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A Study of the Silicon and Aluminium Interaction with Cerebrospinal Fluid Proteins*

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A precipitation experiment was performed with human cerebrospinal fluid (CSF) by adding to it aluminium and/or silicate ions. The aim was to elucidate their possible role in extracellular protein aggregation. Three quite different pathological conditions were simulated, such as predominance of Al (as in renal failure), predominance of Si (as in silicosis), and coexistence of Al and Si (as in Alzheimer's disease). In all cases, precipitates formed immediately, decreasing IgG (up to 34%) and lowering the albumin content in CSF in the order silicate >> aluminium > aluminosilicate. Corresponding quantities of these proteins were not found in the water soluble proteinaceous part of precipitates, suggesting that they are firmly embedded in inorganic matrices identified by FTIR.

INTRODUCTION

Controversy surrounds the involvement of both β-amyloid protein and aluminium in the etiology of Alzheimer's disease (AD).[1] AD is characterized neuropathologically by the development of a significant number of senile plaques (SP), neurofibrillary tangles (NFT) and loss of neurons in selectively vulnerable brain regions such as the cortex, hippocampus and amygdala.[2,3] The major constituent of the senile plaques core is polypeptide β-A4 (~ 4 kD) which forms stable amyloid fibrils.[4] Overexpression of the precursor β-A4

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protein (APP) does not lead to amyloid formation[5] and the presence of soluble monomeric and oligomeric forms of β-A4 in cerebrospinal fluids[6] and in AD meningeal blood vessel walls[7] indicates that β-A4 produced in the brain may be present in these forms without formation of fibrils. Production of senile plaques thus appears to be dependent not only on the presence of β-A4, but also on some factors that can influence the formation of fibrils from the existing β-A4, such as the recently reported apolipoprotein (Apo E4)[8] or amorphous aluminosilicates.[9] Also, it takes about 30 years to generate mature plaques[10] when some or many cortical plaques become associated with numerous other »inflammatory« and extracellular matrix macromolecules.[10,11] Further, there is now general agreement that Al is neurotoxic at the cellular level,[1] but its accumulation in the brain tissue in various pathological conditions, such as AD, or renal failure has produced different neuropathological features. Raised Al content in the form of aluminosilicates has been reported to be a specific feature of the mature senile plaque core in AD, Down's syndrome and some elderly subjects.[12,13] However, in brains of renal dialysis patients, mainly immature plaques and numerous focal accumulation of Al have been found,[14] without any evidence of Si accumulation, as discussed in ref.[15] The question arises about the significance of silicon co-existence with Al for the development of mature plaques.

Conditions of the formation of aluminosilicates in senile brain, containing both octahedral and tetrahedral aluminium, have remained an unsolved problem.[12] It was concluded from experiments in model saline that this type of aluminosilicates required a »silica rich« environment[16] for its formation at pH = 7.4. Although Al and Si normally coexist in serum, ordinarily in small molar excess of Si over Al,[17,18] some reports have demonstrated a significantly higher Si/Al ratio even in the control human brains[19,20] but not in rat brain.[21] Among 19 trace elements in cerebrospinal fluids (CSF) of autopsy-confirmed AD patients, considerably elevated Si concentrations (up to 20 fold) and »age-dependent« Si accumulation in control CSF samples have been reported.[22]

The present study is a continuation of our work[23] in which we have characterized Al and/or Si precipitates from human serum. This time we used human CSF, in spite of being aware that CSF is not identical with the extracellular fluid of the brain which is still of unknown composition. However, CSF could serve as a suitable model of extracellular fluid since it is an available system which contains many known components of »mature« SP deposits, *i.e.* β-A4, α_1-antichymotrypsin, complement factors, antibodies[10,11] or Apo E as a possible »enhancing factor«.[8] As the real chemical composition in such »focal« pathology as in AD or Al-induced encephalopathy is unknown, we have simulated three quite different pathological conditions such as the predominance of Al (as in renal failure), predominance of Si (as in silicosis) and co-existence of Al and Si (as in AD). In our experiments, performed in CSF

at 37 °C, we have used the minimal Al and Si concentrations that produced precipitation effects (within 48 hours) without the decomposition of organic compounds caused by ageing. These Al and Si concentrations are potentially unrealistic with respect to physiological conditions, but can approximate conditions at potential »focal« target sites, such as in senile plaques[12] and in neurofibrillary tangle-bearing hippocampal neurons.[24]

MATERIALS AND METHODS

Two available pools of cerebrospinal fluids we used as model solution. Pool 1 and pool 2 differed in their total protein content (see Table II).

