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LEGENDS

Fig. 1. Ion-exchange chromatography of proteoglycans on DEAE-Sephacel.

The extract from yellow ligament obtained with 4 M guanidine-HCl buffer (pH 5.8, solution A) was dialyzed against 7 M urea buffer (pH 7.0, solution B) and applied to a DEAE-Sephacel column (1.0 x 15.0 cm). The column was eluted with a linear gradient of NaCl(0 - 1.0 M) in solution B. The flow rate was 15 ml/min, and 1.5-ml fractions were collected. The proteoglycan fractions (*bars*) were pooled. ● and ○, absorbance at 530 nm and 595 nm, respectively; a, 10s; b, 30s; c, 50s; d, 70s.

Fig. 2 Gel chromatography of proteoglycans on Sepharose CL-4B.

Proteoglycan fractions from DEAE-Sephacel (*bars* in Fig. 1) were concentrated and applied to a Sepharose CL-4B column (1.0 x 133 cm). The column was eluted with 4 M guanidine-HCl buffer (pH 5.8, solution A) at a flow rate of 15 ml/min, and 1.5-ml fractions were collected. ●, absorbance at 530 nm; V_o, void volume; V_t, total volume; a, 10s; b, 30s; c, 50s; d, 70s.

Fig. 3. Two-dimensional electrophoresis on cellulose acetate membranes of GAGs from small PGs.

Small PG from each age group was digested with Actinase E. The GAGs obtained were subjected to electrophoresis on cellulose acetate membrane (Separax, 10 x 10 cm). The first dimension was obtained in 0.1 M pyridine/0.47 M formic acid buffer (pH 3.0) at 1 mA/cm for 1 h, and the second dimension in 0.1 M barium acetate buffer (pH 8.0) at 1 mA/cm for 4 h. The resulting membranes were stained with 0.1% alcian blue in 70% ethanol. a, 10s; b, 30s; c, 50s; d, 70s.

Fig. 4. Size-fractionation h.p.l.c. of PA-GAGs before and after chondroitinase digestion.

PA-GAGs derived from small PGs were analysed by h.p.l.c. on a Shodex OHpak KB-803 column (0.8 x 30 cm) before (*lane* i) and after digestion with chondroitinase AC-II (*lane* ii), B (*lane* iii), and ABC (*lane* iv). The column was eluted with 0.2 M NaCl at a flow rate of 0.5 ml/min, and the column temperature was 30°C. For detection of PA-GAGs, an excitation wavelength of 320 nm and an emission wavelength of 400 nm were used. *Line* a, 10s; *line* b, 30s; *line* c, 50s; *line* d, 70s; 1, ΔGlcAβ1-3GalNAc(±SO_4)β1-4GlcAβ1-3Galβ1-3Galβ1-4Xyl-PA; 2, ΔGlcAβ1-3Galβ1-3Galβ1-4Xyl-PA.

Fig. 5. Ion-exchange h.p.l.c. of PA-GAGs before and after chondroitinase digestion.

PA-GAGs derived from small PGs were analysed by h.p.l.c. on a TSKgel SAX column (0.6 x 15 cm) before (*lane* i) and after chondroitinase AC-II (*lane* ii) and ABC (*lane* iii) digestion. The column was eluted with a linear gradient of NaCl (0 - 2.0 M) for 30 min in 0.1 M sodium acetate buffer (pH 6.0) at a flow rate of 0.8 ml/min, and a column temperature of 30°C. For detection of PA-GAGs, an excitation wavelength of 320 nm and an emission wavelength of 400 nm were used. *Line* a, 10s; *line* b, 30s; *line* c, 50s; *line* d, 70s.

Fig. 6. Affinity h.p.l.c. of PA-GAGs on Hydroxyapatite-MP.

PA-GAGs derived from small PGs were analysed on a Hibar Hydroxyapatite-MP column (0.8 x 10 cm). The column was eluted with a sodium phosphate linear gradient (5 - 100 mM) at a flow rate of 1.0 ml/min, and a column temperature of 30°C. For detection of PA-GAGs, an excitation wavelength of 320 nm, and an emission wavelength of 400 nm were used. a, 10s; b, 30s; c, 50s; d, 70s; e, standard PA-DS; f, standard PA-CS.

