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Equilibrium of octadecylsilica gel with sodium dodecyl sulphate

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ABSTRACT

The retention times of tyrosine (Tyr), 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylethylamine (DA), norepinephrine (NE), epinephrine (E), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxymandelic acid (VMA), tryptophan (Trp), 5-hydroxytryptophan (5-HTP), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) on octadecylsilica (ODS) gel gradually changed when the ODS gel column was washed with buffer containing sodium dodecylsulphate (0.003%). To reach the steady state, 3200 column volumes of buffer were required. The retention properties of this column correlated with the hydrophobicity of the retained materials, including Tyr, DOPA, DA, NE, E, HVA, DOPAC, VMA, Trp, 5-HTP, 5-HT and 5-HIAA. These observations are of practical importance in preparing reproducible ODS gel columns for the determination of trace amounts of materials in biological fluids.

INTRODUCTION

A solvent-generated (dynamic) ion-exchange system facilitates the separation of catecholamines, indolamine and their precursors or metabolites in biological fluids[1,2]. In the separation of materials by "soap chromatography"[2], sodium dodecyl sulphate (SDS), an anionic detergent, is adsorbed by the reversed-phase surface to form an anionic layer, thereby exhibiting properties similar to those of an ion exchanger. In experiments with an octadecyl (C_{18}) silica (ODS) gel column and a buffer containing SDS, the selectivity and resolution of some eluates on ODS gel, treated with SDS, could not be explained by the properties of an ion exchanger. For this reason, we examined the equilibration process of the ODS gel, treated with SDS, and observed a relationship between the hydrophobicity of the eluites and their retention times on the ODS gel.

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EXPERIMENTAL

Materials

L-Tyrosine (Tyr), 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylethylamine (DA), norepinephrine (NE), epinephrine (E), homovanillic acid (HVA), DL-3-methoxy-4-hydroxymandelic acid (VMA), L-tryptophan (Trp), 5-hydroxytryptophan (5-HTP), serotonin creatinine sulphate (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were purchased from Nacalai Chemicals (Kyoto, Japan) and 3,4-dihydroxyphenylacetic acid (DOPAC) from Sigma (St. Louis, MO, U.S.A.). Each compound was dissolved in 0.1 *M* perchloric acid to give a 100-nmol/ml solution; the solutions were stored at −80°C and, just before use, were diluted to 5 nmol/ml with 0.1 *M* perchloric acid. The reversed-phase resin, Partisil-5 (5-μm, octadecylsilica gel), was obtained from Whatman (Maidstone, U.K.). SDS was purchased from Nacalai Chemicals. All other chemicals were of analytical-reagent grade and were used without further purification.

High-performance liquid chromatography (HPLC)

The HPLC system consisted of a sample injector (Rheodyne, Cotati, CA, U.S.A.), a Model L-6200 intelligent pump (Hitachi, Tokyo, Japan), a guard column

