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Analysis of low-molecular-mass proteins in cerebrospinal fluid by sodium dodecyl sulfate capillary gel electrophoresis

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Abstract

Proteins of low molecular mass (M_r) in human cerebrospinal fluid (CSF) were analyzed by capillary electrophoresis in a sodium dodecyl sulfate-containing polymer solution. Under the conditions employed, peaks of β_2-microglobulin (βMG) (M_r: 11 700), γ-trace protein (12 300), myelin basic protein (18 000), β-trace protein (βTP) (23 000 to 30 000) and α_1-acid glycoprotein (42 000) were detected on the electropherograms. The concentrations of βMG and βTP were determined based on the peak area relative to that of an internal standard, Orange G, which was added at a constant amount as the front marker. It was demonstrated that their levels in CSF change under various pathological conditions in the central nervous system. © 1997 Elsevier Science B.V.

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1. Introduction

Cerebrospinal fluid (CSF) has been biochemically studied in connection with neuropsychiatric diagnosis. CSF contains several minor proteins that are produced in the central nervous system (CNS) and major proteins that penetrate through the blood–brain–CSF barrier [1]. β-Trace protein (βTP) is the most abundant component among those CSF proteins originating from the CNS. Although βTP was discovered by Clausen in 1960 [2], the structure, functions and the site of production have remained unknown until recently. Recent biochemical and molecular biological studies revealed that βTP is identical to lipocalin-type prostaglandin D (PGD) synthase [3,4] and is a sialoglycoprotein with a mean molecular mass (M_r) of 27 000 [5]. The enzyme is produced in the leptomeninges and oligodendrocytes [6,7], secreted into the CSF as βTP, and is proposed to be involved in the regulation of physiological sleep [8,9]. Several other CSF proteins of low M_r, such as β_2-microglobulin (βMG, M_r: 11 700), γ-trace protein (γTP, M_r: 12 300) and myelin basic protein (MBP, M_r: 18 000), are also produced and

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play important roles in the CNS [10,11]. It is, therefore, considered that the qualitative and quantitative changes in those CSF proteins of low M_r reflect pathological alterations in the function of the CNS.

Capillary electrophoresis (CE) is a rapid and simple microanalytical technique for the separation and determination of a variety of biomedical substances, including proteins in human body fluids. CE in sodium dodecyl sulfate (SDS)-containing gels or polymer solutions (SDS–CGE) requires far less sample solution to be injected and a shorter analysis time than conventional SDS–polyacrylamide gel electrophoresis (SDS–PAGE) [12–15]. In this study, we applied SDS–CGE to the analysis of low M_r proteins in the CSF from patients with various neurological disorders, including psychiatric diseases.

2. Experimental

2.1. Subjects

CSF samples were taken by lumbar puncture from 25 male and 23 female patients with ages ranging from 18 to 74 years of age with the following disorders: cerebral infarction (eight cases), cerebroarteriosclerotic dementia and/or Parkinsonism (three), meningitis and meningoencephalitis (four), Alzheimer's disease (two), Parkinson's disease (three), multiple sclerosis (MS) (four), epilepsy (six), schizophrenia (three), manic-depressive illness (two), neurosis (five) and peripheral neuropathy (eight). Informed consent was obtained from all patients in this study.

2.2. Preparation of samples

A constant volume (2.5 ml) of lumbar CSF was centrifuged at 2000 *g* for 30 min at 4°C in a Centricon-50 miniconcentrator (M_r cut-off=50 000; Amicon, Beverly, MA, USA). The ultrafiltrate (2 ml) was then concentrated to 50 μl using a Centricon-10 (M_r cut-off =10 000; Amicon). The concentrated CSF fractions, which contained proteins with M_rs ranging from 10 000 to 50 000, were used for further analyses. The samples were stored at −20°C until experiments could be performed.

2.3. Chemicals

All the reagents used were of analytical grade. Authentic human samples of α_1-acid glycoprotein (αAGP) and βMG were purchased from Sigma (St. Louis, MO, USA). Human 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 [16]. The flow-through fraction was also recovered as the βTP-free CSF.

