Separation of enantiomers on a chiral stationary phase based on ovoglycoprotein

VII. Comparison of chiral recognition ability of ovoglycoprotein from chicken and Japanese quail egg whites

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Abstract

Ovoglycoproteins from chicken and Japanese quail egg whites (OGCHI and OGJPQ, respectively) were isolated, and characterized by isoelectric focusing, high-performance capillary electrophoresis, reversed-phase HPLC and matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) MS. The isoelectric point (pI) of natural OGCHI was 4.37–4.51 by isoelectric focusing, while natural OGJPQ showed two discrete bands at pI 4.68 and 4.77. The average molecular masses of natural OGCHI and OGJPQ were estimated to be about 30 000 and 27 400 by MALDI-TOF-MS. Both natural OGCHI and OGJPQ were either fully or partially glycosylated with a ratio of ca. 3:1. Next, natural OGCHI and OGJPQ were bound to aminopropyl-silica gels activated with N,N'-disuccinimidylcarbonate to compare retentive and enantioselective properties of the two columns. The OGCHI column is suitable for chiral resolution of basic compounds, while the OGJPQ column is suitable for that of acidic compounds. With regard to chiral resolution of neutral compounds, it is dependent on a compound resolved which column could be suitable. Differences in the retentivity and enantioselectivity between OGCHI and OGJPQ columns are due to differences in the enantioselective binding properties. The results obtained reveal that chiral recognition of various solutes could be efficiently attained by using both columns complementarily. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chiral stationary phases based on a glycoprotein have included α_1-acid glycoprotein (AGP) [1],

avidin [2], ovomucoid (OMCHI) [3] and ovoglycoprotein (OGCHI) [4] from chicken egg whites, and cellobiohydrolase I [5]. They can separate a lot of enantiomeric forms, and are stable against repeated injections and eluent changes. Recently, we found that OMCHI used in previous studies was crude, and that crude OMCHI included about 10% of other glycoprotein [4]. In addition, we

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isolated the glycoprotein from the crude OMCHI preparations and chicken egg whites [4]. The glycoprotein, OGCHI, had an average molecular mass of 29 700 and sugar content of 25%. Further, pure OMCHI and OGCHI, respectively, were bound to aminopropyl-silica gels to evaluate the chiral recognition ability. The OMCHI gave no chiral recognition ability, while the OGCHI showed higher chiral recognition abilities than those of crude OMCHI reported by Miwa et al. [3]. This revealed that chiral recognition ability of crude OMCHI reported previously came from the OGCHI.

On the other hand, the elution order was reversed between chiral stationary phases based on bovine and human serum albumins (BSA and HSA, respectively); on the HSA-based stationary phases (*S*)-warfarin eluted before (*R*)-warfarin whereas on the BSA-based stationary phases the opposite elution order was observed [6]. It is well-known that enantioselective binding property is different among species [7]. In this study, we compared the retentivity and enantioselectivity of OGCHI and ovoglycoprotein from Japanese quail egg whites (OGJPQ). We isolated OGJPQ, and characterized it as well as OGCHI by isoelectric focusing (IEF), high-performance capillary electrophoresis (HPCE), reversed-phase high-performance liquid chromatography (HPLC) and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Next, OGCHI and OGJPQ were bound to aminopropyl-silica gels. The chiral recognition abilities of two columns were compared, and the enantioselective binding properties of two columns were discussed.

2. Experimental

2.1. Materials

Ketoprofen and ibuprofen were kindly donated by Chugai Pharmaceuticals (Tokyo, Japan) and Kaken Pharmaceuticals (Tokyo, Japan), respectively. Hexobarbital, tolperisone hydrochloride and oxprenolol hydrochloride were donated by Teikoku Chemicals (Osaka, Japan), Eisai (Tokyo, Japan) and Novartis (Tokyo, Japan), respectively. Chlorpheniramine maleate, alprenolol hydrochloride and propranolol

hydrochloride were purchased from Sigma–Aldrich Japan (Tokyo, Japan). Benzoin was purchased from Wako (Osaka, Japan). D-Glucosamine hydrochloride was purchased from Nacalai Tesque (Kyoto, Japan). *N,N*′-Disuccinimidylcarbonate (DSC) was obtained from Tokyo Chemical Industries (Tokyo, Japan). SP Sepharose FF and Sephadex G-25 (fine) were purchased from Amersham Pharmacia Biotech (Tokyo, Japan). The silica gels (Ultron-12, 5 μm diameter, 12 nm pore size) were from Shinwa (Kyoto, Japan). Other reagents and solvents of analytical- or HPLC-grade were obtained from Wako and used without further purification.

Water, purified using a Nanopure II unit (Barnstead, Boston, MA, USA), was used for the preparation of the eluent and the sample solution.