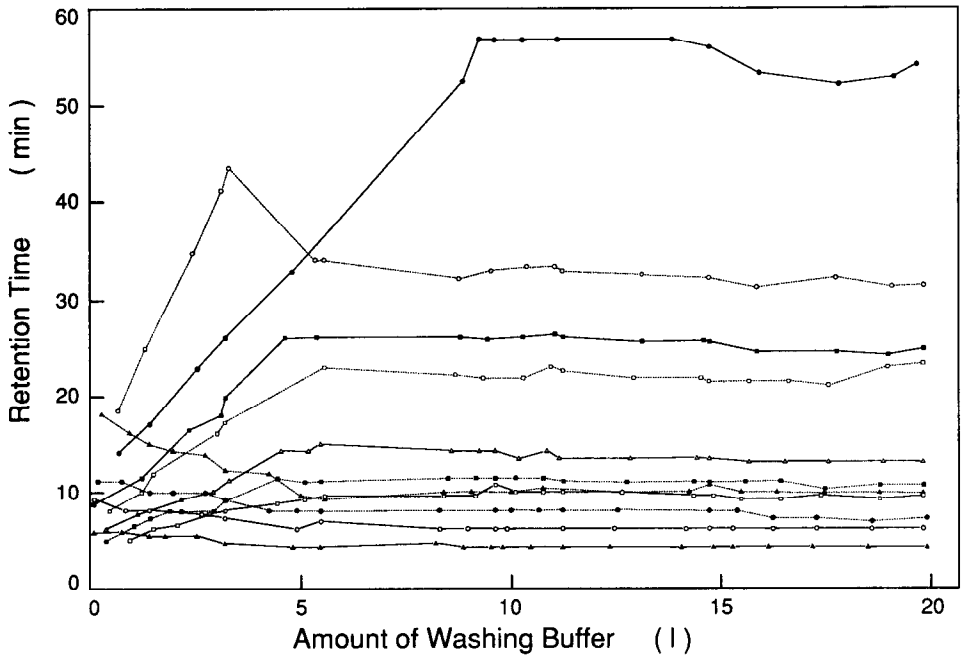

Fig. 1. Equilibrium of octadecysilica (ODS) gel column with sodium dodecyl sulphate (SDS). Stationary phase, octadecylsilica (ODS); mobile phase, 0.02 *M* citrate–sodium citrate buffer (pH 2.1)–3% (v/v) 1-propanol–0.2 *M* sodium perchlorate–0.003% SDS; flow-rate, 1 ml/min; temperature, 25°C. ●———● = 5-HT; ○- - - -○ = 5-HTP; ■———■ = DA; □- - - -□ = Tyr; △———△ = DOPA; ■- - - -■ = E; ▲- - - -▲ = NE; □———□ = HVA; ●- - - -● = 5-HIAA; ○———○ = DOPAC; ▲———▲ = VMA.

(10 mm × 4 mm I.D.), an isolation column (250 mm × 4 mm I.D.) with a column oven (25°C), a Model E-308 amperometric detector (Irica Instrument, Kyoto, Japan), a Model 7000A data analyser (System Instrument, Tokyo, Japan) and a floppy disc Model FD-10A recorder (System Instrument). The guard and isolation columns were filled with reversed-phase resin (Partisil-5) using a slurry-packing system (Nihon Seimitsu Kagaku, Tokyo, Japan). The potential of the glassy carbon working electrode of the amperometric detector was set at +0.95 V *vs.* Ag/AgCl. This relatively high potential was necessary to detect Trp with the same sensitivity as that obtained for 5-HT and 5-HIAA (data not shown). The mobile phase was 0.02 *M* citrate–sodium citrate buffer (pH 2.1)–3% (v/v) 1-propanol–0.2 *M* sodium perchlorate–0.003% SDS.

Determination of hydrophobicity

The hydrophobicity of the materials was calculated using the hydrophobic fragmental constants[3].

RESULTS AND DISCUSSION

Equilibrium of ODS gel with SDS

The retention times on the ODS gel of catcholamines (DA, NE, E), indolamine (5-HT), their precursors (Tyr, DOPA, Trp, 5-HTP) and their metabolites (DOPAC, HVA, VMA, 5-HIAA) were gradually changed by washing the ODS gel column with the above mobile phase, as shown in Fig. 1. The amount required to attain a steady state varied depending on the material to be separated by ODS gel column

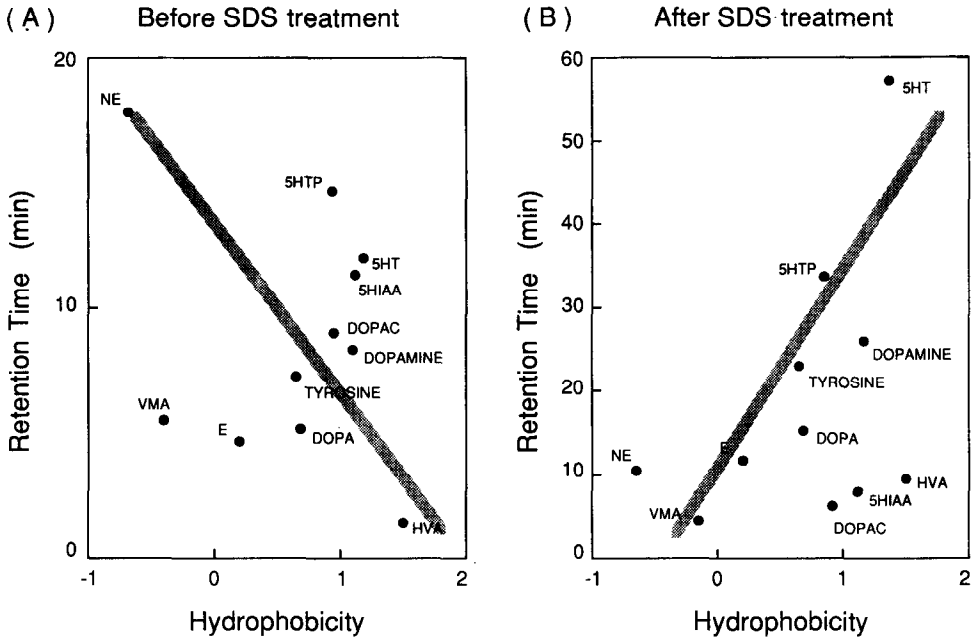

Fig. 2. Relationship between the retention time of an eluite on octadecylsilica (ODS) gel and its hydrophobicity. Stationary phase: (A) untreated ODS; (B) ODS treated with SDS. Mobile phase as in Fig. 1.