2

Age-related changes in chemical structure and affinity for hydroxyapatite of glycosaminoglycan chains from small proteoglycan of human yellow ligament

ヒト黄色靭帯低分子プロテオグリカンを構成するグリコサミノグリカン糖鎖の構造とそのヒドロキシアパタイトに対する結合性の加齢に伴う変化

[表と図]

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Age-related changes in chemical structure and affinity for hydroxyapatite of glycosaminoglycan chains from small proteoglycan of human yellow ligament

Tables & Figures

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Table 1. Chondroitin/dermatan sulphate compositions of GAGs.

The CS/DS compositions were calculated from the yields of the unsaturated disaccharides obtained from exhaustive digestion with chondroitinase ABC and AC-II. The yields of unsaturated disaccharides were analysed by reverse-phase h.p.l.c. on a DC-613 column.

Age group	Compositions (%)	
	Dermatan sulphate	Chondroitin sulphate
10s	73.3	26.7
30s	81.5	18.5
50s	60.9	39.1
70s	57.3	42.7

Table 2. Unsaturated disaccharide compositions of GAGs.

Unsaturated disaccharides obtained after exhaustive digestion with chondroitinase ABC were analysed by h.p.l.c. on a DC-613 column.

Age group	Compositions (%)			
	ΔDi-4S	ΔDi-6S	ΔDi-0S	ΔDi-diS_B
10s	86.1	11.0	2.0	0.9
30s	85.1	9.6	3.7	1.6
50s	70.0	26.6	1.6	1.8
70s	68.7	28.6	1.6	1.1

