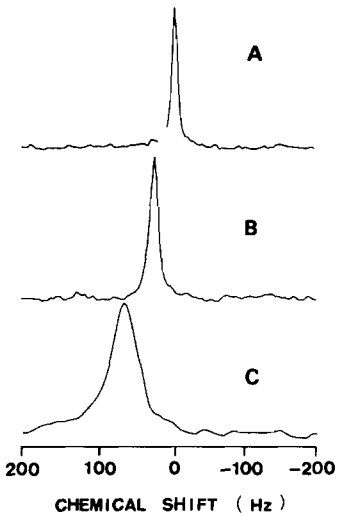

Fig. 1. ^{19}F NMR spectra of: (A) 0.1 mM TFP in 0.1 M MES–KOH buffer (pH 7.0), containing 0.1 mM EGTA; (B) 0.1 mM TFP–0.1 mM S100 in 0.1 mM EGTA solution; (C) 0.1 mM TFP–0.1 mM S100 in 2.0 mM $CaCl_2$–0.1 mM EGTA solution; sweep width, 4000 Hz; number of scans, 8×10^3 for (A), 2×10^4 for (B), 3×10^4 for (C); exponential line broadening, 4Hz for (A) and (B), 16 Hz for (C); pulse width, 25 μs (45° pulse); acquisition time, 0.27 s.

presence of 2.0 mM $CaCl_2$ or 0.1 mM EGTA. On addition of S100 (0.1 mM) to the TFP (0.1 mM) solution in the absence of Ca^{2+}, the ^{19}F NMR signal of TFP shifted downfield by 27 Hz and the half-bandwidth increased a little from 9 Hz to 13 Hz (Fig. 1A, B). In the presence of 2.0 mM $CaCl_2$, the change of the chemical shift of TFP caused by adding S100 was more pronounced (70 Hz) than that in its absence, and the half-bandwidth of TFP was also increased to 33 Hz by adding S100 (Fig. 1C). These ^{19}F NMR changes seemed to be caused by the binding of TFP to S100 both in the presence and absence of Ca^{2+}, although equilibrium dialysis studies have suggested that S100 has no TFP binding activity [7].

Figure 2A shows the effect of adding S100 on the chemical shift and the half-bandwidth of TFP. The extent of changes in the chemical shift of TFP with the addition of S100 in the presence of Ca^{2+} were different from those in its absence. When Ca^{2+} (2.0 mM) was present, the ^{19}F NMR signal abruptly shifted downfield with an increasing concentration of S100, and the chemical shift became constant at S100 concentrations greater than 0.1 mM. On the other hand, in the absence of Ca^{2+} the chemical shift gradually changed until the S100 concentration reached 0.3 mM. The half-bandwidth of TFP in the presence of Ca^{2+} hyperbolically increased by adding S100 in much the same way as in its absence (Fig. 2B), although their saturated half-bandwidths were different from each other. From these titration curves (Fig. 2A), the maximum K_d value for TFP with S100