2.4. Conditions for SDS–CGE

The concentrated CSF low M_r protein fraction (25 μl) was mixed with 50 μl of 120 m*M* Trishydroxymethylaminomethane (pH 6.6)–1% (w/v) SDS, 5 μl of 0.1% (w/v) Orange G (OG) as the front marker, 2.5 μl of 2-mercaptoethanol and 20 μl of deionized water. The mixture was heated at 95°C for 5 min. After cooling, a constant volume (360 nl) of the mixture was injected by pressure at 3.4 kPa for 60 s into a fused-silica capillary (47 cm×100 μm I.D.) filled with an SDS polymer solution, the gel buffer of an eCAP SDS 14–200 kit (Beckman, Fullerton, CA, USA). The CSF low M_r proteins were separated at 25°C for 30 min at 14.1 kV using a Beckman P/ACE 2000 unit to which the capillary was attached in the reversed polarity mode. The detector was operated at 214 nm. The correlation between the migration time (t_M) and M_r was evaluated using co-analyzed marker proteins: βMG (11 700), carbonic anhydrase (31 000), αAGP (42 000), ovalbumin (45 000), bovine serum albumin (66 200) and phosphorylase b (92 000).

2.5. Identification of CSF low M_r proteins

βTP, βMG and αAGP were identified by spiking the analytes with the authentic human proteins. For further identification of βTP, βTP-free CSF and a standard βTP sample (obtained as described in Section 2.3) were also analyzed. Other low M_r proteins in the CSF were tentatively assigned from

their reported M_r values [10] and from calculations of their migration times.

2.6. Determination of CSF low M_r proteins

Quantification of βTP and βMG was accomplished on the basis of peak area relative to OG as the internal standard (I.S.). For confirmation of linearity between the area of peaks and the concentration in CSF, various amounts of βTP (0, 1.5, 3 and 6 μg) or βMG (0, 0.25, 0.5 and 1 μg) were added to a constant volume (25 μl) of the concentrated low M_r protein fraction (200 μl) from pooled CSF (10 ml) and were analyzed by SDS–CGE (see Section 2.4). Consequently, of the mixtures analyzed, three had higher βTP levels, by 60, 120 and 240 μg/ml, and another three had greater βMG concentrations, by 10, 20 and 40 μg/ml, than the control sample, to which neither of the proteins had been added. The levels of other components were tentatively determined on the basis of peak area relative to the I.S., because authentic samples of the human proteins were not available. All of the data used for determination of proteins were analyzed using System Gold software (Beckman).

3. Results and discussion

3.1. Identification and assignment of CSF low M_r proteins

Fig. 1 shows typical electropherograms of CSF low M_r proteins from a patient with cerebral infarction (Fig. 1A), a standard sample of βTP (Fig. 1B) and of the βTP-free CSF (Fig. 1C). As shown in Fig. 1A, peaks of CSF low M_r proteins were detected within 20 min of the front marker, OG. Among them, a complex of three or four overlapping peaks (M_r: 23 000–30 000) was the must abundant (the group of peaks that is labelled "d" in Fig. 1A). The migration times of these peaks, including the highest one (M_r 26 000), agreed well with those of a standard sample of βTP (Fig. 1B). They were enhanced by spiking them with a standard sample of βTP and were absent on the electropherogram of βTP-free CSF (Fig. 1C). Recent studies with SDS–PAGE and two-dimensional gel electrophoresis revealed that βTP has a molecular heterogeneous M_r and isoelectric point [17,18], due to differences in the oligosaccharide side chains, although its mean M_r was estimated to be 27 000 [5]. Thus, these peaks were identified as a complex of βTP subfractions.