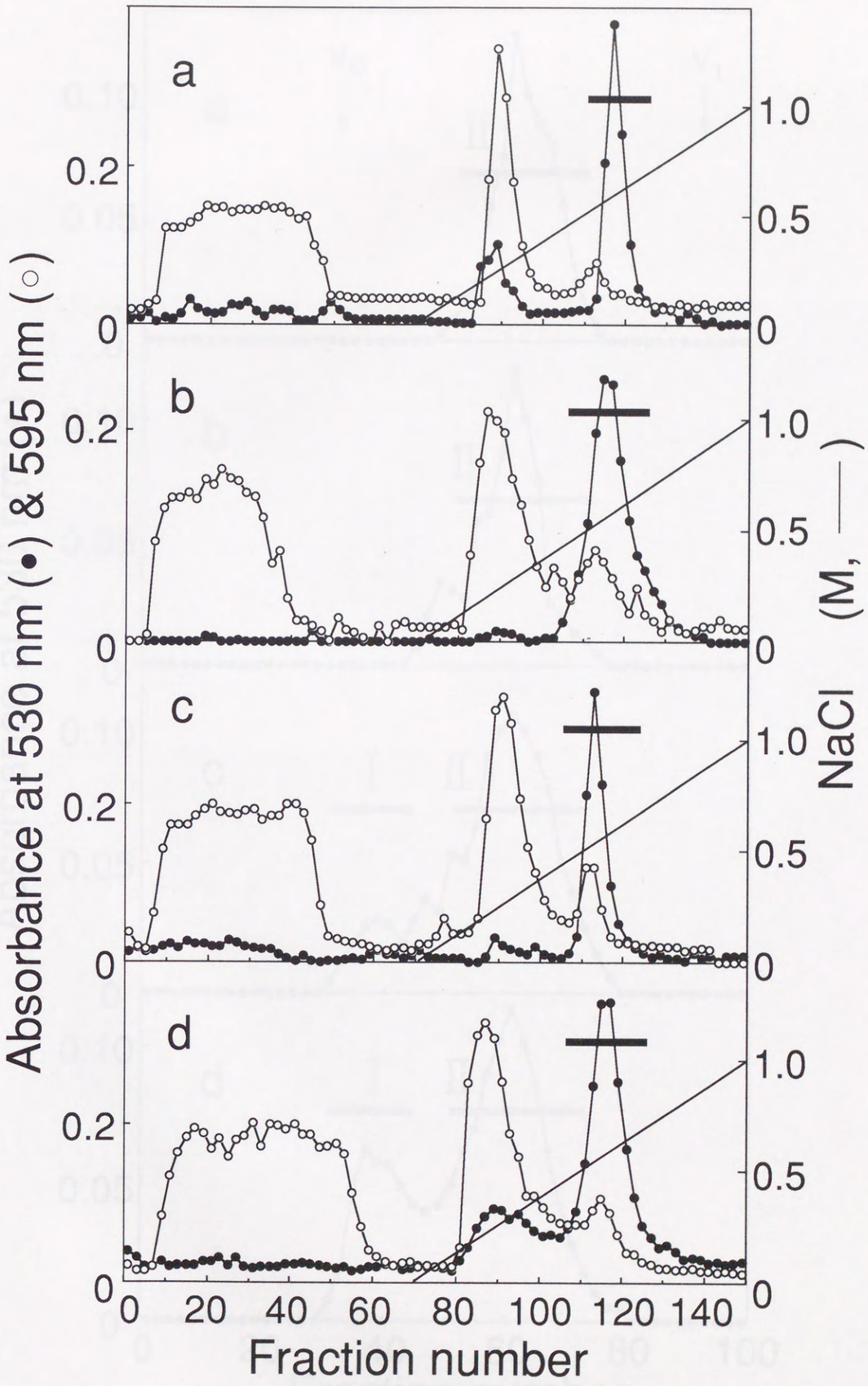
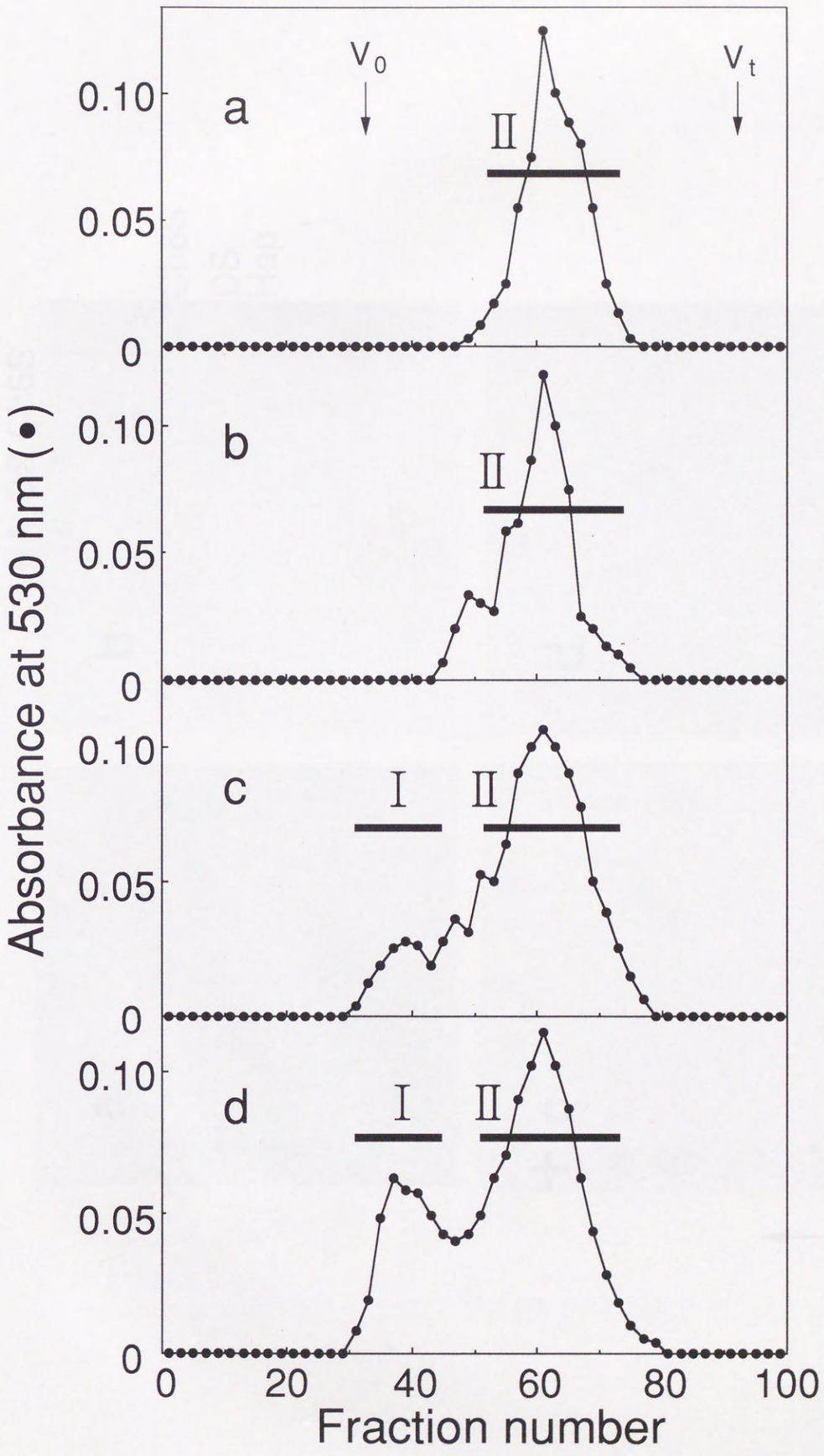