Aluminium chloride, $AlCl_3 \cdot 6H_2O$ (p.a., Merck, Darmstadt, FRG) was dissolved in double-distilled water and the Al(III) content of this solution was determined as described by Öhman and Sjöberg.[25] Silicic acid solution was prepared by dissolving $Na_2SiO_3 \cdot 9H_2O$ (p.a., Baker Chemicals B.V., Deventer, The Netherlands)., in double-distilled water. The final pH was adjusted to 7.4 using either hydrochloric acid or sodium hydroxide (Merck, p. a.).

Chemicals used for the molecular mass analysis of proteins were acrylamide, bisacrylamide, sodium dodecyl sulfate (SDS), supplied by LKB AB (Broma, Sweden) 2-mercaptoethanol, *N,N,N',N'*-tetramethyl-ethylenediamine, ammonium persulfate, and silver nitrate were supplied by Sigma (St. Louis, M.O.) and Gel-Bond polyacrylamide gel (PAG) film and calibration kits for the molecular mass determination were supplied by Pharmacia Fine Chemicals AB (Uppsala, Sweden).

Five samples were obtained by precipitation from pooled human cerebrospinal fluid by adding aluminium, silicate and aluminium with silicate ions and letting the solutions age for 24 hrs at 37.0 + 0.1 °C. The total Al and Si concentrations used in these *in vitro* studies are presented in Table I.

TABLE I.

Conditions for precipitate formation from the system $AlCl_3$-Na_2SiO_3-HCl/NaOH-cerebrospinal fluid (37 °C, 24 h).

Sample number	CSF	Concentration of precipitation components in mmol/L			
		$AlCl_3$	Na_2SiO_3	NaOH	HCl
1	Pool 1	4.67	0	1.87	0
2	Pool 1	0	7.30	0	19.71
3	Pool 1	3.90	7.80	0	10.29
4	Pool 2	9	7.83	0	21.70
5	Pool 2	3.96	7.92	0	8.87

TABLE II.

Determination of IgG and albumin in the CSF pools and in the obtained precipitates, *after adding Si, Al or Al+Si.

	Albumin g/L	Decreasing of albumin concentration in CSF pools. (%)	IgG g/L	Decreasing of IgG concentration in CSF pools. (%)
Pool 1. (native)	0.227		0.059	
Supernatants:				
–pool 1 + Si	0.194	14.5	0.039	33.9
–pool 1 + Al	0.205	9.7	0.052	11.8
–pool 1 + Si + Al	0.207	8.8	0.055	5.6
Precipitates from:				
–pool 1 + Si	Ø		0.006	
–pool 1 + Al	Ø		Ø	
–pool 1 + Si + Al	0.026		Ø	
Pool 2. (native)	0.141		0.026	
Supernatants:				
–pool 2 + Si	0.120	14.9	0.021	19.5
–pool 2 + Si + Al	0.133	5.6	0.025	3.8
Precipitates from:				
–pool 2 + Si	Ø		Ø	
–pool 2 + Si + Al	Ø		Ø	

*Prepared as 1% aquatic homogenate (see Ch. Materials and Methods); Ø Below detection

The samples were centrifuged in a Sorvall (Norwalk, Connecticut) centrifuge at 7970 x g and dried in a desicator over silica gel, prior to the protein and infrared spectral analysis.

For protein analysis, the formed precipitates of the five samples from Tabe I were homogenized with buffered saline as 10 g/L homogenates.

Total protein concentrations in CSF pools (pool 1, 0.48 g/L; pool 2, 0.22 g/L) were determined in accordance with the colorimetric method described by Rieder.[26]

Concentrations of albumin and IgG were determined using LC-immunodiffusion plates (Behringwerke AG, Marburg, FRG) in CSF pools, supernatants and the precipitates formed (homogenated in saline).