2.2. Isolation of OGCHI and OGJPQ from egg whites

OGCHI and OGJPQ were isolated as reported previously [8]. Briefly, crude OMCHI and Japanese quail ovomucoid (OMJPQ) were precipitated from the corresponding egg whites with ethanol according to the procedures modified slightly from those of Fredericq and Deutsch [9]. The obtained crude OMCHI and OMJPQ were further purified by cation-exchange chromatography. A mass of 2 g of crude OMCHI or OMJPQ was applied to a SP Sepharose FF column (12×5 cm I.D.) that was equilibrated with 10 mM CH_3COONH_4 (pH 4.6) applying a linear gradient to 700 mM CH_3COONH_4 (pH 4.6) for 6 h at flow-rate of 100 ml/h, and then the eluent was changed to 1000 mM CH_3COONH_4 (pH 4.6). The eluent was monitored at 280 nm with a Model AC-500 spectrophotometric monitor (Atto, Tokyo, Japan). The separation was performed at 4°C. The OGCHI or OGJPQ fraction was collected and lyophilized. The lyophilized OGCHI and OGJPQ was desalted with a Sephadex G-25 (fine) column (20×5 cm I.D.) using 15 mM NH_4HCO_3 (pH 9.0) as the eluent with an average flow-rate of 120 ml/h. The eluate was collected and lyophilized.

2.3. Preparation of OGCHI and OGJPQ materials

Aminopropyl-silica gels were prepared from silica gels, and activated by DSC as reported previously

[4]. OGCHI and OGJPQ were bound to amino-propyl-silica gels activated by DSC as described previously [4]. Briefly, 1 g of the DSC-activated silica gels was slurried in 20 mM sodium phosphate buffer (pH 6.8). A mass of 2.67 μmol of OGCHI or OGJPQ, which corresponds to 80 or 72.9 mg, was dissolved in 20 ml of the same buffer. Then the solution was slowly added to the silica gel slurry at room temperature for 1 h by adjusting pH to 6.6, and the resulting mixture was further stirred for 15 h at 30°C. The reaction mixture was washed with water, dissolved in 20 ml of a blocking solution (pH 6.6) including 300 mM D-glucosamine and stirred slowly for 1 h at room temperature. Then the reaction mixtures were filtered using a glass filter, and washed with water and water–ethanol (90:10, v/v). The obtained materials were packed into a stainless steel column (100×2.0 mm I.D.) using a slurry packing method. The slurry and packing solvent was water–ethanol (90:10, v/v).

2.4. Determination of the amount of OGCHI and OGJPQ bound to silica gels

The bound amounts of OGCHI and OGJPQ to DSC-activated aminopropyl-silica gels were determined as follows. After reaction with a respective protein, the materials were washed with water. All washing solutions were collected, and their volumes were measured. The concentration of each protein was determined using a reversed-phase chromatographic method as described below, and the unbound amounts of the protein were estimated. The bound amounts of a protein were calculated by subtraction of the unbound amounts of the protein from the reacted amounts.

2.5. Evaluation of chiral recognition abilities of OGCHI and OGJPQ columns

For chiral resolution of racemic solutes on the OGCHI and OGJPQ columns, the HPLC system used consisted of an LC-10A pump, an SPD-6A spectrophotometer, a Rheodyne 7125 injector with a 5-μl loop and a C-R6A integrator (all from Shimadzu, Kyoto, Japan). The flow-rate was maintained at 0.2 ml/min. Detection was performed at 210 nm. Retention factors were calculated from the equation $k=(t_R-t_0)/t_0$, where t_R and t_0 are retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured by injecting a solution whose organic modifier content was slightly different from that of the eluent used. The enantioseparation factor is calculated from the equation $\alpha=k_2/k_1$, where k_1 and k_2 are the retention factors of the first and second eluted enantiomers, respectively. Resolution is calculated from the equation $R_s=2(t_{R2}-t_{R1})/(w_1+w_2)$, where t_{R1} and t_{R2} are the retention times of the first and second eluted enantiomers, respectively and w_1 and w_2 are the baseline peak widths of the first and second eluted enantiomers, respectively. All separations were carried out at 25°C using a water bath (Thermo Minder Lt-100, Taitec, Saitama, Japan). The eluents used are specified in the legends of figures and tables.

The significance of difference in chiral recognition abilities between the OGCHI and OGJPQ columns was analyzed by a paired t-test. The 0.05 level of probability was accepted as significant.

2.6. Isoelectric focusing

IEF of proteins was carried out on a Multiphor II electrophoresis unit (Amersham Pharmacia Biotech), thermostated at 10°C using a Multitemp II thermostatic circulator. A Multidrive XL electrophoresis constant power supply was used in conjunction with a volt–hour integrator. Ampholine PAG plate pH 3.5–9.5 (Amersham Pharmacia Biotech) was used at 1500 V, 50 mA for 30 min with 1 M phosphoric acid as anolyte and 1 M sodium hydroxide as catholyte. After IEF was completed, the gel was removed and stained with Coomassie Brilliant Blue R-250.

2.7. High-performance capillary electrophoresis

HPCE separations were performed with a Beckman P/ACE system 5500 equipped with a diode array detector (Fullerton, CA, USA). A linear polyacrylamide-coated capillary [37 cm (effective length 30 cm)×75 μm I.D.] was prepared by the method of Hjertén [10] and was used for separations. All capillaries were thermostated at 23°C by using a liquid coolant.