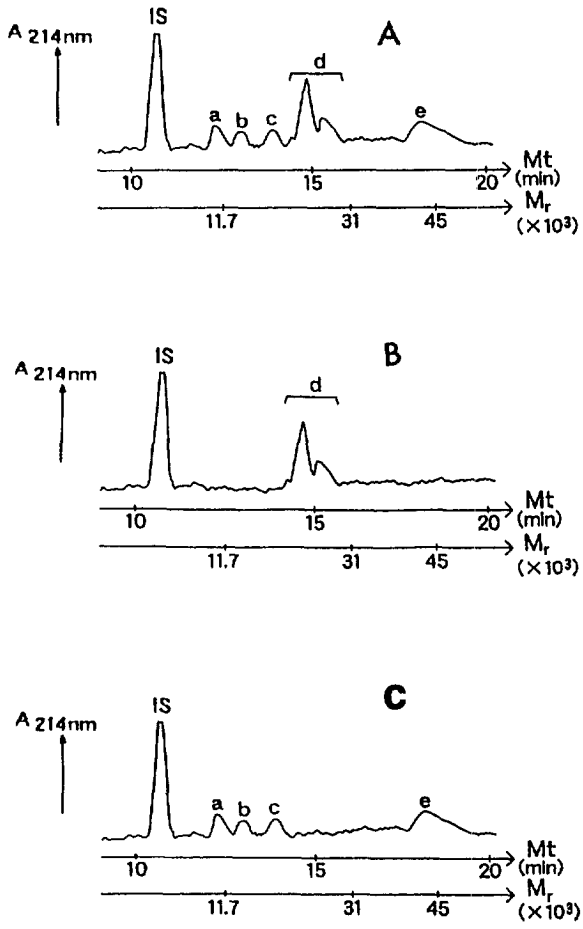

Fig. 1. SDS–CGE electropherograms of CSF low-molecular-mass proteins (A), a standard sample of βTP (B) and βTP-free CSF (C). I.S., OG as the front marker (see Section 2.4); peaks: a=βMG; b=γTP; c=MBP; d=βTP; e=the α_1-globulin peak (see Section 3.1 of text); Mt=migration time (min); M_r=molecular mass.

A small peak with a M_r of 11 500 was observed on electropherograms of all of the samples examined (peak "a" in Fig. 1A and Fig. 1C). It was identified as βMG (M_r: 11 700) by spiking it with authentic sample.

A small broad peak with a M_r of 41 000–48 000 (peak "e" in Fig. 1A and Fig. 1C) was detected in

43 out of the 48 samples examined. The authentic sample of αAGP (M_r: 42 000) had the same mobility as some of the components in this peak. However, this peak seemed to contain some other CSF component(s), presumably α_1-antitrypsin (αAT), which has a M_r of 45 000–55 000. This was, therefore, named the α_1-globulin peak. Two other small peaks with M_r values of 12 500 and 18 000 (peaks "b" and "c") were also detected in 42 and 36 samples, respectively. They were tentatively assigned as γTP (M_r: 12 300) and MBP (M_r: 18 000), respectively, based on their M_r values [10], since authentic samples of the human proteins were not available.

3.2. Determination of low M_r proteins in CSF

3.2.1. Reproducibility in their determination by SDS–CGE

The peak of the I.S. (OG) did not overlap with those of any CSF components when monitored at 214 nm. The concentration of βTP was obtained as the sum of its subfractions. Reproducibility of the data in the determination of βTP and βMG was confirmed by the fact that coefficients of variation were 11.1 and 11.8%, respectively, in the within-run tests using the concentrated low M_r protein fraction from the CSF of a neurosis patient (Table 1).

In the experiments performed for calibration of the system (see Section 2.6), linearity was observed between the peak area and the βTP or βMG concentrations within the range of 0–600 μg/ml for standard βTP and 0–80 μg/ml for authentic βMG, respectively (Fig. 2). The slopes of the dose-dependent curves were identical both in the presence or absence of the concentrated CSF low M_r protein fraction. The detection limits of these proteins in samples injected ranged between 5 and 10 μg/ml.

Table 1
Results of a within-run reproducibility test for determination by SDS–CGE of the βTP and βMG concentrations in CSF

Component	Mean±S.D. (n=6)[a] (μg/ml)	C.V.[b] (%)
βTP	207±23	11.1
βMG	17±2	11.8

[a]The levels in the concentrated CSF low M_r protein fraction from a neurosis patient (see Section 2.2).
[b]Coefficient of variation.

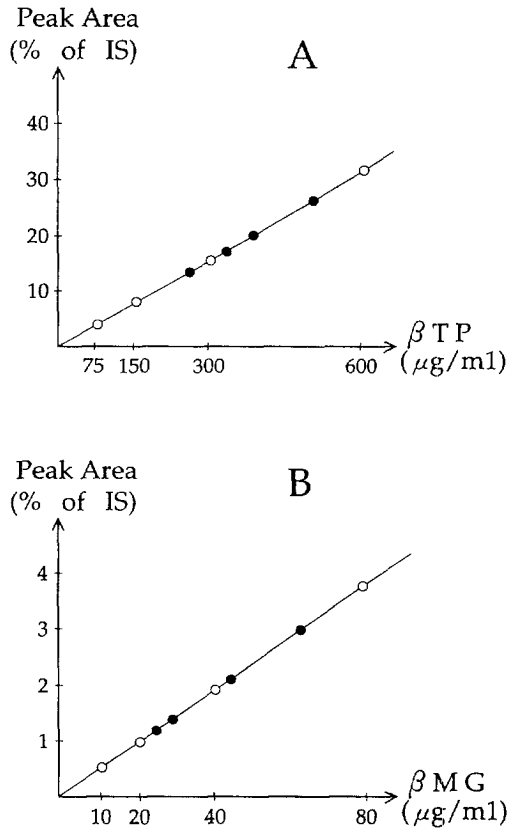

Fig. 2. Correlations between the peak area relative to the I.S. (% values) and the levels of βTP (A) and βMG (B) (μg/ml in the concentrated CSF low M_r protein fraction). Various amounts of authentic βTP or βMG were analyzed by SDS–CGE in the presence (●) or absence (○) of the CSF low M_r proteins. The levels of βTP and βMG in the CSF low M_r protein fraction sample were 273 and 24 μg/ml, respectively (see Section 2.6 of text).

