Effect of Trifluoperazine-Binding on Aggregates of S-100 Proteins

Sadayuki MATSUDA
Hokkaido University of Education at Asahikawa, 9, Hokumoncho, Asahikawa-shi, Hokkaido 070
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Synopsis. Aggregates (oligomers) of both S-100b and a mixture of S-100a and S-100a′ (S-100a.a′) were characterized using HPLC of gel filtration. The addition of trifluoperazine to S-100a.a′ resulted in the disappearance of the aggregates irrespective of zinc ion binding. The aggregates of S-100b dissociated into dimeric form of S-100b only in the presence of EDTA.

S-100 proteins are Ca^{2+}-binding proteins found mainly in brain such as calmodulin, and exist as a mixture of three dimer isoforms, S-100a, S-100a′, and S-100b, with the subunit of $\alpha\beta$, $\alpha'\beta$, and $\beta\beta$, respectively.[1—3] S-100 proteins form complexes with Zn^{2+} more tightly than with Ca^{2+}.[4] Taking into account the concentrations of Zn^{2+} and Ca^{2+} in brain, Zn^{2+}-binding of S-100 proteins seems to be more significant rather than Ca^{2+}-binding of S-100 proteins.[4] It is known that each isoform has approximately the same molecular weight (21000). Although the characteristic properties of the S-100a and S-100a′ are very similar,[3] those of the S-100b are different.[3,5] The physiological function of S-100 proteins is still not disclosed. The binding of trifluoperazine (TEP) to calmodulin causes a conformational change of the modulating protein.[6—8] It is also known that S-100 proteins interact with TFP.[9,10] On the other hand, it has been reported that S-100 proteins associate with each other at a concentrations above 2 mg ml^{-1}.[11—13] Further, the investigations by means of small-angle X-ray scattering (SAXS) technique has suggested that the association of S-100 protein is influenced by the addition of TFP.[11,12]

The highest molecular weight component in the association reaction system can be observed by SAXS technique, but the SAXS technique does not give direct information about the association number and the ratio of aggregates per total S-100 protein. The gel filtration of HPLC, on the other hand, can give the information of the degree of aggregations. In the present paper, the effect of TEP on the aggregation of S-100 proteins is studied by means of the HPLC technique, and characteristic behaviors of the aggregation of S-100a.a′ and S-100b proteins with or without Zn^{2+} are discussed.

Experimental

Materials. S-100 proteins were prepared from bovine brain by the method of Isobe et al,[1] and separated into S-100a.a′ and S-100b by phenylsepharose chromatography.[14] The purity of S-100a.a′ and S-100b were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis: No other protein bands were observed. The concentration of S-100a.a′ and S-100b was determined by means of molar extinction coefficient as reported by Isobe et al.[1] The reagents of analytical grade were used without further purification.

HPLC Experiments. HPLC of gel filtration was done at room temperature using a Toso HLC-803D chromatograph equipped with a Waters Protein Pak-125 column (7.8×300 mm). The fractionation range of this column was 2000 to 80000 in molecular weight. The following buffer systems were: (1) 0.2 M $(NH_4)_2SO_4$, 0.1 M KH_2PO_4–K_2HPO_4 (pH=6.5), 1 mM EDTA, and 2 mM β-mercaptoethanol (βME), (2) 0.2 M $(NH_4)_2SO_4$, 0.1 M 3-morpholino-1-propanesulfonic acid (MOPS)–NaOH (pH=6.5), 0.5 mM $ZnSO_4$, and 2 mM βME (1M=1 mol dm^{-3}). The addition of 0.2 M $(NH_4)_2SO_4$ enhanced the reproducibility of the retention time. Flow rate was 0.45 ml min^{-1}. Injected volume and concentration of S-100a.a′ or S-100 were 15 μl and 250 μM, respectively. To test the effect of TFP on the degree of aggregation of S-100a.a′ and S-100b, TFP was added in the molar ratio of TFP/S-100 protein dimer to both running buffer and injected sample solution. Absorbance at 280 nm was monitored.

Results and Discussion

Figure 1 shows the elution curves of S-100a.a′ and S-100b in the presence of 1 mM EDTA. The time dependent shift of base line is probably due to the insufficient time to equilibrate the column with the buffer. Two large peaks (the first and second peaks in Fig. 1) were observed for all the case of S-100a.a′ and S-100b. These correspond to the two types of aggregation. The third

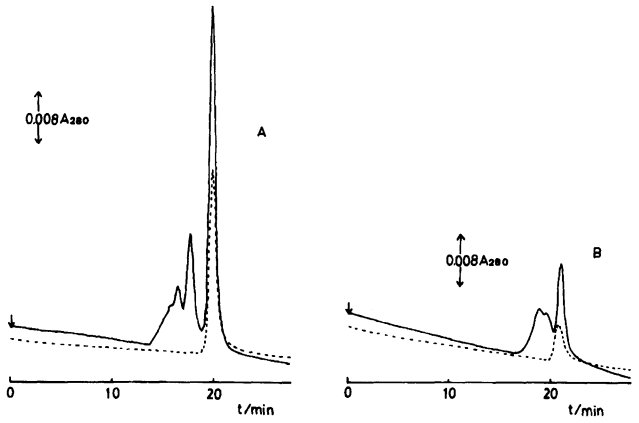

Fig. 1. HPLC of S-100a.a′ (A) and S-100b (B) on Protein Pak-125 column in the presence of 1 mM EDTA. Each sample is indicated at the time indicated by an arrow. Detailed conditions are described in Experimental. The solid line and broken line show the chromatograms in the absence of TFP and in the presence of TFP, respectively.