Electrophoretic buffer (running buffer) solutions used in this study were 50 m*M* sodium dihydrogenphosphate–disodium hydrogenphosphate (pH 5.0). The running buffer solutions were filtered though a 0.45-μm membrane filter (Gelman Sciences Japan, Tokyo, Japan) and degassed with a Branson Model B-2200 ultrasonic bath (Yamato, Tokyo, Japan) prior to use. The capillary was rinsed with water for 1 min, 50 m*M* phosphoric acid–sodium dihydrogenphosphate (pH 2.5) for 3 min, water for 1 min and the running buffer for 2 min prior to the run. The sample concentration was 4 mg/ml. The sample solution was injected from the cathodic end at ca. 3500 Pa for 0.5 s. Both ends of the capillary were dipped into the running buffer solution, and a constant voltage of −12.5 kV was applied for the separation. Detection was performed at 214 nm.

2.8. Reversed-phase HPLC

For reversed-phase chromatographic separations of OGCHI and OGJPQ, the same HPLC system as described above was used expect that two pumps were used for gradient elution. The eluents used are as follows: eluent A, water–acetonitrile (80:20, v/v) including 0.1% trifluoroacetic acid (TFA); eluent B, water–acetonitrile (20:80, v/v) including 0.1% TFA; linear gradient from 0% eluent B at 0 min to 100% eluent B at 90 min. The column used was Cosmosil 5C18-AR (250×4.6 mm I.D., Nacalai Tesque). Detection was carried out at 280 nm. The flow-rate was 1.0 ml/min. Loaded amounts of a protein were 100 μg. All separations were performed at 30°C using a CO-1093C column oven (Uniflows, Tokyo, Japan).

2.9. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry

MALDI-TOF mass spectra were measured using a Vision 2000 reflector-type TOF instrument (Thermoquest, Tokyo, Japan) equipped with an N_2 laser operating at a wavelength of 337 nm with a pulse duration of 3 ns. The ions generated were accelerated to a potential of 5 kV in the ion source and postaccelerated to a potential of 20 kV for detection with a secondary ion multiplier. The MALDI-TOF mass spectra represent the accumulation of 15 to 20 single laser shots. They were calibrated externally by a standard sample (BSA, molecular mass 66 430) that was placed on the same target. The matrix used was 2,5-dihydroxybenzoic acid, dissolved in a 2:1 mixture of 0.1% aqueous TFA and acetonitrile at a concentration of 50 m*M*. Samples were dissolved in a water at a concentration of 10^{-6} *M*. A 0.5-μl portion of the sample solution was mixed with an equal volume of the matrix solution on the target, resulting in a used sample amount of 500 fmol. After deposition on the stainless steel target, the sample was air-dried and introduced into the mass spectrometer.