3.2.2. Determination of βTP and βMG in CSF from patients with various neuropsychiatric diseases

The mean (±S.D.) levels of βTP and βMG in the concentrated CSF low M_r protein fraction from all subjects were 236±83 μg/ml (n=48, range: 122–373 μg/ml) and 19±8 μg/ml (n=48, range: 10–36 μg/ml), respectively. Based on the assumption that βTP and βMG in CSF were completely recovered in the concentrated CSF low M_r protein fraction during the preparation procedure (Section 2.2), their levels in CSF were calculated to be 5.9±2.2 μg/ml (n=48) and 0.5±0.2 μg/ml (n=48), respectively. The calcu-

lated CSF levels were slightly lower than those previously reported from the immunological determination of CSF βTP [19–21] and βMG [22], possibly due to adsorption of these proteins to the filters.

The data obtained using this method for various diseases and disease groups are summarized in Table 2. The βTP concentration in the CSF of patients with organic diseases in the CNS (6.7±1.9 μg/ml), such

Table 2
CSF βTP and βMG levels in various neurological disorders

Disease or disease group		CSF levels (μg/ml) of	
		βTP Mean±S.D. (Range)	βMG Mean±S.D. (Range)
I. Organic diseases in the CNS	(*n*=30)	6.7±1.9 (4.5–9.3)	0.5±0.2 (0.3–0.9)
Cerebrovascular diseases	(*n*=11)	7.2±1.1 (4.7–8.6)	0.5±0.2 (0.3–0.7)
Cerebral infarction	(*n*=8)	7.6±0.8 (6.0–8.6)	0.5±0.2 (0.3–0.7)
Cerebroarteriosclerotic dementia and/or Parkinsonism	(*n*=3)	5.9±1.1 (4.7–7.4)	0.5±0.2 (0.4–0.5)
Inflammatory disorders of the brain and/or meninges	(*n*=4)	6.5±1.0 (5.4–7.8)	0.7±0.2 (0.5–0.9)
Degenerative and autoimmune diseases in the CNS	(*n*=9)	6.8±1.8 (5.1–9.3)	0.4±0.1 (0.3–0.6)
Alzheimer's disease	(*n*=2)	(5.1–7.1)	(0.3–0.4)
Parkinson's disease	(*n*=3)	6.1±0.4 (5.6–6.6)	0.4±0.1 (0.3–0.5)
Multiple sclerosis	(*n*=4)	7.5±1.2 (6.2–9.3)	0.5±0.1 (0.3–0.6)
Epilepsy	(*n*=6)	5.6±1.1 (4.5–7.1)	0.5±0.1 (0.3–0.6)
controlled	(*n*=3)	5.4±0.8 (4.5–6.4)	0.4±0.1 (0.3–0.5)
uncontrolled	(*n*=3)	5.9±0.9 (5.2–7.1)	0.5±0.1 (0.3–0.6)
II. Other neuropsychiatric disorders	(*n*=18)	4.6±1.4 (3.1–6.3)	0.4±0.1 (0.3–0.9)
Psychiatric disorders	(*n*=5)	4.9±1.0 (3.8–6.3)	0.4±0.1 (0.3–0.6)
Schizophrenia	(*n*=3)	5.2±0.8 (4.2–6.3)	0.4±0.1 (0.3–0.6)
Manic-depressive illness	(*n*=2)	(3.8–5.9)	(0.3–0.5)
Neurosis	(*n*=5)	4.6±1.1 (3.1–6.3)	0.4±0.1 (0.3–0.6)
Peripheral neuropathy	(*n*=8)	4.6±1.0 (3.5–6.1)	0.5±0.1 (0.3–0.6)
Total	(*n*=48)	5.9±2.2 (3.1–9.3)	0.5±0.2 (0.3–0.9)