a
b
c
d
0.2
0
1.0
0.5
0
0 20 40 60 80 100 120 140
Fraction number
Absorbance at 530 nm (•) & 595 nm (○)
NaCl (M, ——)

Fig. 1


Absorbance at 530 nm (•)
Fraction number
a
b
c
d
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II
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V_t
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0.05
0
0
20
40
60
80
100

Fig. 2

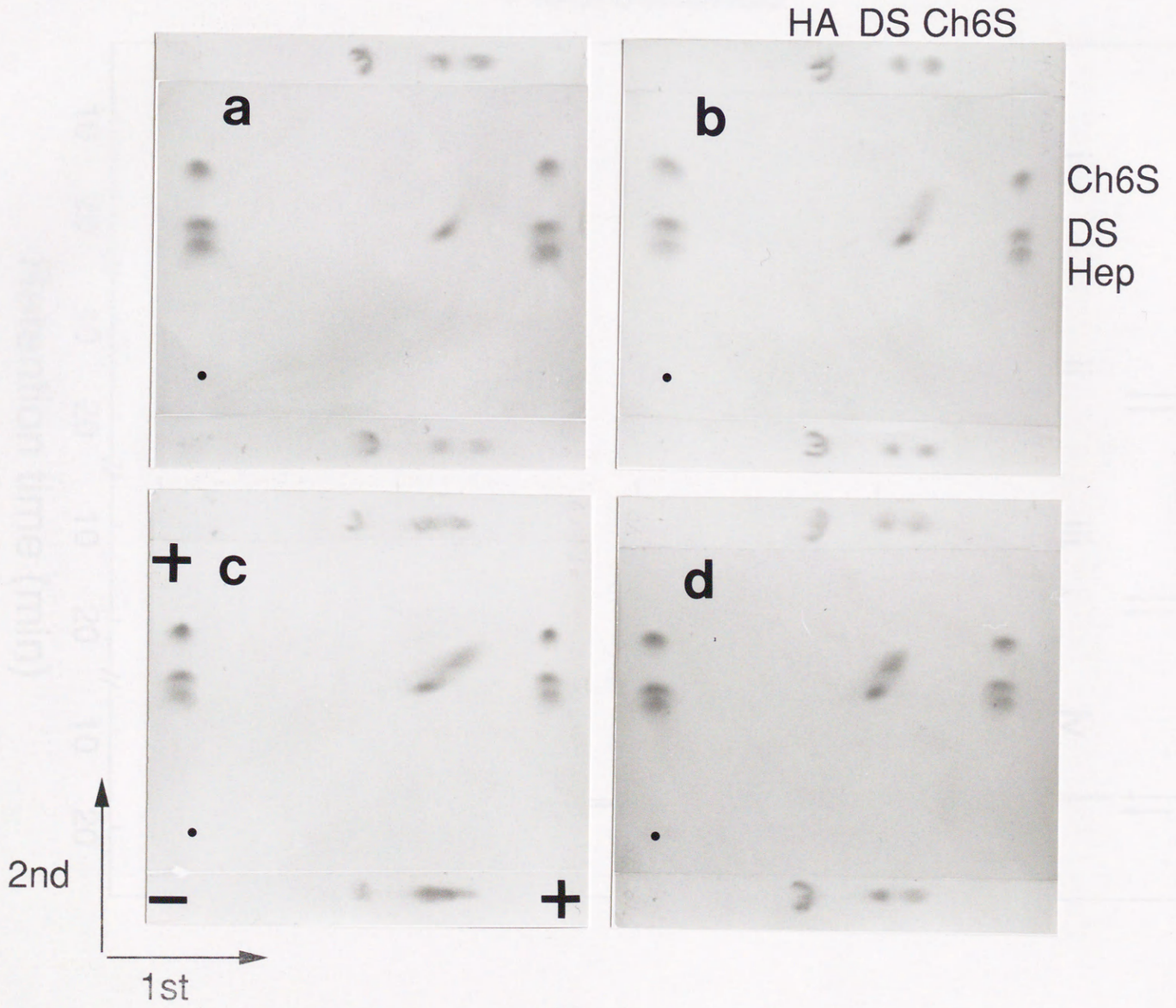


Fig. 3

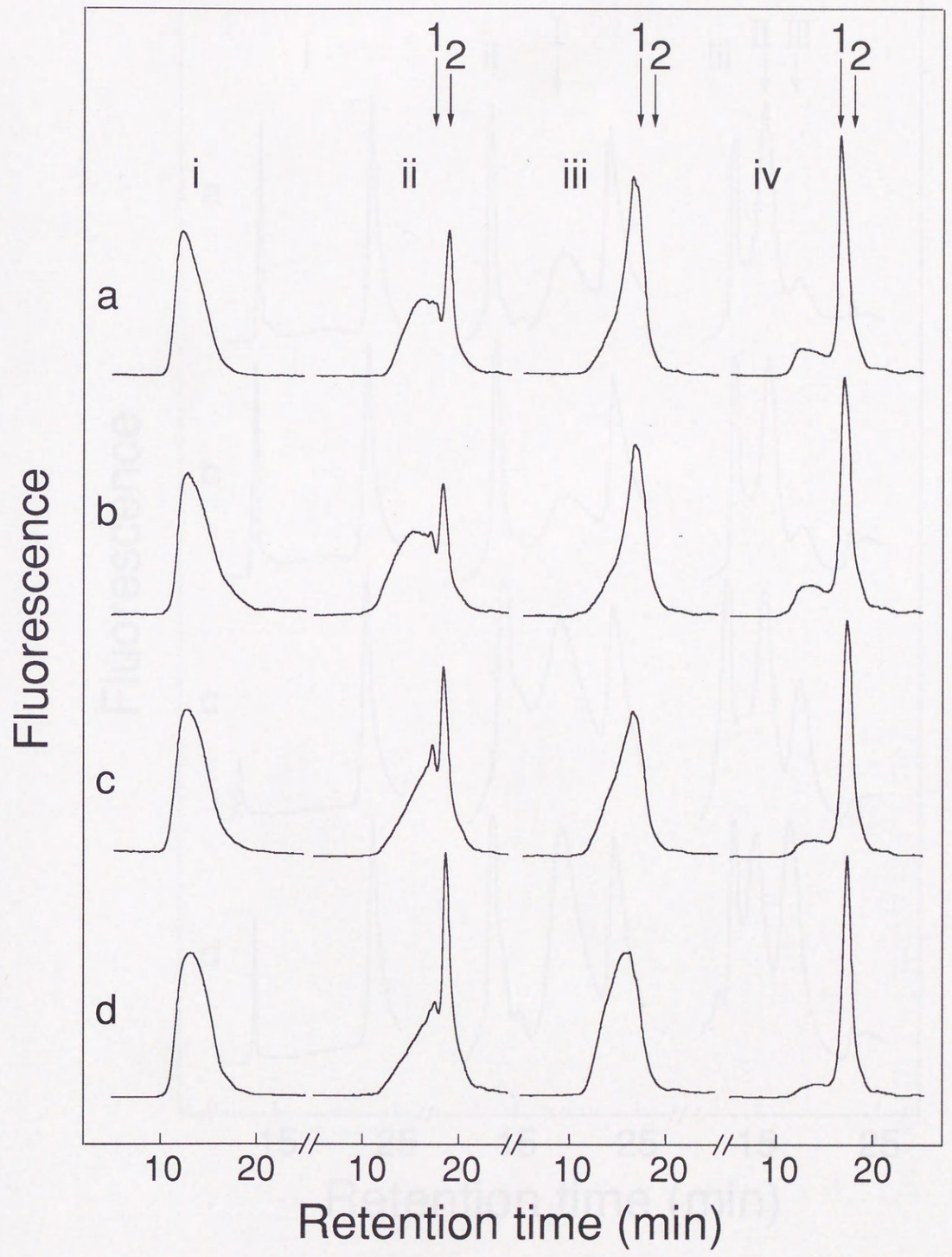

1
2
1
2
1
2
i
ii
iii
iv
a
b
c
d
Fluorescence
10
20
10
20
10
20
10
20
Retention time (min)