All samples for SDS-PAGE analysis were diluted a) with 10 g/L SDS and incubated for 60 min at 37 °C (mild denaturating conditions), or b) with 25 g/L

of SDS and 50 ml/L of β-mercaptoethanol and incubated for 10 min in boiling water (strong denaturating conditions). After extensive washing of precipitates with SDS/β-mercaptoethanol, further solubilization of proteins that might have remained bound to inorganic matrix after treatment b) was performed with a solution containing 35% acetonitrile and 0.1% trifluoroacetic acid. This solution was proposed by Yankner *et al.*,[27] for solubilization of β-amyloid fragments. Molecular-mass analysis of proteins was performed in horizontal SDS polyacrylamide gel electrophoresis (SDS-PAGE) in an ultra-thin layer (0.5 mm) of home-made pore-gradient polyacrylamide gel T = 4–20%, C = 4%) with highly sensitive silver-nitrate staining as described by Trbojević-Čepe .[28]

An LKB 2117 Multiphor System with constant power supply was used for SDS-PAGE. A home-made gradient mixer was used to prepare the pore-gradient gels. Molecular masses were determined by using proteins from calibration kits.

Infrared spectra of untreated precipitates, of those treated with SDS at 37 °C, and of selected silicate precipitates treated with SDS at 100 °C and then extensively washed were recorded on a Perkin – Elmer (Norwalk, CT) Fourier – transform infrared spectrophotometer Model 1720, with KBr discs.

RESULTS

1. Characterization of proteins and protein fragments bound to the studied inorganic matrices

Table II presents quantitative analyses of albumin and IgG in pooled CSF supernatants obtained after removal of precipitates and in precipitates themselves (homogenates in saline) formed by the conditions from Table I. Results show a 3.8 – 34% loss of proteins in CSF supenatants relative to native CSF samples. The highest decrease of IgG and loss of albumin were observed in supernatants of silicate precipitates, a somewhat smaller decrease in supernatants of aluminium precipitate and the lowest decrease in the supernatant of alumosilicate precipitate. Concentrations of albumin and IgG in homogenates of the formed precipitates were around or below the sensitivity limit of the applied method (0.008 g/L for IgG, 0.05 g/L for albumin). it should be emphasized that quantitative results in Table II apply to water soluble proteins only. Insoluble proteins imbedded into inorganic matrices could be determined by the applied procedure.

For further characterization of proteins in the formed precipitates, a more sensitive SDS-PAGE/silver-staining method was used.

