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Sodium dodecyl sulfate–capillary gel electrophoretic analysis of molecular mass microheterogeneity of β-trace protein in cerebrospinal fluid from patients with central nervous system diseases

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Abstract

Molecular mass (M_r) microheterogeneity of β-trace protein (βTP) in cerebrospinal fluid (CSF) from patients with various neurological disorders was analyzed by sodium dodecyl sulfate capillary gel electrophoresis. Under the conditions employed, βTP with a M_r distribution of 23 000–30 000 was roughly separated into two subfractions containing the major peaks with M_r of 26 000 and 28 500, respectively. The peak area ratios of the two subfractions on the electropherograms varied among the samples examined, and elevation in the total βTP level in the CSF from patients with organic diseases in the central nervous system (CNS) was often accompanied by changes in the ratios of the subfractions. The quantitative changes in the subfraction level in CSF βTP are considered to reflect the pathological alterations in the CNS. © 1998 Elsevier Science B.V.

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1. Introduction

Various compounds in cerebrospinal fluid (CSF), including proteins, have been analyzed as aids in the biochemical diagnosis of neurological disorders. Among the CSF proteins, β-trace protein (βTP), which was discovered by Clausen in 1960 [1], is the most abundant in the components originating not from the blood plasma but from the central nervous system (CNS). Its structure, functions and the producing sites have remained unknown for a long time, although it has recently been revealed by some biochemical and molecular biological studies that it is a sialoglycoprotein with a mean molecular mass (M_r) of 27 000 [2] and is identical to lipocalin-type prostaglandin D_2 (PGD) synthase [3,4]. It has also been reported that βTP is synthesized in the leptomeninges and oligodendrocytes [5,6], and secreted into the CSF. As PGD is one of the substances causing physiological sleep, the role of βTP is considered to be involved in the regulation of sleep [7,8]. Earlier workers who employed the immuno-

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logical techniques demonstrated that βTP in CSF increased non-specifically in various organic diseases in the CNS [9–11]. Essentially the same results were also obtained in our previous papers using ordinary capillary zone electrophoresis (CZE) [12,13]. Recent studies with sodium dodecyl sulfate–polycrylamide gel electrophoresis (SDS–PAGE) and two-dimensional electrophoresis suggested that βTB has heterogeneities in charge and M_r [14,15] possibly due to differences in the structure of oligosaccharide side chains. However, we have virtually no knowledge on the changes in subfraction level of CSF βTP in various CNS diseases.

CZE in SDS-containing gels or polymer solutions (SDS–CGE) requires a far smaller amount of a sample solution to be injected and a shorter analysis time than conventional SDS–PAGE [16–19]. Recently, we applied SDS–CGE to the analysis of CSF proteins, and succeeded in analyzing some low-M_r proteins including βTP based on the differences in their M_r values (11 700–42 000) [13,20]. In these papers, CSF βTP with a M_r distribution of 23 000–30 000 was roughly separated into two subfractions on the electropherograms [13,20], and the total levels of βTP were determined as the sum of those two subfractions [20]. In this study, we quantitatively analyzed the βTP subfractions with the different M_r values in CSF from patients with a variety of neurological disorders by SDS–CGE.

2. Experimental

2.1. Subjects

CSF samples were taken by lumbar puncture from 26 male and 29 female patients of 20 to 73 years of age who were divided into 8 groups based on the differences in properties of their diseases: 13 cases with cerebrovascular diseases consisting of 8 with cerebral infarction, 2 with transient ischemic attack (TIA), and 3 with cerebroarteriosclerotic dementia and/or Parkinsonism (group A), 6 with infectious and inflammatory disorders in the brain and/or meninges consisting of 4 with meningitis and meningoencephalitis, and 2 with Guillain-Barré syndrome (group B), 9 with degenerative diseases in the CNS consisting of 4 with Alzheimer's disease and senile dementia of Alzheimer type (SDAT), 3 with Parkinson disease, and 2 with amyotropic lateral sclerosis (ALS) (group C), 5 with multiple sclerosis (MS) (group D), 6 with epilepsy (group E), 5 with psychotic disorders consisting of 3 with schizophrenia and 2 with depressive illness (group F), 5 with neurosis and tension headache (group G), and 6 with peripheral neuropathy having no abnormality detected by CSF routine laboratory tests in the hospital (group H). CSF samples were taken immediately after admittance into the hospital, and routine laboratory tests, such as measurement of the total protein content and cell counting were performed. Informed consent was obtained from all patients in this study.