Fig. 4

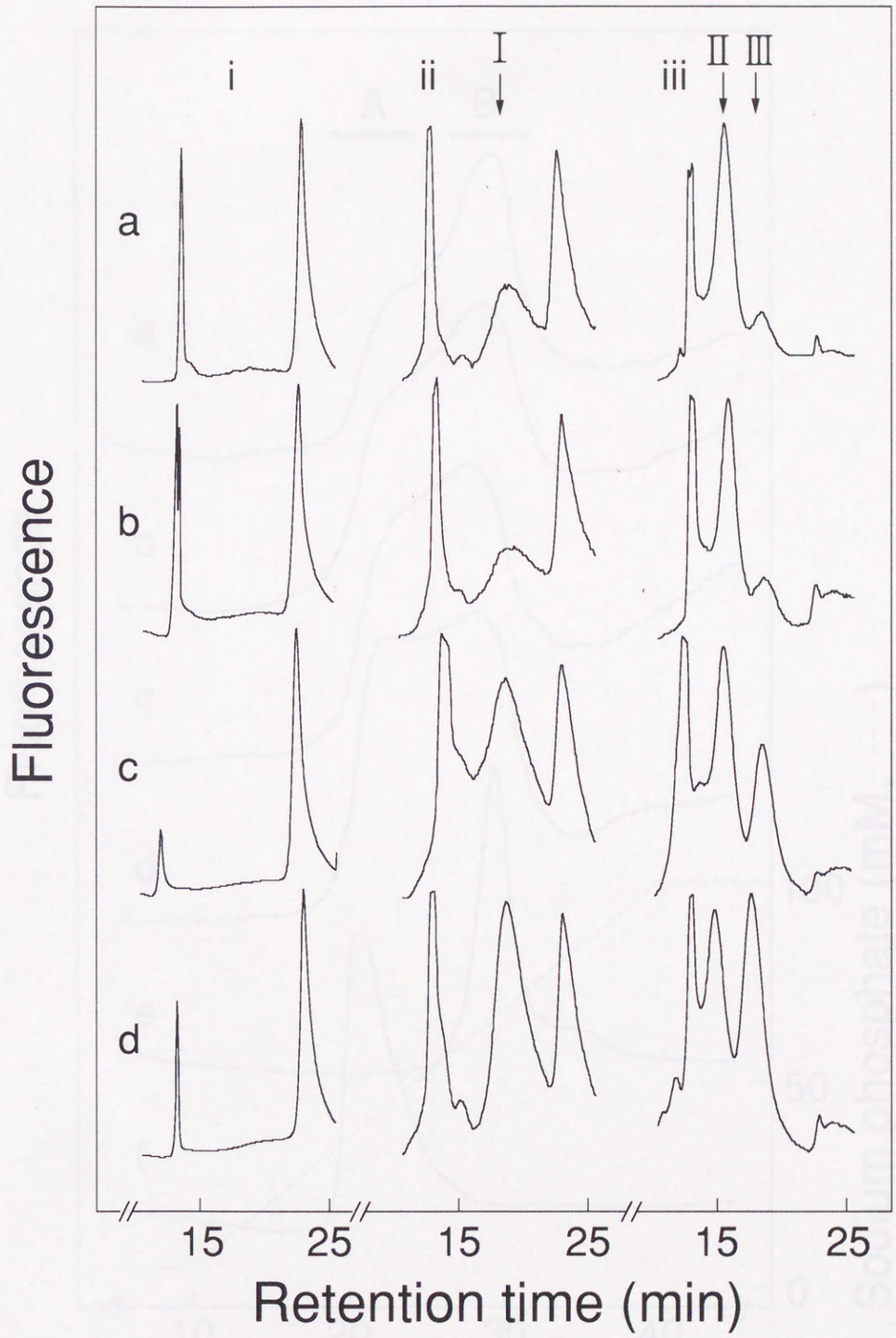

i
ii
I
iii
II
III
a
b
c
d
Fluorescence
15
25
15
25
15
25
Retention time (min)