3. Results and discussion

3.1. Characterization of OGCHI and OGJPQ

OGCHI and OGJPQ were characterized by IEF, HPCE, reversed-phase HPLC and MALDI-TOF-MS. The isoelectric point (p*I*) of natural OGCHI was in the range 4.37 to 4.51 by IEF, while natural OGJPQ showed two discrete bands at p*I* 4.68 and 4.77. OGJPQ had a slightly higher p*I* value than OGCHI. With regard to the p*I* of OMCHI, it was in the range 3.93 to 4.49. Previously, it was reported that the p*I* values of OGCHI and OMCHI were about 3.9 [11]. We isolated OGCHI from crude OMCHI preparations, which included about 10% OGCHI, by cation-exchange chromatography using ammonium acetate buffer (pH 4.6) as an eluent. OMCHI and OGCHI were eluted in this order. There is good agreement between the elution order and the p*I* values measured by us. Fig. 1A and B show electropherograms of natural OGCHI and OGJPQ, respectively. Since linear polyacrylamide-coated capillaries were used for the separation of these proteins, the electroosmotic flow was negligible [11]. In addition, we used 50 m*M* sodium dihydrogenphosphate–disodium hydrogenphosphate (pH 5.0) as a running buffer solution. The p*I* values of OGCHI and OGJPQ being below 5.0, OGCHI and OGJPQ electrophoretically migrated toward the anodic end. Further, as OGJPQ had a slightly higher p*I* value than OGCHI, the former migrated slower than the latter. Broad peaks of OGCHI should be due to the microheterogeneity of sugar moieties of OGCHI. On the other hand,
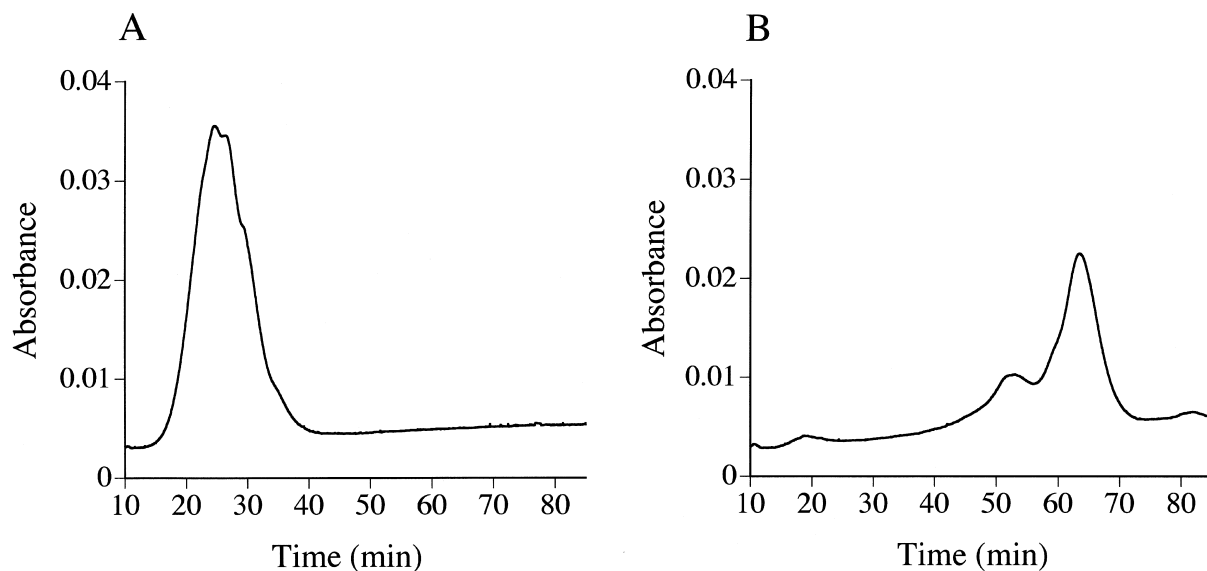
Fig. 1. Electropherograms of natural OGCHI (A) and OGJPQ (B). HPCE conditions: capillary, linear polyacrylamide-coated capillary (effective length 30 cm×75 μm I.D.); running buffer solution, 50 mM sodium dihydrogenphosphate–disodium hydrogenphosphate (pH 5.0); sample, 4 mg/ml each protein; applied voltage, -12 kV. Other conditions as in Experimental.
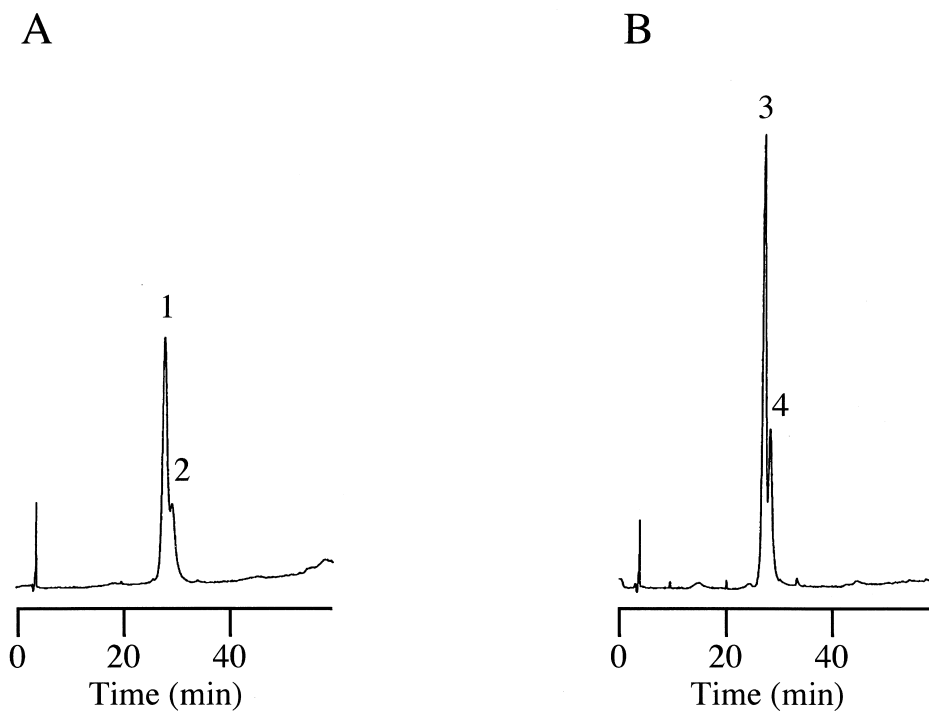

Fig. 2. Reversed-phase chromatograms of natural OGCHI (A) and OGJPQ (B). Eluents: eluent A, water–acetonitrile (80:20, v/v) including 0.1% TFA; eluent B, water–acetonitrile (20:80, v/v) including 0.1% TFA; linear gradient from 0% eluent B at 0 min to 100% eluent B at 90 min. Flow-rate: 1.0 ml/min. Detection: 280 nm. Loaded amount: 100 μg. Peaks: 1=fully glycosylated OGCHI; 2=partially glycosylated OGCHI; 3=fully glycosylated OGJPQ; 4=partially glycosylated OGJPQ.