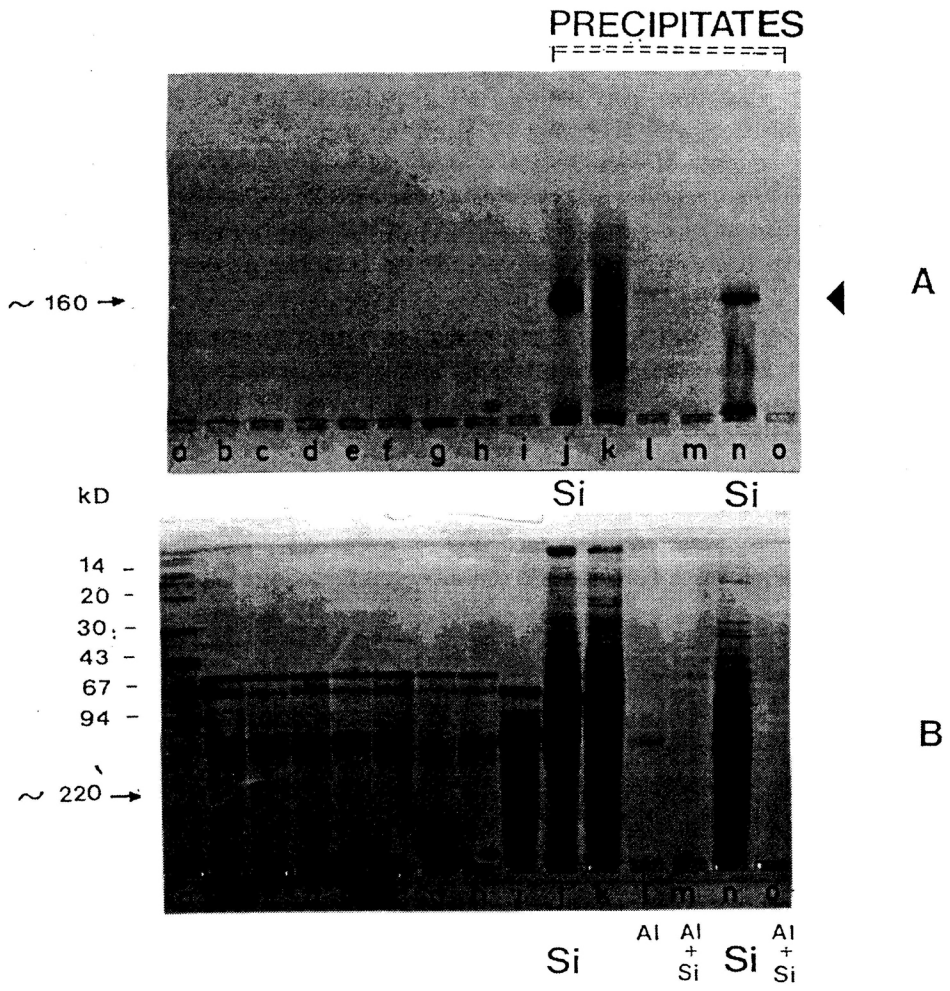

Figure 1. SDS-PAG electrophoresis of proteins under mild denaturation conditions at 37 °C of CSF pools 1 and 2, CSF supernatants and the formed precipitates, containing silicate, aluminium or aluminosilicate (see Table II) followed by a single colouring with silver (A) and after recycled staining procedure (B).
a, i – standards of mol. mass; b, f – pools 1 and 2; c – supernatant (pool 1 +Si); d – supernatant (pool 1+Al); e – supernatant (pool 1 +Si +Al); g – supernatant (pool 2 +Si); h – supernatant (CSF pool 2 +Si +Al); j – precipitate (pool 1 +Si); l – precipitate (pool 1 +Al); m – precipitate (pool 1 + Si +Al); n – precipitate (pool 2 +Si); o – precipitate (pool 2 + Si +Al). The samples were treated with 1% SDS at 37 °C. The sample k – precipitate (pool 1 +Si) was treated with 2.5% SDS and 5% β-mercaptoethanol at 100 °C. Isolated precipitates were prepared as 1% homogenates.

Figure 1 shows protein profiles of native CSF (pools 1 and 2), CSF supernatants and of the obtained precipitates using the SDS-PAGE method under mild denaturation conditions at 37 °C. The results obtained after single colouring with silver (A) and after recycled colouring with silver (B) are presented. Applying procedure (A), one can observe in lines j and n an intensively coloured broad protein band in the region of γ-globulins (150-170 kD) in both silicate containing precipitates (pool 1 +Si and pool 2 +Si, Table II). By increasing the sensitivity of the method with recycled colouring (B), it is only in silicate precipitates, the numerous other lower molecular mass protein bands can be also observed. Further, in silicate precipitates obtained from CSF with a higher protein concentration (pool 1+ Si, Table II), a protein of approx. 200–220 kD molecular mass was also observed (line j, Figure 1B). A protein of similar molecular mass was also demonstrated in our experiments with human serum.[23]

Protein profiles of precipitates containing aluminium (line 1) and aluminosilicates (lines m, o) show only traces of SDS soluble proteins. However, at the start, a remaining protein can be observed in all precipitates, suggesting that it is still firmly embedded in inorganic matrices.

In protein profiles of supernatants after removal of silicate and loss of aluminosilicate precipitates (lines c, e, g, h), one can observe a significantly decreased band of IgG (arrow). These results support the quantitative results presented in Table II.