2.2. Chemicals

All the reagents were of analytical grade. The standard human sample of βTP, i.e., lipocalin-type PGD synthase, was purified from human CSF by chromatography with an immunoaffinity resin conjugated with monoclonal antibody against the enzyme [21]. The flow-through fraction was also recovered as the βTP-free CSF.

2.3. Preparation of samples

The concentrated fractions, which were prepared to contain CSF low-M_r proteins with M_r from 10 000 to 50 000 [13,20], were used for the analysis. The samples were stored at −20°C until experiments could be performed.

2.4. Analytical conditions

The procedure for preparation of samples to be injected and the electrophoretic conditions using Beckman eCAP kit (Beckman, Fullerton, CA, USA) were the same as those for the SDS–CGE analysis in our previous studies [13,20]. The correlation between the migration time (t_m) and M_r was evaluated with co-analyzed marker proteins: β_2-microgobulin (βMG) (11 700), carbonic anhydrase (31 000), α_1-acid glycoprotein (αAGP) (42 000), ovalbumin (45 000) and bovine serum albumin (66 200).

2.5. Identification and determination of βTP and its subfractions

Identification of βTP was performed as in our preliminary research of the SDS–CGE analysis of CSF low-M_r proteins [20].

Determination of the total βTP level in CSF was accomplished on the basis of peak area relative to OG as the internal standard (I.S.), and the absolute concentration (μg/ml) for the sum of the subfractions in individual sample was obtained. All the data were analyzed with a System Gold software (Beckman).

Identification and assignment of the CSF low-M_r proteins other than βTP were also carried out as described previously [20].

3. Results and discussion

3.1. Identification of βTP

The SDS–CGE electropherograms of CSF low-M_r proteins obtained in this study resembled those in our previous studies [13,20]. Fig. 1 shows typical runs of a patient with schizophrenia (Fig. 1A), a standard sample of βTP (Fig. 1B), and the βTP-free CSF (Fig. 1C). A set of three or four overlapping peaks including two major ones (M_r 26 000 and 28 500) was identified as a complex of βTP subfractions. The two peaks indicated as 'd' consisted of peak 'I' with a M_r distribution of 23 000–27 500 and 'II' with the M_r range of 27 500–30 000 (see Fig. 1A and B), which was in agreement with preliminary research on the analysis of CSF low-M_r proteins by SDS–CGE [20]. Some other low-M_r proteins in CSF, such as βMG, γ-trace protein, myelin basic protein, and αAGP (peaks 'a'–'c' and 'e', respectively), were also detected. Changes in their CSF levels detected by SDS–CGE were published in Ref. [20].

3.2. Determination of the total βTP concentration in CSF

The mean (±S.D.) value of the total βTP level in the concentrated CSF low-M_r protein fraction (Section 2.3) from all subjects was 311±127 μg/ml (n=55, range: 125–521 μg/ml). The total βTP concentration in CSF was therefore calculated to be 7.8±3.2 μg/ml (n=55, range: 3.1–13.0 μg/ml) on the assumption that the protein was completely recovered during the preparation procedure (Section 2.3). The total βTP level in the CSF from 39 patients belonging to groups A–E (Section 2.1) was 8.5±3.1 μg/ml (mean±S.D., n=39, range: 3.5–13.0 μg/ml), which was significantly greater ($p<0.05$) than that in the CSF from patients of groups F–H (Section 2.1) (5.2±1.6 μg/ml, n=16, range: 3.1–7.3 μg/ml). These results showed that the total CSF βTP level was elevated in various organic diseases in the CNS, agreeing with those in our previous works with