peak, which is the main peak in Figs. 1A and 1B, is thought to correspond to the dimer from the molecular weight (21000) of S-100 protein. The calibration curve for molecular weight estimation in this condition is shown in Fig. 2. It is difficult to obtain the exact association number of S-100a.a′ or S-100b at present, because the retention time of the dimer of S-100a.a′ or S-100b deviates from the calibration curve. The aforesaid aggregates disappeared by the addition of TFP to both proteins. The dimer peak in the presence of TFP was apparently smaller than that in the absence of TFP (Figs. 1A and 1B). It is due to the adsorption of S-100 proteins on packing material in the presence of TFP.

The elution curves obtained in the presence of 0.5 mM Zn^{2+} are shown in Fig. 3. As described above, the aggregates of S-100a.a′ or S-100b were noticed. The ratio of aggregates to dimer was larger than that in the presence of EDTA (Fig. 1). The addition of TFP to S-100a.a′ caused the dissociation of aggregates, and only the dimer fraction wad detected. Contrary to S-100a.a′, the aggregates of S-100b did not dissociate even in the presence of TFP. Thus, the different behavior between S-100a.a′ and S-100b was clearly demonstrated. Similar results as described above were also obtained in the presence of 0.1 M NaCl, 0.1 M MOPS–NaOH, 1 mM $CaCl_2$, and 2 mM βME, while the data were not shown here.

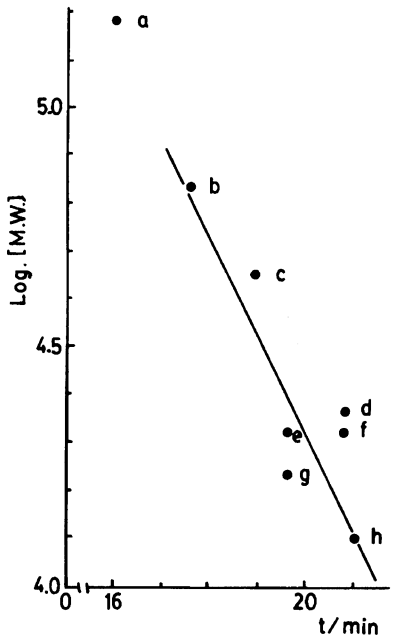

Fig. 2. Calibration curve for the dimers of S-100a.a′ and S-100b obtained by Protein Pak-125 column in the presence of 0.2 M $(NH_4)_2SO_4$ and 1 mM EDTA. The retention time of standard proteins were plotted versus logarithm of their molecular weight: (a), alcohol dehydrogenase; (b), bovine serum albumin; (c), egg albumin; (d), chymotrypsinogen; (e), dimer of S-100a.a′; (f), dimer of S-100b; (g), calmodulin; (h), cytochrome c.

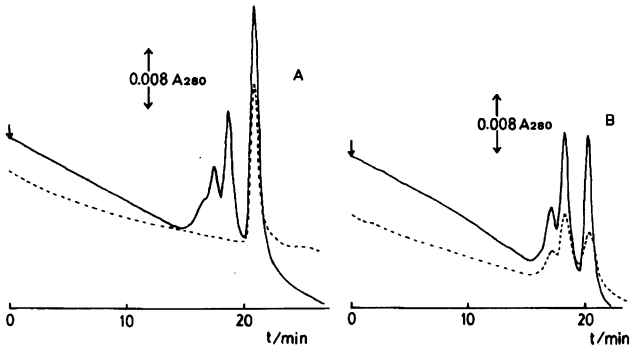

Fig. 3. HPLC of S-100a.a′ (A) and S-100b (B) in the presence of 0.2 M $(NH_4)_2SO_4$ and 0.5 mM Zn^{2+}. The solid line and broken line show the chromatograms in the absence of TFP and in the presence of TFP, respectively.

Although the association number of aggregates were not exactly determined, the occurrence of tetramer and other oligomer (hexamer or octamer) could be reasonably postulated. Thus, the data of SAXS, for example, showed that the radius of gyration for S-100a.a′ changed from 31.8 to 20.0 Å by the addition of TFP.[11,12]

The concentration of S-100a.a′ or S-100b (250 μM) may be much higher than their in vivo concentrations. However, the localization of S-100 protein in membranes has been reported recently by Donato et al.[15—18] At present, it is experimentally difficult to determine the association number of S-100 protein in membranes. S-100 protein may exist as aggregates or dimer in membranes.

Besides chemical equilibrium, hydrophobic interaction must have important role for the formation of aggregates, particularly in the presence of Zn^{2+}. Several authors pointed out that the hydrophobicity of S-100a.a′ and S-100b certainly differ, and they increase due to metal ion binding.[19—21]

The addition of TFP to S-100b in the presence of Zn^{2+} caused no dissociation of aggregates. However, the aggregates of S-100a.a′ dissociated into the dimer protein. These result shows that the effect of TFP on the S-100 protein may be summarized as follows: (1) The aggregates of S-100a.a′ dissociate into the dimer protein irrespective of Zn^{2+}-binding. (2) The aggregates of S-100b dissociate into the dimer protein only in the presence of EDTA.

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