A

30144

59673

90101

B

27308

55132

82242

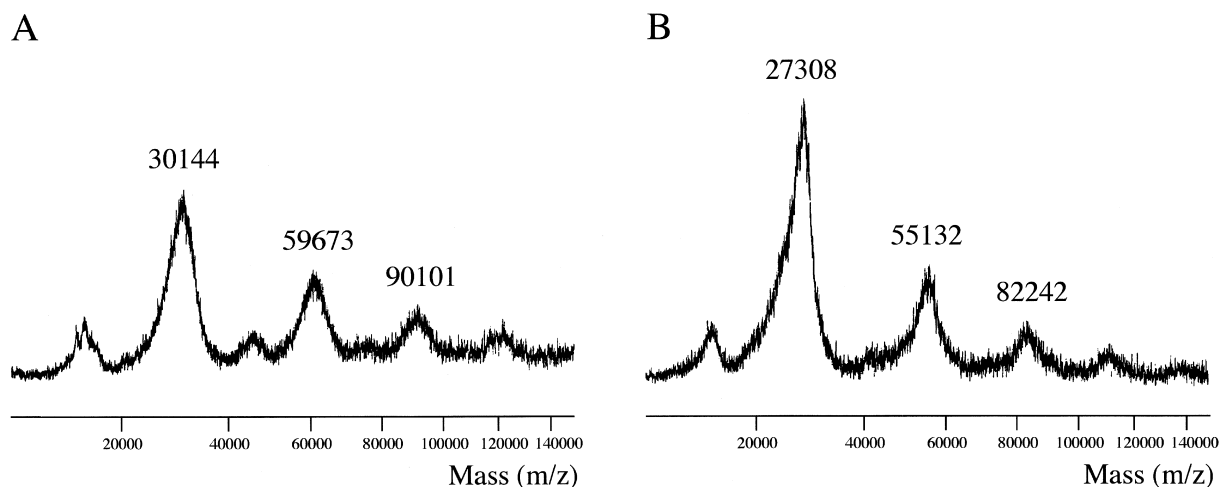Mass (m/z)   Mass (m/z)

Fig. 3. MALDI-TOF mass spectra of natural OGCHI (A) and OGJPQ (B). Flight mode; reflector-type. Matrix; 2,5-dihydroxybenzoic acid. Other conditions as in Experimental.

Table 1
Bound amounts of OGCHI and OGJPQ to DSC-activated aminopropyl-silica gels, and the bound ratio[a]

Column	Reacted protein amount (μmol/g silica)	Bound protein amount $\pm$SD (μmol/g silica)	Bound ratio $\pm$SD (%)
OGCHI	2.67	2.40$\pm$0.04	89.9$\pm$1.66
OGJPQ	2.67	2.46$\pm$0.05	92.2$\pm$1.83

[a] Average of three replicates.

Table 2
Batch reproducibility of OGCHI and OGJPQ columns[a]

	Hexobarbital			Ketoprofen			Tolperisone		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
OGCHI									
Batch 1	1.28	1.31	1.47	15.8	1.18	1.61	0.93	2.43	3.83
2	1.28	1.32	1.27	15.3	1.16	1.52	0.99	2.39	3.66
3	1.34	1.32	1.34	15.7	1.18	1.36	1.04	2.40	3.83
Average$\pm$SD	1.30$\pm$0.04	1.32$\pm$0.01	1.36$\pm$0.10	15.6$\pm$0.30	1.17$\pm$0.01	1.50$\pm$0.13	0.99$\pm$0.05	2.41$\pm$0.02	3.77$\pm$0.10
OGJPQ									
Batch 1	1.06	1.55	2.04	13.1	1.31	2.54	1.22	1.28	0.95
2	1.05	1.56	1.63	12.9	1.31	2.03	1.23	1.29	0.79
3	1.10	1.56	1.69	13.7	1.35	2.28	1.25	1.30	0.85
Average$\pm$SD	1.07$\pm$0.03	1.56$\pm$0.01	1.79$\pm$0.22	13.2$\pm$0.42	1.32$\pm$0.02	2.28$\pm$0.25	1.24$\pm$0.01	1.29$\pm$0.01	0.86$\pm$0.08

[a] HPLC conditions: column size, 100$\times$2.0 mm I.D.; eluent, [20 m*M* sodium dihydrogenphosphate–disodium hydrogenphosphate (pH 5.1)]–ethanol (90:10, v/v); flow-rate, 0.2 ml/min; detection, 210 nm; temperature, 25°C; loaded amount, 100 ng.

Table 3

Comparison of the retention factor, enantioseparation factor and resolution of neutral, acidic and basic compounds between OGCHI and OGJPQ columns[a]

Compound	Column					
	OGCHI			OGJPQ		
	k_1	α	R_s	k_1	α	R_s
Neutral						
Hexobarbital	1.30*[b]	1.32	1.36	1.07	1.56*	1.79*
Benzoin	8.03*	3.01*	10.28*	7.27	2.26	6.84
Acidic						
Ketoprofen	15.6*	1.17	1.50	13.2	1.32*	2.28*
Ibuprofen	6.49*	1.34	2.76	6.12	1.42*	3.06*
Basic						
Tolperisone	0.99	2.41*	3.77*	1.24*	1.29	0.86
Chlorpheniramine	4.28	2.10*	5.40*	5.73*	1.14	0.76
Alprenolol	11.8	1.10*	0.58*	15.9*	1.00	
Oxprenolol	5.84	1.20*	1.32	6.76*	1.08	0.30
Propranolol	34.0	1.12	0.78	42.1*	1.14*	0.97*

[a] HPLC conditions as in Table 2. Data given are average values obtained with three different batch columns.
[b] Significantly larger ($P<0.05$).