as cerebrovascular diseases, inflammatory disorders in the brain and/or meninges, degenerative and autoimmune diseases in the CNS, and epilepsy, was significantly higher ($p<0.05$) than that in other neuropsychiatric disorders (4.6±1.4 μg/ml), such as psychotic diseases, neurosis and peripheral neuropathy. The highest βTP concentration (9.3 μg/ml) was obtained in the CSF from an MS patient. In the CSF from patients with cerebrovascular diseases, the βTP concentration tended to be greater in patients with cerebral infarction than in those with cerebroarteriosclerotic dementia and/or Parkinsonism. Among epileptic patients, those suffering from uncontrolled seizures exhibited a higher CSF βTP level than those whose seizures had been suppressed by the action of anti-epileptic drugs. These data show that the levels of βTP in the CSF increase nonspecifically in a variety of organic diseases of the CNS, especially in those that cause severe physical damage to brain tissue, as reported previously [19–21]. Such a trend was also observed in our previous investigation on CSF proteins, which was conducted with an ordinary capillary zone electrophoresis (CZE) system [23]. Our previous method lacked resolution and sensitivity in the analysis of βTP subfractions and other low M_r proteins, including βMG. However, in this study, we found that the peak patterns of βTP subfractions on the electropherograms differed between the acute and recovery phases in cerebral infarction and between the active and inactive phases in MS (data not shown). These changes in βTP subfractions in CSF seem to reflect pathological alteration in CNS function. An investigation on the clinical significance of changes in βTP subfractions in CSF is now in progress.

The βMG level in CSF did not differ significantly for organic diseases in the CNS (0.5±0.2 μg/ml) and other neuro-psychiatric diseases (0.4±0.1 μg/ml). The highest βMG level in CSF was observed in inflammatory disorders in the brain and/or meninges (0.7±0.2 μg/ml). A remarkable elevation of the CSF βMG level was reported in patients with brain tumor [22]. βMG is a major component of the β-globulin fraction of plasma proteins. βMG in CSF has, therefore, been considered to originate from the blood plasma. In this study, three out of four patients with inflammatory disorders in the CNS had a CSF total protein content that was higher than the normal upper limit (40 mg/dl), indicating a disturbance in the function of the blood–brain–CSF barrier. However, the CSF βMG level was not correlated with the CSF total protein content. In the CSF of a meningoencephalitis patient, the βMG level was the highest (0.9 μg/ml), whereas the total protein content was only slightly raised (50 mg/dl) in this patient. In a patient with meningitis, the CSF total protein content was the highest (190 mg/dl), in spite of the fact that the CSF βMG level was moderate (0.45 μg/ml). Therefore, the increased levels of βMG in the CSF of those patients was considered to be derived from the CNS and not from the blood plasma. This conclusion is in agreement with the recent report that βMG, as well as several other proteins including βTP, is produced in the CNS [11].

3.2.3. Other low M_r proteins

γTP was detected in 42 CSF samples out of the 48 examined (the % values of peak area relative to I.S. ranged between 0 and 1.3). CSF from five out of six patients with cerebrovascular diseases did not contain a detectable amount of γTP, suggesting that reduction in the CSF content of γTP might reflect some pathological changes in the CNS associated with these diseases, although the clinical significance of the CSF γTP level has not been established.

MBP was detected in 36 CSF samples. The MBP content was high (the area value above 0.8% of the I.S. value) in the CSF from two patients with cerebral infarction and in two with MS; the highest value (1.1%) was found in an MS patient. This finding is consistent with an earlier report that MBP in CSF increased only when acute and severe demyelination occurred [24]. In this study, those patients with high CSF MBP levels also showed elevated CSF βTP levels (7.4–9.3 μg/ml). Among them, one MS patient expressed the highest MBP and βTP levels. Elevation of both MBP and βTP levels in the CSF seemed to occur in diseases that caused severe organic damage to brain tissue, such as MS and cerebral infarction.

The α_1-globulin peak was detected in 43 of the 48 CSF samples examined. The α_1-globulin level was high (>2% of the I.S. value) in three patients with meningitis. These patients also showed an increase in the CSF total protein content that was beyond the normal upper limit (40 mg/dl), and one meningitis

patient showed the highest CSF levels of both α_1-globulin (2.5% of the I.S.) and total protein content (190 mg/dl). As this fraction contained αAGP and αAT, major components of the α_1-globulin fraction of plasma proteins, an increase in this fraction in the CSF may be useful as a sign of functional disturbance of the blood–brain–CSF barrier.

3.3. Conclusion

In this study, we showed that SDS–CGE, which requires far less sample and affords a faster analysis time than SDS–PAGE, is a useful tool for the simultaneous analysis of CSF low M_r proteins. We demonstrated by this method that the CSF levels of low M_r proteins, especially βTP and βMG, were increased in various diseases and disease groups. Such changes seem to reflect some pathological conditions in the CNS, although data from genuine controls were not available, due to the difficulty involved in obtaining consent from healthy subjects to sample their CSF.

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