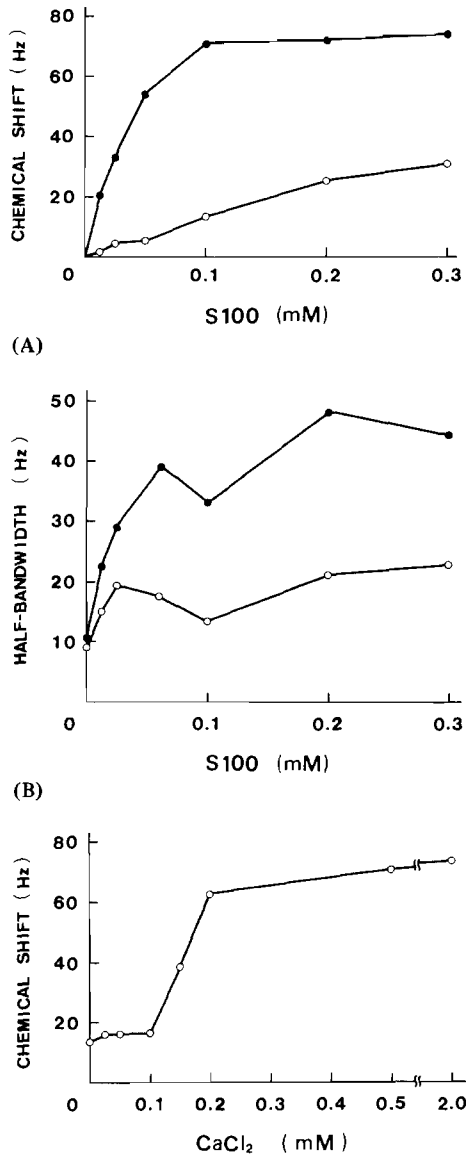

Fig. 2. ^{19}F NMR spectral changes for 0.1 mM TFP caused by adding S100 in: 0.1 mM EGTA (—○—); 2.0 mM $CaCl_2$–0.1 mM EGTA (—●—) solutions. (A) Spectral changes in terms of chemical shift in Hz. (B) Spectral changes in terms of the half-bandwidth in Hz. (C) ^{19}F NMR spectral changes of the 0.1 mM TFP–0.3 mM S100 solution by adding $CaCl_2$ in 0.1 M MES–KOH buffer (pH 7.0). Other experimental conditions were the same as in Fig. 1.

in the presence of 2.0 mM $CaCl_2$ was estimated to be nearly 20 μM, while in the absence of Ca^{2+} it was nearly 85 μM. Figure 2A also indicates that a stoichiometry of TFP to S100 is 1 mol/S100 in the presence of 2.0 mM $CaCl_2$. As can be seen in Fig. 2C, the changes in chemical shift of TFP caused by the addition of S100 increased with increasing concentration of Ca^{2+} in the solution, giving the K_d value for

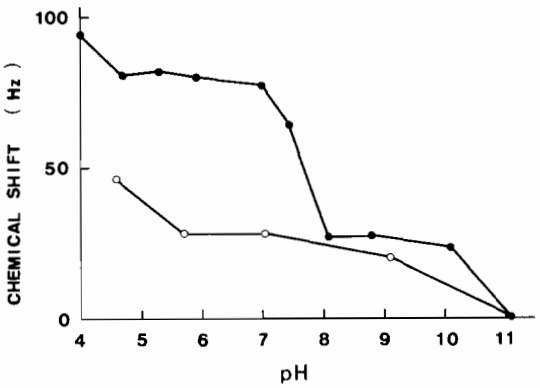

Fig. 3. Effects of pH on ^{19}F NMR spectra of 0.1 mM TFP–0.1 mM S100 in: 0.1 mM EGTA (—○—); 2.0 mM $CaCl_2$–0.1 mM EGTA (—●—) solutions. The pH of the solutions were adjusted by either 0.1 M NaOH or 0.1 M HCl. Other experimental conditions were the same as in Fig. 1.

$CaCl_2$ as nearly 0.15 mM. These results suggest that the conformation of S100 is changed by Ca^{2+} and thus the interaction of TFP with S100 is strengthened.