Figure 2. shows the protein profiles of native CSF (pools 1 and 2), CSF supernatants after removal of precipitates and of precipitates themselves, using the SDS-PAGE method at 100 °C (strong denaturation conditions) and recycled colouring with silver. The results show that, through the application of higher SDS concentrations and β-mercaptoethanol (strong denaturation conditions) , the intensively coloured band in silicate precipitates, studied at 37 °C (Figure 1A, lines j and n), disappeared at 100 °C. These results also suggest that the intensively coloured broad band in silicate precipitates belongs to immunoglobulins (compare lines j and k, Figure 1A). However, the appearance of the 110–120 kD protein band in the silicate precipitate of CSF pool 1, treated at 100 °C, is probably related to the disappearance of the 220 kD band found in the same samples treated at 37 °C (compare lines j and k, Figure 1B). It is interesting to note that the soluble BAPP of similar molecular mass (100 kD and 218 kD) was detected in cell culture supernatants.[29] The trace amount of proteins that remained at the start of silicate precipitates (lines i, l, Figure 2.) can be completely washed out with SDS/β-mercaptoethanol solution. Further solubilization of precipitates with a solution containing 35% acetonitrile and 0.1% trifluoroacetic acid and applying the SDS-PAGE method did not reveal any additional protein bands, and, therefore, this SDS-PAGE figure was not presented.

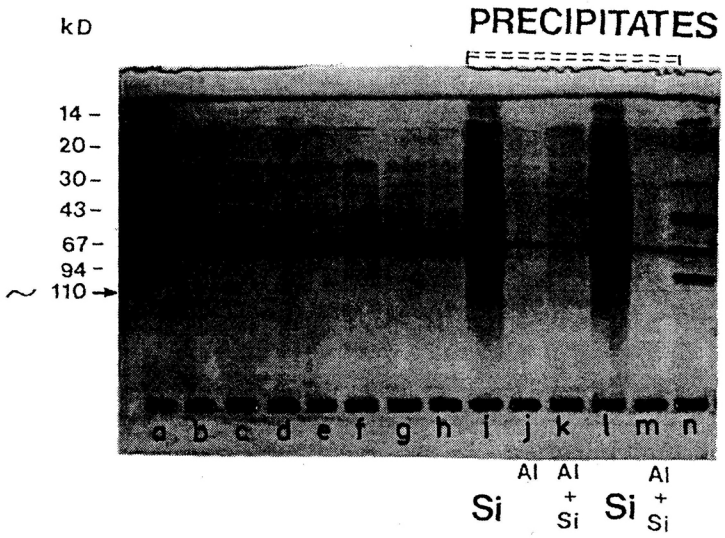

Figure 2. SDS-PAG electrophoresis of proteins under completely reducing conditions at 100 °C of CSF pools 1 and 2, CSF supernatants and the formed precipitates containing silicate, aluminium or aluminosilicate followed by recycled colouring with silver. a, n – standards of mol. masses b, f – pools 1 and 2; c – supernatant (pool 1 +Si); d – supernatant (pool 1 +Al); e – supernatant (pool 1 +Si +Al); g – supernatant (pool 2 +Si); h – supernatant (pool 2 +Si +Al); i – precipitate (pool 1 +Si); j – precipitate (pool 1 +Al); k – precipitate (pool 1 +Si +Al) l – precipitate (pool 2 +Si); m – precipitate (pool 2 +Si +Al). All samples were treated with 2.5% SDS and 5% β-mercaptoethanol at 100 °C. Isolated precipitates were prepared as 1% homogenates.

2. FTIR Spectral Characteristics in the 4000–400 cm^{-1} Region

Figures 3 A, B. C present spectra of untreated precipitates from samples 1, 2 and 3 (Table I), respectively. The spectra are very different and will be qualitatively described using the conventions of Bellamy.[30] The presence of protein is observable only in spectrum A of aluminium precipitate (sample 1, Table I). In the other two spectra, B and C, the inorganic matrix predominates. All three precipitates show a broad and strong band with the minimum at 3468 cm^{-1} of decreased OH^- stretching frequency, indicating hydrogen bonding.