Table 4

Influence of eluent pH on the retention factor, enantioseparation factor and resolution of hexobarbital, ketoprofen and tolperisone enantiomers on the OGCHI and OGJPQ columns[a]

Eluent pH	Column					
	OGCHI			OGJPQ		
	k_1	α	R_s	k_1	α	R_s
pH 4.0						
Hexobarbital	1.08*[b]	1.35	1.23	0.87	1.48*	1.20
Ketoprofen	59.7*	1.29	2.85	47.5	1.54*	3.87*
Tolperisone	–[c]			–		
pH 5.1						
Hexobarbital	1.30*	1.32	1.36	1.07	1.56*	1.79*
Ketoprofen	15.6*	1.17	1.50	13.2	1.32*	2.28*
Tolperisone	0.99	2.41*	3.77*	1.24*	1.29	0.86
pH 6.0						
Hexobarbital	1.36	1.42	1.72	1.17	1.65*	2.05
Ketoprofen	3.26*	1.09	0.33	2.74	1.21*	0.96*
Tolperisone	5.54	1.63*	3.90*	8.64*	1.00	
pH 6.9						
Hexobarbital	1.43	1.58	2.43	1.28	1.77*	2.54
Ketoprofen	0.79	1.00		0.78	1.00	
Tolperisone	24.3	1.34*	2.77*	35.0	1.15	1.21

[a] HPLC conditions is as in Table 2 except that the eluent used is a mixture of 20 mM sodium dihydrogenphosphate–disodiumhydrogen phosphate and ethanol (90:10, v/v). Data given are average values obtained with three different batch columns.
[b] Significantly larger ($P<0.05$).
[c] Not retained.

OGJPQ showed two discrete peaks as observed with IEF, and both peaks were broad because of the microheterogeneity of sugar moieties of OGJPQ.

Fig. 2A and B show the reversed-phase chromatograms of natural OGCHI and OGJPQ, respectively. In a previous communication [12], we reported that natural OGCHI was present as fully and partially glycosylated OGCHIs with a ratio of ca. 4:1, which correspond to peaks 1 and 2, respectively, in Fig. 2A. With regard to OGJPQ, it also gave two peaks with a ratio of ca. 3:1 on a reversed-phase chromatogram as shown in Fig. 2B. These two peaks might be due to fully and partially glycosylated OGJPQ.

Fig. 3A and B show MALDI-TOF mass spectra of natural OGCHI and OGJPQ, respectively. The aver-age molecular masses, averaged from $(M+H)^+$, $(2M+H)^+$ and $(3M+H)^+$ ions, are 30 004±156 for natural OGCHI and 27 308±130 for natural OGJPQ. It was reported that OMCHI and OMJPQ had five and four sugar chains, respectively [13,14]. Similarly, if assumed that OGCHI had the larger number of sugar chains than OGJPQ, it is plausible that the average molecular mass of OGCHI is larger than that of OGJPQ. The sugar moieties of OGCHI and OGJPQ were completely cleaved by the treatment with *N*-glycosidase, following denaturation by sodium dodecyl sulfate [12]. The average molecular masses of completely deglycosylated OGCHI and OGJPQ, estimated by MALDI-TOF-MS, were 21 362±40 and 21 516±73, respectively. Thus, dif-

A   B

0.032 Au

0   5   0   5

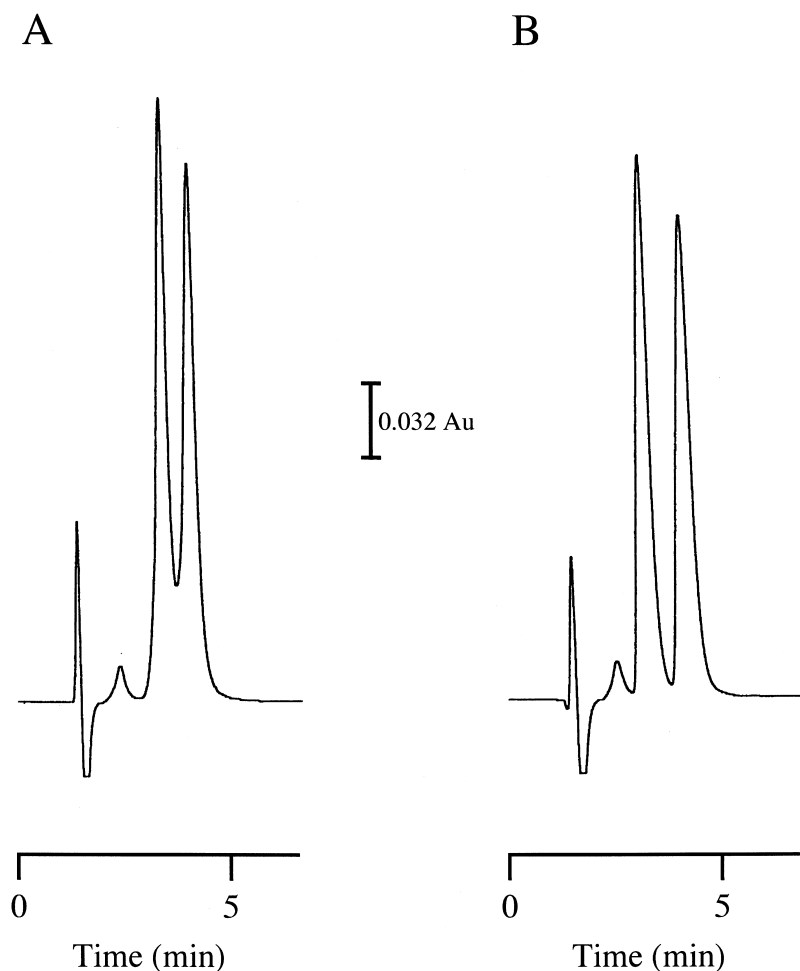Time (min)   Time (min)