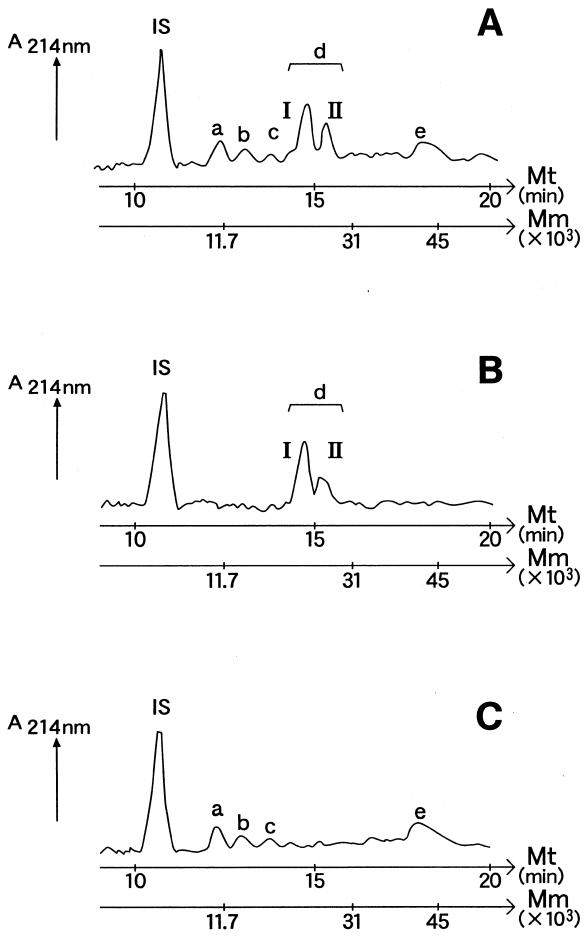

Fig. 1. SDS–CGE electropherograms of CSF low-molecular-mass proteins. (A), a standard sample of βTP (B), and the βTP-free CSF (C). I.S., OG as the front marker (see Section 2.4 of text); a, βMG; b, γ-trace protein; c, myelin basic protein; d, β-trace protein; e; αAGP; t_m=migration time; M_r=molecular mass.

ordinary CZE [12,13] and SDS–CGE [20] and also reports by earlier workers with the immunological techniques [9–11].

3.3. Subfraction patterns of βTP in CSF

As described in 3.1, βTP in CSF was separated roughly into two major subfractions, I (M_r 23 000–27 500) and II (M_r 27 500–30 000), respectively (Fig. 1A).

The mean (±S.D.) values of the ratios of peak area of these βTP subfractions in CSF from all subjects were 59.4±18.7% (n=55, range: 31.6–81.3%) for I and 40.6±15.4% (n=55, range: 18.7–68.4%) for II, respectively. The peak area of I relative to II (I/II) was 1.5±0.9 (mean±S.D., n=55, range: 0.5–4.3). The value of I/II in the CSF from patients of groups A–E and F–H (Section 2.1) were 1.4±0.8 (n=39, range: 0.5–4.3) and 1.8±0.5 (n=16, range: 1.1–3.1), respectively. Changes in the ratios of the subfractions, as well as the total βTP level, in CSF from patients so far examined are summarized in Table 1. These data suggested that, in patients of groups F–H (those without organic damage in the CNS), the value of I/II was maintained within a rather narrow range, but in those with various

Table 1
The total βTP level and ratios of βTP subfractions (I/II)[a] in CSF from patients with various neurological disorders

Disease or disease group	Mean±S.D. of	
	the total βTP level (μg/ml) (Range)	I/II[a] (Range)
Organic diseases in the CNS (n=39)	8.5±3.1 (3.5–13.0)	1.4±0.8 (0.5–4.3)
A. Cerebrovascular diseases (n=13)	9.4±2.8 (6.3–13.0)	2.2±0.9 (1.2–3.9)
Cerebral infarction (n=8)	10.1±2.5 (7.2–13.0)	1.9±0.7 (1.6–3.9)
TIA[a] (n=2)	(8.7–10.5)	(1.9–3.3)
Cerebroarteriosclerotic dementia and/or Parkinsonism (n=3)	(6.3–9.3)	(1.2–2.8)
B. Infectious and inflammatory disorders in brain and/or meninges (n=6)	8.3±2.4 (5.1–11.7)	2.7±0.6 (1.7–4.3)
Meningitis and meningo encephalitis (n=4)	7.7±2.3 (5.1–11.7)	2.3±0.5 (1.7–3.2)
Guillan–Barré syndrome (n=2)	(8.4–10.8)	(2.6–4.3)
C. Degenerative disorders In the CNS (n=9)	7.6±3.2 (3.5–12.1)	0.9±0.2 (0.6–1.4)
Alzheimer's disease and SDAT (n=4)	8.9±2.2 (6.5–11.6)	0.8±0.2 (0.6–1.0)
Parkinson's disease (n=3)	(5.4–9.7)	(0.8–1.2)
ALS[a] (n=2)	(3.5–6.8)	(1.1–1.4)
D. MS[a] (n=5)	9.1±.21 (6.0–12.4)	0.6±0.2 (0.5–0.9)
E. Epilepsy	6.9±2.0 (4.2–9.7)	1.2±0.3 (0.7–1.6)
Other neuropsychiatric disorders giving no organic damage to the CNS tissue (n=16)	5.2±1.6 (3.1–7.3)	1.8±0.5 (1.1–3.1)

[a] See text.

organic diseases in the CNS (groups A–E), elevation of the total CSF βTP level described in Section 3.2 was often accompanied by changes in the ratios of the subfractions.