The effect of the pH of the TFP–S100 solution on the ^{19}F NMR chemical shift was examined in the presence and absence of Ca^{2+} (Fig. 3). In the absence of Ca^{2+}, the effect of pH on the chemical shift of the TFP–S100 solution was relatively small. On the other hand, the chemical shift of TFP–S100 solution in the presence of 2.0 mM $CaCl_2$ showed a pronounced pH dependence: the δ value decreased abruptly at pHs between 7 to 8. Since the chemical shift of TFP alone hardly showed a pH dependence (data not shown), such a change as that observed for the TFP–S100 solutions as a function of pH may be associated with protonation (or deprotonation) of the amino acid residue(s) of proteins which have pK_a value(s) between 7 and 8. This protonation or deprotonation of amino acid residue(s) will be affected by Ca^{2+}. The pH effects of the TFP–S100 solution is in contrast with those observed for the TFP–CaM solution in that changes in the ^{19}F NMR chemical shift for the Ca^{2+}-free TFP–CaM solution are more marked than those for the Ca^{2+}-containing TFP–CaM solution [13]. It has been reported that *N*-(6-aminohexyl)-5-chloro-1-naphthalensulfon-amide (W-7) can bind to CaM and suppresses the activity of CaM [14]. The binding site of CaM to W-7 was thought to be a hydrophobic region of the CaM molecule, which is exposed by binding of Ca^{2+} [12]. It has also been reported that the hydrophobicity of S100 increases and tyrosine residues of S100 are exposed by the binding of Ca^{2+} [15, 16]. Therefore, it is suggested that environments of the TFP-binding site(s) of the Ca^{2+}-bound S100 molecule may be different from those of the Ca^{2+}-free S100 molecule. This environmental difference will be related to a conformational change caused by Ca^{2+}, e.g. exposure of tyrosine residue(s) of S100 [15, 16]. From a fluorescence study [17] it has been reported that Zn^{2+} also bind to S100. ^{19}F NMR spectral changes caused by adding bivalent cations other than Ca^{2+} to the TFP-S100 solutions were studied (Table I). Addition of 1.0 mM $ZnCl_2$ to TFP–S100 solution caused a marked change in chemical shift of ^{19}F NMR (74.22 Hz), and the ^{19}F NMR bandwidth also became broad (54 Hz). The effect of Zn^{2+} on the interaction of TFP with S100 was similar to that of Ca^{2+}. However, the addition of 5.0 mM $MgCl_2$ did not change the chemical shift and bandwidth of ^{19}F NMR signal of the TFP–S100 solution.

The effect of KCl on the ^{19}F NMR spectra of TFP–S100 solutions in the presence and absence of Ca^{2+}, Mg^{2+} and Zn^{2+} were examined (Table I). By adding 0.1 M KCl the changes in the ^{19}F NMR chemical shift of the TFP–S100 solution caused by adding 2 mM $CaCl_2$ (70.31 Hz) decreased to 31.25 Hz and the ^{19}F NMR bandwidth also became more narrow. On the other hand, the chemical shift changes induced by Zn^{2+} decreased a little by the addition of 0.1 M KCl (66.41 Hz). These results suggested that K^+ may bind to the binding site of Ca^{2+} on the S100 molecule, and that Ca^{2+} and Zn^{2+} may affect the interaction of TFP with S100 in a different way.

Recently, it has been reported that a proteinous factor, that is present in bovine brain extracts or is

TABLE I. ^{19}F NMR Spectra of TFP and TFP–S100 Complex

	Metal cations	Chemical shift (Hz)	Half-bandwidth (Hz)
TFP (0.1 mM)	0.1 mM EGTA	0	9.16
TFP (0.1 mM)–S100 (0.1 mM)	0.1 mM EGTA	27.34	13.35
	2.0 mM $CaCl_2$	70.31	33.25
	1.0 mM $ZnCl_2$	74.22	54.00
	5.0 mM $MgCl_2$	27.34	29.00
	0.1 mM EGTA–0.1 M KCl	27.34	12.35
	2.0 mM $CaCl_2$–0.1 M KCl	31.25	12.35
	1.0 mM $ZnCl_2$–0.1 M KCl	66.41	34.79
	5.0 mM $MgCl_2$–0.1 M KCl	31.25	13.94

released from cultures of C6 glioma cells, has several properties which are in common with S100b such as electrophoretic mobility in the presence of SDS, amino acid composition and primary sequence, and stimulation of neurite growth in cultured neuronal cells [18]. Therefore, it will be very interesting to investigate the effect of TFP in such neuronal system in order to reveal the biological function of S100.

Conclusion

(1) TFP interacts with S100 both in the absence and presence of Ca^{2+}, but the interaction is strengthened by Ca^{2+}.

(2) Environments of the TFP-binding site(s) of the Ca^{2+}-bound S100 molecule may well be different from those of the Ca^{2+}-free S100 molecule.

(3) Zn^{2+} also enhances the binding of TFP to S100.

(4) KCl binds to Ca^{2+}-binding site(s) in the S100 molecule, changing the interaction of TFP with S100.

The high utility of ^{19}F NMR for studying the drug–protein interactions should be emphasized.

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