Fig. 4. Chromatograms of hexobarbital enantiomers on OGCHI (A) and OGJPQ (B) columns. HPLC conditions as in Table 2.

ferences in masses observed between OGCHI and OGJPQ, 2696, might correspond to one sugar chain.

From the results of reversed-phase HPLC, we assumed that natural OGJPQ as well as OGCHI were either fully or partially glycosylated. To verify this, we isolated fully and partially glycosylated OGCHIs (which correspond to peaks 1 and 2, respectively) and fully and partially glycosylated OGJPQs (which correspond to peaks 3 and 4, respectively) in Fig. 2, and estimated their average molecular masses by off-line MALDI-TOF-MS. The average molecular masses for fully and partially glycosylated OGCHIs were estimated to be $30\,470\pm135$ and $28\,448\pm352$, respectively. Similarly, those for fully and partially glycosylated OGJPQs were estimated to be $28\,115\pm183$ and $26\,448\pm136$, respectively. Differences in masses observed between fully and partially glycosylated OGCHIs were 2022, and those observed between fully and partially glycosylated OGJPQs were 1667. These results reveal that natural OGCHI and OGJPQ are either fully or partially glycosylated, and that differences in partially or fully

glycosylated OGCHI and OGJPQ might be deficient in one sugar chain or not.

3.2. Comparison of retentivity, enantioselectivity and resolution of racemic solutes on OGCHI and OGJPQ columns

Next, natural OGCHI and OGJPQ, respectively, were bound to DSC-activated aminopropyl-silica gels in order to compare chiral recognition properties of OGCHI and OGJPQ. Since linear correlation is obtained between the retention factor of each enantiomer and the bound amount of OGCHI [8], it is important to immobilize the same molar amount of OGCHI or OGJPQ. The reaction amounts of OGCHI and OGJPQ per 1 g of the activated silica gel were 2.67 μmol, which corresponds to 80 mg and 72.9 mg for OGCHI and OGJPQ, respectively. Table 1 shows bound amounts of OGCHI and OGJPQ, and the bound ratio. Similar molar amounts of OGCHI and OGJPQ were reproducibly bound to aminopropyl-silica gels. Previously, we reported that the OGCHI
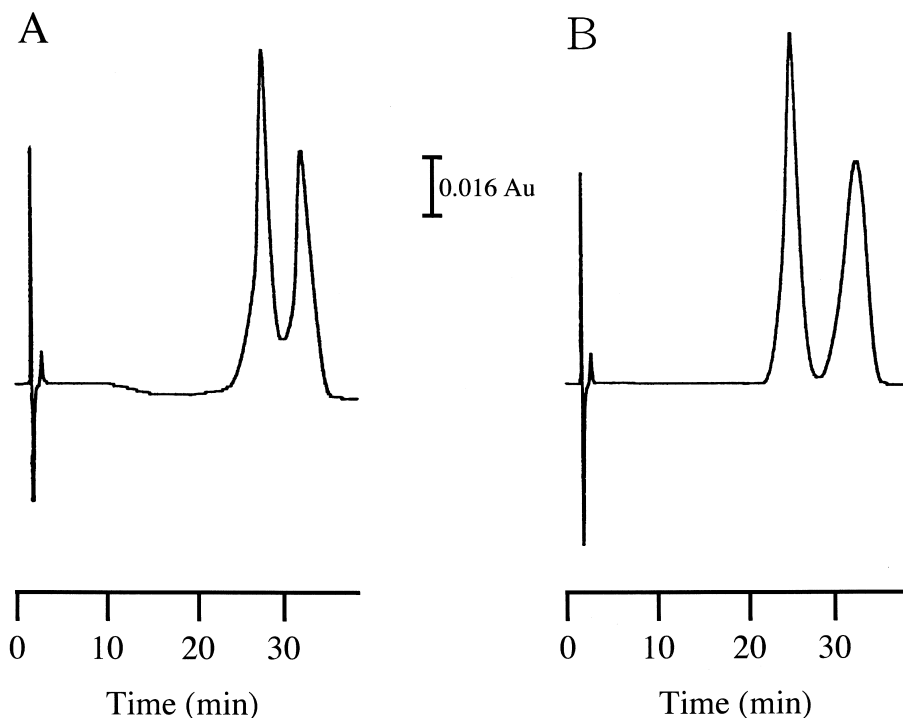

Fig. 5. Chromatograms of ketoprofen enantiomers on OGCHI (A) and OGJPQ (B) columns. HPLC conditions as in Table 2.

column prepared via DSC-activated aminopropyl-silica gels gave excellent batch reproducibility for the retentivity and enantioselectivity of various solutes [15]. As shown in Table 2, both OGCHI and OGJPQ columns showed excellent batch reproducibility.