As shown in Table 1, the value of I/II in the CSF from group B patients was 2.7±0.6 (n=6, range: 1.7–4.3), which was significantly higher (p<0.05) than that of group F–H patients as the control (1.8±0.5, n=13, range: 1.1–3.1). The value in the CSF of group A patients also tended to be raised (2.2±0.9, n=13, range: 1.2–3.9). On the other hand, the value of I/II in the CSF from patients of groups C (0.9±0.2, n=9, range: 0.6–1.4), D (0.6±0.2, n=5, range: 0.5–0.9) and E (1.2±0.3, n=6, range: 0.7–1.6) were significantly lower (p<0.05) than the control (in the cases of groups C and D) or tended to be reduced (in the case of group E). The highest and lowest values of I/II (4.3 and 0.5) were found in the CSF from a Guillain–Barré syndrome patient of group B and an MS case of group D, respectively. Their electropherograms are shown in Fig. 2. These data revealed that elevation in the total βTP level in the CSF from group A and B patients was given mainly by an increase of the subfraction I and that

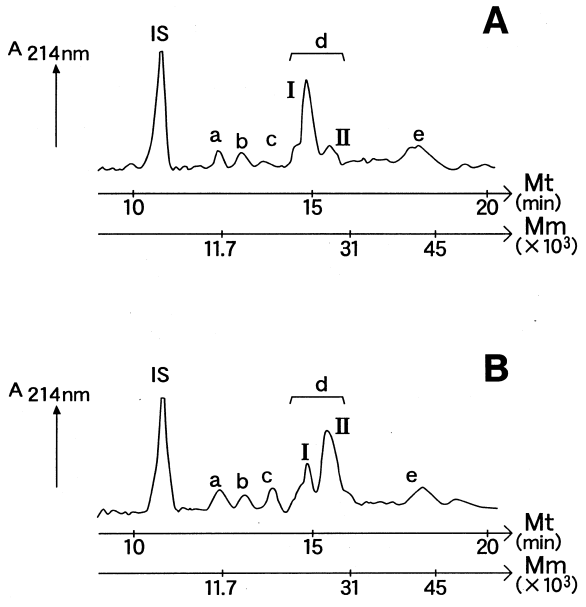

Fig. 2. SDS–CGE electropherograms of CSF from a Guillain–Barré syndrome patient (A) and an MS patient (B) exhibiting the highest ratios of the subfractions I and II, respectively.

increase of βTP in the CSF from patients of groups C–E was caused predominantly by elevation of the subfraction II level, respectively. It was therefore suggested that quantitative changes in the subfraction level in CSF βTP can reflect different pathological alterations in the CNS.

3.4. Correlation between the ratios of βTP subfractions and the total protein content in CSF

Elevation in the CSF total protein content beyond the upper normal limit of 40 mg/dl (up to 185 mg/dl) was found in 12 patients belonging to groups A (4 cases, 50–75 mg/dl), B (4: 60–185 mg/dl), C (2: 50–60 mg/dl), and D (2: 45–50 mg/dl). In the CSF from patients of groups A and B, an increase of βTP given mainly by that of the subfraction I was generally associated with elevation of the total protein content, while in the CSF from group C–E patients, elevation of the βTP level caused predominantly by that of the subfraction II level was generally independent of changes in the total protein content (data not shown). Indeed, the highest value of I/II (4.3) was exhibited by a CSF sample having the highest total protein level of 185 mg/dl (Section 3.3). However, in CSF so far examined, no significant correlation was present between the total protein content and the βTP level.

3.5. Conclusion

As the major part of CSF proteins is derived from the blood plasma [22], it is generally accepted that elevation in the total CSF protein content is mainly due to disturbance in the function of the blood–brain–CSF barrier. However, only a trace amount of βTP is contained in blood plasma [14,15]. So, it is difficult to conclude that, in patients with both elevated total CSF levels of protein and βTP, a part of βTP (subfraction I) in blood plasma appeared in CSF by accelerated penetration through the barrier membrane under the pathological state. It was therefore speculated that accelerated production and/or secretion into the CSF of the βTP subfraction I occurred in the CNS of these patients with cerebrovascular diseases and infectious and inflammatory disorders in brain and/or meninges giving disturbance in the function of the blood–brain–CSF

barrier. The mechanisms for the increase of βTP subfraction II in the CSF of patients with degenerative diseases in the CNS, MS and epilepsy were considered to be different from those giving disturbance in the function of the blood–brain–CSF barrier.

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