chromatography. To reach a steady state, 3200 column volumes of the buffer were required. The retention times of Tyr, DOPA, DA, E, HVA, Trp, 5-HTP and 5-HT were increased by SDS treatment, whereas those of DOPAC, NE, VMA and 5-HIAA decreased. In contrast to the data of Crombeen *et al.*[4], the retention time of NE decreased with SDS treatment and that of HVA increased. In the separation of materials by "soap chromatography"[2], SDS (anionic detergent) is adsorbed by the reversed-phase surface to form an anionic layer, thus exhibiting properties similar to those of an ion exchanger. As an enormous amount of the buffer containing SDS was required to attain a steady state on the ODS gel column, a change in the solid phase (ODS gel) by SDS was suspected. To examine the participation of SDS as a paired ion, the hydrophobicity of the eluite was determined and the results were compared with the retention times on the ODS gel.

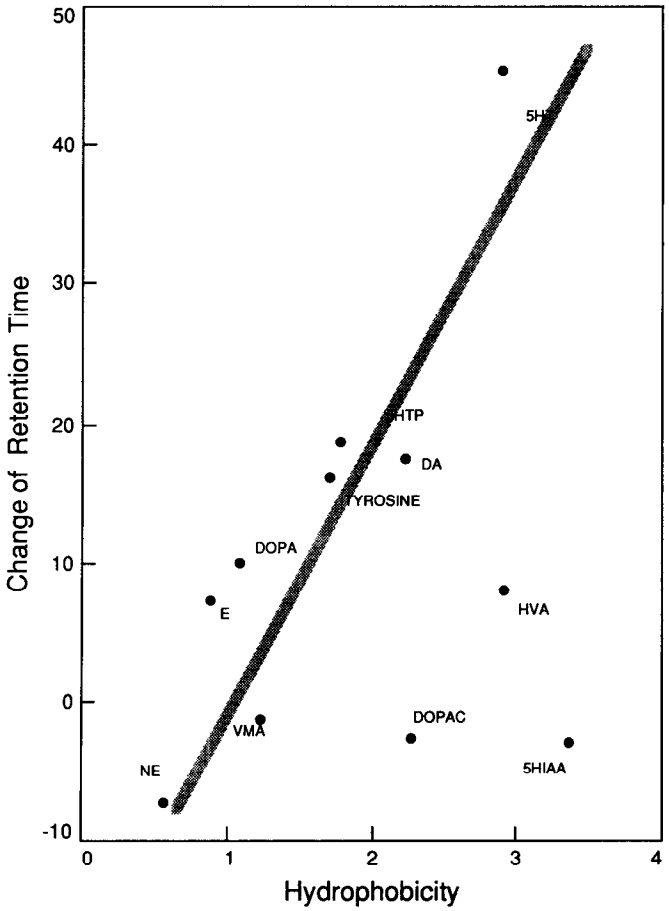

Fig. 3. Relationship between the change in retention time of an eluite on octadecysilica (ODS) gel and its hydrophobicity. The change in retention time was calculated by subtracting of the retention time of each eluite in Fig. 2A from that in Fig. 2B.