Table 3 shows comparison of the retention factor, enantioseparation factor and resolution of neutral, acidic and basic compounds between OGCHI and OGJPQ columns, where [20 mM sodium dihydrogenphosphate–disodium hydrogenphosphate (pH 5.1)]–ethanol (90:10, v/v) was used as an eluent. Figs. 4–6 show chromatograms of hexobarbital, ketoprofen and tolperisone enantiomers on the OGCHI and OGJPQ columns. With regard to the retentivity of the solutes tested, the retention factors

of neutral and acidic solutes on the OGCHI column were significantly larger than those on the OGJPQ column. On the other hand, the retention factors of basic compounds on the OGJPQ column were significantly larger than those on the OGCHI column. With regard to the enantioselectivity, the enantioseparation factors and resolution of the acidic compounds on the OGJPQ column were significantly higher than those on the OGCHI column. Moreover, the enantioseparation factors and resolution of basic compounds on the OGCHI column were significantly higher than those on the OGJPQ column, except for propranolol. With regard to neutral compounds, benzoin showed significantly higher enantioseparation factor and resolution on the OGCHI column than on the OGJPQ column, while hexobarbital
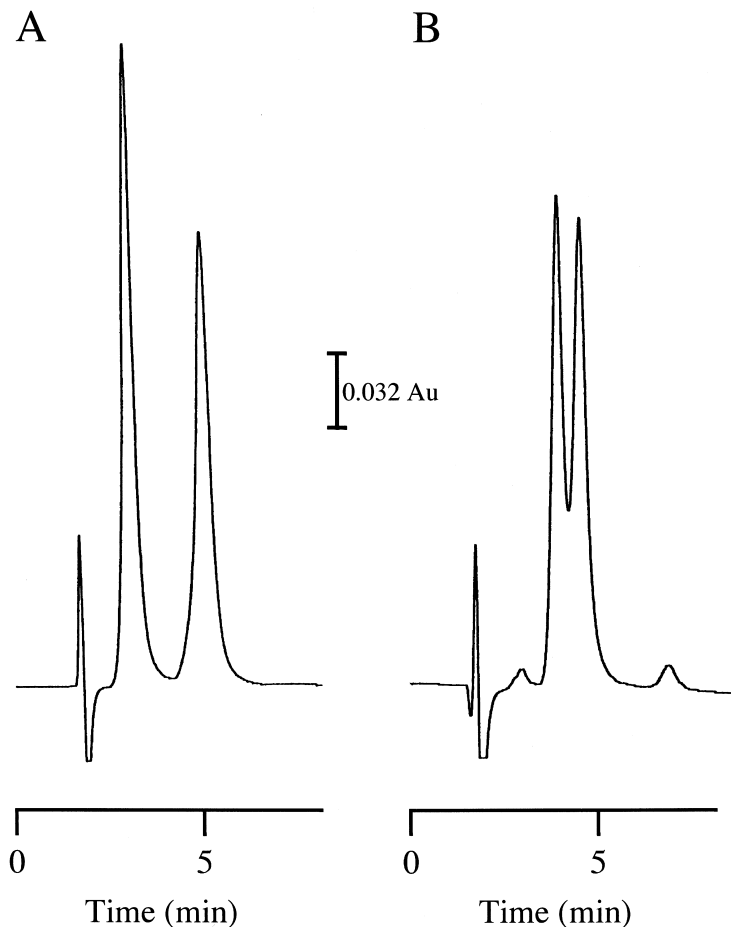

Fig. 6. Chromatograms of tolperisone enantiomers on OGCHI (A) and OGJPQ (B) columns. HPLC conditions as in Table 2.

showed significantly higher enantioseparation factor and resolution on the OGJPQ column.

Table 4 shows influence of eluent pH on the retention factor, enantioseparation factor and resolution of hexobarbital, ketoprofen and tolperisone enantiomers on the OGCHI and OGJPQ columns. As reported previously [16], hydrophobic and ionic interactions played an important role in the retentivity and enantioselectivity of these solutes. The tendencies for differences in the retentive and enantioselective properties of both columns were independent of eluent pH, as shown in Table 4. This indicates that differences in the retentivity and enantioselectivity between OGCHI and OGJPQ columns are not due to differences in the pI values, but due to differences in the enantioselective binding properties.

The OGCHI column is suitable for chiral resolution of basic compounds, while the OGJPQ column is suitable for that of acidic compounds. With regard to chiral resolution of neutral compounds, it is dependent on a compound resolved which column is suitable. The results obtained above reveal that chiral recognition of various solutes should be efficiently attained by using both columns complementarily.

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