Two weak side bands at 2948 and 2859 cm^{-1}, due to C-H stretching vibrations which provide a good indication of the protein presence, are visible only in spectrum A. The amide I and II bands at 1551 and 1541 cm^{-1} in spectrum A can be attributed to the CO and NH deformation modes. The band at 1422 cm^{-1} of the COO^{-1} vibration is rather pronounced, which indicates that the protein is negatively charged. There is no peak at 1743 cm^{-1} found

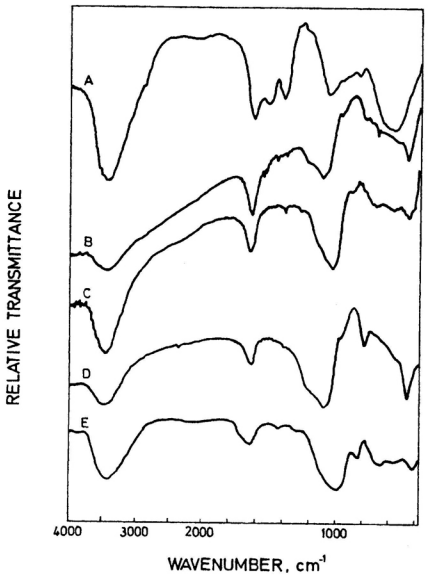

Figure 3. Fourier – transform infrared spectra of untreated precipitates. A. Precipitate formed in sample 1, Table I, from $AlCl_3$, and human cerebrospinal fluid. B. Precipitate formed in sample 4, Table I, from Na_2SiO_3, and human cerebrospinal fluid. C. Precipitate in sample 3, Table I, from $AlCl_3$, Na_2SiO_3 and human cerebrospinal fluid. D. Reference spectrum of H_4SiO_4 formed from Na_2SiO_3 and HCl (1:2). E. Reference spectrum of $NaAl(OH)_4 \cdot H_4SiO_4$ formed from $AlCl_3$, $H_2C_2O_4$ and Na_2SiO_3 (10:50:2) at pH = 7.78 (see Bilinski *et al.*[31]).

in the aluminosilicate precipitated from human serum, which was assigned to CO of the un-ionized carboxyl group.[23] Amide III bands at 1318 and 1262 cm^{-1} are present only as shoulders in spectrum A, at 1385 cm^{-1} as a weak band in spectra B and C and as shoulders at 1228 cm^{-1} in spectrum B, and at 1270 cm^{-1} in spectrum C. The strong and broad band at 1089 cm^{-1} in spectrum A, at 1119 cm^{-1} in spectrum B and at 1029 cm^{-1} in spectrum C is in the range characteristic of Al-OH and Si-OH bending.

The strong and broad peak at 591 cm^{-1} in spectrum A is an overlap of possible Al-OH stretching and of the broad band in the same region typical of proteins, assigned to the amide V band. Spectrum A does not resemble the aluminium hydroxide matrix. Spectrum B is rather similar to the one of H_4SiO_4 with regard to the main asymmetric stretch frequency near 1100 cm^{-1} and the sharp peak at 471 cm^{-1}. Spectrum D of H_4SiO_4 is added to support this discussion of spectrum B. Spectrum C is rather similar to the one reported for $NaAl(OH)_4 \cdot H_4SiO_4$ (see Figure 1, spectrum g, Bilinski *et al.*[31]) Spectrum E, added in Figure 3, corresponding to $NaAl(OH)_4 \cdot H_4SiO_4$ is also added to make the discussion easier.

Precipitates from samples 1–3 (Table II) were treated with SDS at 37 °C, then they were separated and dried prior to FTIR measurement. All three precipitates displayed a very similar and pronounced spectrum of an insoluble protein or protein fragment, containing pronounced amide I, II, III bands and the band of COO^- vibration, as presented in Figure 4 A, B, C. In the treatment at 100 °C, the selected silicate precipitate (Sample 2, Table I) displays a modified spectrum in which amide II band is decreased.