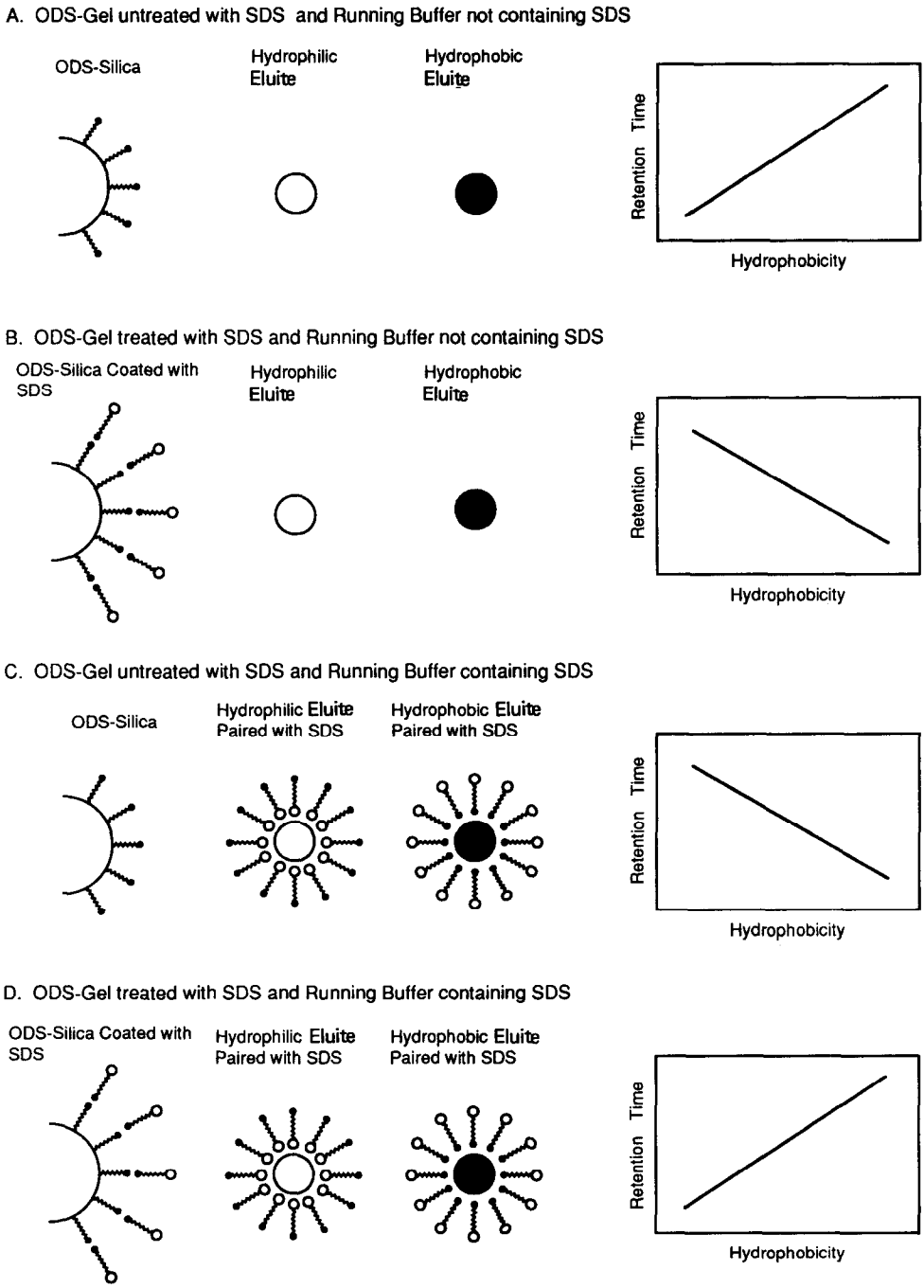

Fig. 4. Hypothetical model for the status of octadecylsilica (ODS) and eluites in solvent.

Correlation between hydrophobicity of eluites and their retention times on the ODS gel

Before treatment of the ODS gel with SDS, the increase in retention time of the eluites decreased with their hydrophobicity (Fig. 2A). After treatment of the ODS gel with SDS, the retention time increased with their hydrophobicity (Fig. 2B). Further, the difference in retention times obtained after and before SDS treatment was correlated with the hydrophobicity (Fig. 3). To explain these results, four possible conditions of the ODS gel and eluites were considered, as shown in Fig. 4: (A) ODS gel not treated with SDS (hydrophobic surface), hydrophilic and hydrophobic eluites; in this situation, an increase in hydrophobicity of the eluite should increase its retention time on the ODS gel; (B) ODS gel treated with SDS (changed to a hydrophilic surface), hydrophilic and hydrophobic eluites; in this situation, an increase in hydrophobicity of the eluite should decrease its retention time on the ODS gel; (C) ODS gel not treated with SDS (hydrophobic surface), hydrophilic, coated with SDS (changed to hydrophobic), and hydrophobic, coated with SDS (changed to hydrophilic), eluites; in this situation, an increase in hydrophobicity of the eluite should decrease its retention time on ODS gel; (D) ODS gel treated with SDS (changed to hydrophilic surface), hydrophilic, paired with SDS (changed to hydrophobic), and hydrophobic, paired with SDS (changed to hydrophilic), eluites; in this situation, an increase in hydrophobicity of the eluite should increase its retention time on the ODS gel. Our data on the relationship between retention time and hydrophobicity can be explained by conditions C and D. The eluite, dissolved in the buffer containing SDS, is readily paired with SDS, but the ODS gel is not sufficiently coated with SDS when the eluite is injected onto the ODS gel column not treated with SDS. Hence an increase in the hydrophobicity of the eluite decreased its retention time (Fig. 2A). At the steady state of the ODS gel treated with SDS (Fig. 2B), an increase in hydrophobicity of the eluite increased the retention time. Thus the selectivity and resolution on the ODS gel equilibrated with SDS (0.003%) correlates with the hydrophobicity of the eluites. These observations are of practical importance in the preparation of reproducible ODS gel columns for the determination of trace amounts of materials in biological fluids.

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