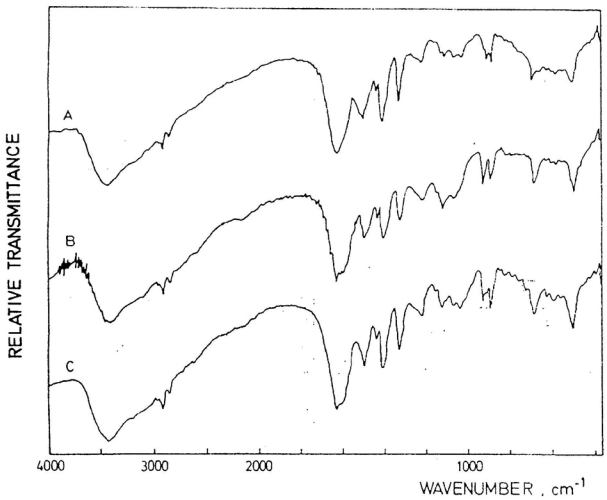

Figure 4. Fourier – transform infrared spectra of the transformed precipitates A, B, C described in Figure 3 after treatment with SDS for 60 min. at 37 °C.

By repeated washing with SDS and then with a solution containing 35% acetonitrile and 0.1% CF_3COOH, all protein fragments were removed from the precipitate, finally characterized by FTIR as silicic acid.

DISCUSSION

The present work with cerebrospinal fluids is a continuation of our work on aluminosilicate precipitation under human serum conditions.[23] Although the chemical composition of CSF is mainly influenced by serum constituents, some differences exist as a result of the blood-brain barrier function, intrathecal metabolism and nervous tissue cells secretion. Our results have demonstrated some quite different effects of Al and/or Si in CSF from those

in the blood serum. Aluminium chloride, when added to human serum, gave no precipitate for weeks. When added to CSF, precipitation occurred within 24 horus, although the total protein content was significantly lower than in the serum sample. It is an indication that CSF does not contain enough ligands that could form soluble complexes with aluminium ions. In serum, one of such ligands is presumably transferrine.[32] Its concentration in CSF samples is about 1% of serum values, but there are also differences in the molecule charges of a number of sialic acid residues.[33]

Si and Al+Si formed precipitates quickly from both the blood serum and CSF, but characterization of proteinaceous residues by electrophoretic methods (SDS-PAGE) showed great differences. Proteinaceous residues of all serum precipitates showed a great similarity to the native human serum protein pattern. Opposite to the blood serum study, Si precipitates obtained from CSF samples showed quite unusual »cascade-like« closely lined-up bands (from about 3 kD to 100 kD), which only in a small proportion belong to immunoglobulins. Numerous lower mol. mass protein bands could be demonstrated which were neither present in serum precipitates, nor are visible in the normal CSF protein profile. It seems that in CSF samples silica can act as a »catcher« for some minor small protein molecules, thus influencing their aggregation state. There was also some similarity. In the Si precipitate from serum, an additional pronounced band was obtained corresponding to mol. mass of about 220 kD. The same band was also demonstrated in the presence of Si in one of the CSF samples which contained a higher protein content (pool 1).

Under the blood serum conditions,[23] an amorphous aluminosilicate formed also contained human serum proteins, cations Na^+, K^+, Ca^{2+}, Mg^{2+}, and anions Cl^-, and $(PO_4)^{3-}$. From the ^{27}Al MAS NMR spectrum, it was observed that 4-coordinated aluminium predominated and that the 6- coordinated aluminium was present in a smaller proportion. Under the CSF conditions, an amorphous aluminosilicate was formed which was characterized only by FTIR spectra since the available amount of precipitate was not sufficient for NMR study. Spectrum C in Figure 3 is similar to those reported for hydrous feldspathoids in different environments,[34,16,31,23] for which group it is characteristic that they have a Si:Al ratio ≥ 1 and a proportion of tetrahedral Al in its structure.

For their information, these compounds require silicic acid concentrations in excess of aluminium ion, Al^{3+} *i.e.* about 150 μM.[16] Such conditions could be obtained also in the presence of any strong complexing ligand even if the total aluminium concentration is higher than that of total silicic acid. This can be concluded from our earlier results when oxalate ligand was used.[31] Formation constants derived for aluminosilicates precipitate from oxalate solutions predict formation of hydrous feldspathoids above pH 6.1 as the most stable phase, which seems to agree with the finding in senile

plaque.[9] Numerous complexing ligands present in the serum and in CSF can diminish the concentration of Al^{3+} ion and make the conditions of silicic acid being in excess over Al^{3+}. In more simplified *in vitro* experiments, such as the saline system,[16] none of organic complexing ligands was available.

The critical concentration for Si polymerization *in vitro* is above 100 μM[35] when Si changes its biological properties, *i.e.* its subsequent interactions with macromolecules were described. It was reported that a small amount of polysilicic acid tends to interact selectively with large amounts of globulins, which may have a very important and irreversible biological effect.[36] In the present study with CSF, we also confirmed this prominent effect. Allafuzzof *et al.*[37] investigated albumin and IgG contents in blood and CSF from patients with multi-infarct dementia (MID) and senile dementia of Alzheimer's type (SDAT/AD). Of interest is the low IgG ratio in the SDAT/AD group, which fell into the so-called biologically irrelevant area. This may indicate some sort of consumption of IgG by the nervous tissue, manifested by the finding of immunoglobulins in senile plaques[38] and vessel walls.[39] The aorta and vascular bed were also organs with the highest concentrations of Si in rats,[40] while Frackowiak *et al.*[7] showed that smooth muscle cells in meningeal and cortical vessels in AD and Down's syndrome are cells which highly accumulate β-amyloid in the cytoplasm or as deposits between cells, as well as monomeric and oligomeric forms of soluble β-44 (3 kD to 17.5 kD). They also presumed these cells to be the most likely source of soluble β-protein in CSF.

Questions arise about the sources of silica exposure, Si-homeostasis and critical concentrations for Si polymerization *in vivo*. Silicon is the most abundant element in the earth's crust and there is a great variability in Si concentration in drinking water, from 30 μM to > 500 μM.[41] Silicon toxicity due to accumulation in various tissues was reported[42,43] but Si investigations in brain tissue are rare. However, investigations carried out as bulk analyses[12,13] have shown Si accumulation in nervous tissue (see also introduction). It is interesting to note that a long-term study was undertaken[47] to determine the effect of diet on the uptake of silicon and aluminium in the brain of rats. It was found that, while the distribution of Al appeared to be rather constant over the brain, silica concentrations were higher in some regions, *i.e.* hippocampus, caudate and lentiform nucleus. Now, it is well known that the hippocampus is the key site of damage in AD.

Although Al and Si seem to go together in inorganic chemistry, we cannot expect Al and Si to behave in the same way in an organic system. Our results on human serum and CSF have confirmed this assumption. Al and/or Si have produced some quite different effects in CSF and the question arises of what they can do in focal brain pathology in which the real environment and concentrations of constituents are quite unknown. The real silica concentration at the target site is a key to its possible biological action. If the

total concentrations of Si during the maturation of plaques (about 30 years) can exceed critical polymerization concentrations (above 100 μM), then it can precipitate with Al as aluminosilicates. Additionally, it could react with numerous other protein ligands, as mentioned above, or shwon in our present work.

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SAŽETAK

Interakcija silicija i aluminija s proteinima moždane tekućine

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Izveden je pokus taloženja u moždanoj tekućini i to dodatkom aluminijevih i/ili silicijevih iona. Željeli smo protumačiti moguću ulogu silicija i aluminija pri stvaranju agregata ekstracelularnih proteina. Simulirana su tri različita patološka stanja: suvišak aluminija (slučaj oštećenja bubrega), suvišak silicija (slučaj silikoze) i koegzistencija aluminija i silicija (slučaj Alzheimerove bolesti). U moždanoj tekućini talozi su u svim slučajevima nastali odmah, uz smanjenje sadržaja IgG (ispod 34%) i nešto manje albumina u redu: silikat >> aluminij > aluminosilikat. Odgovarajuće količine ovih proteina nisu nađene u dijelu taloga, koji se otapa u vodi, što upućuje na zaključak da su oni čvrsto vezani u anorganskim matricama identificiranima pomoću FTIR.