Fig. 5

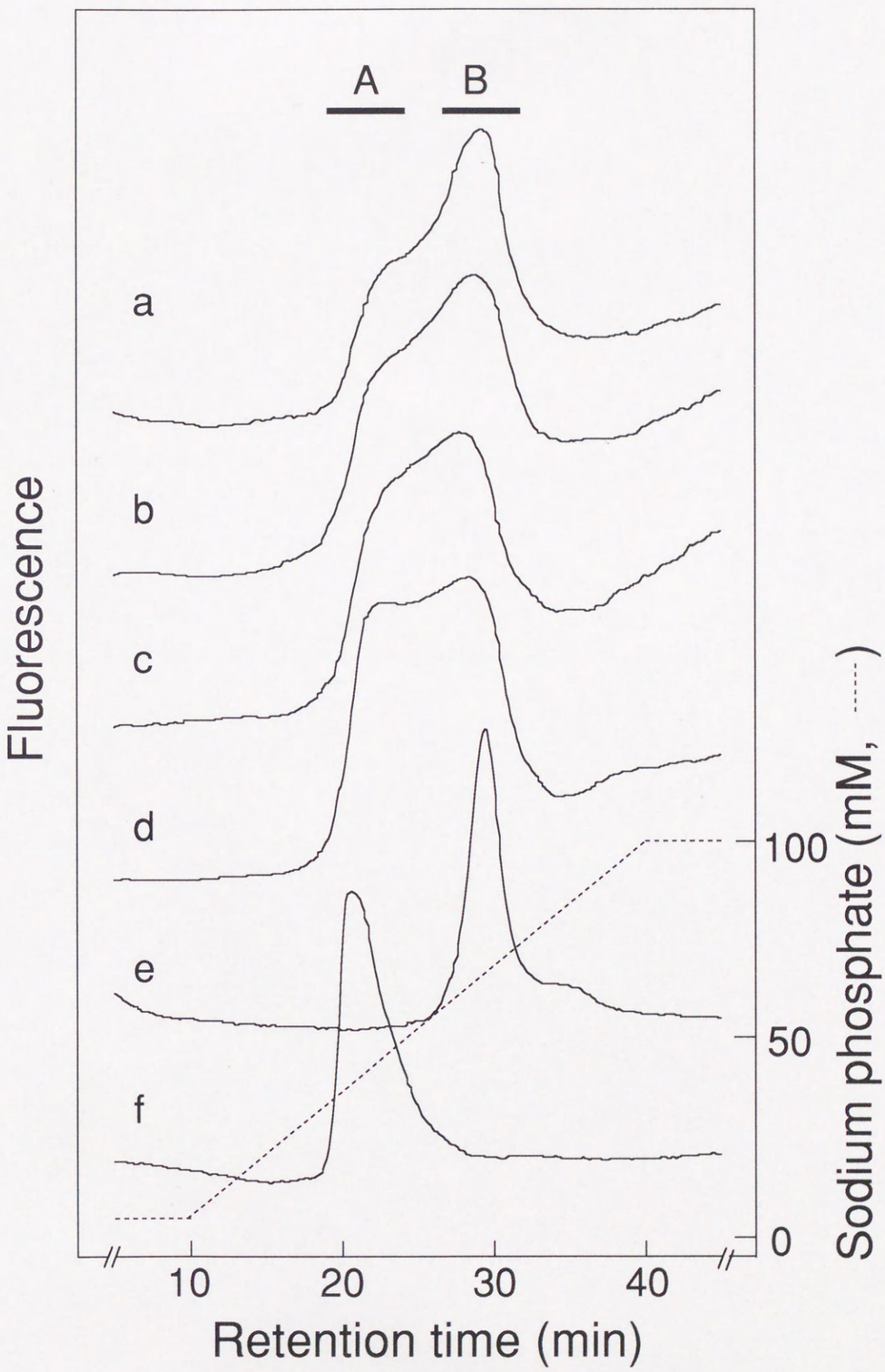
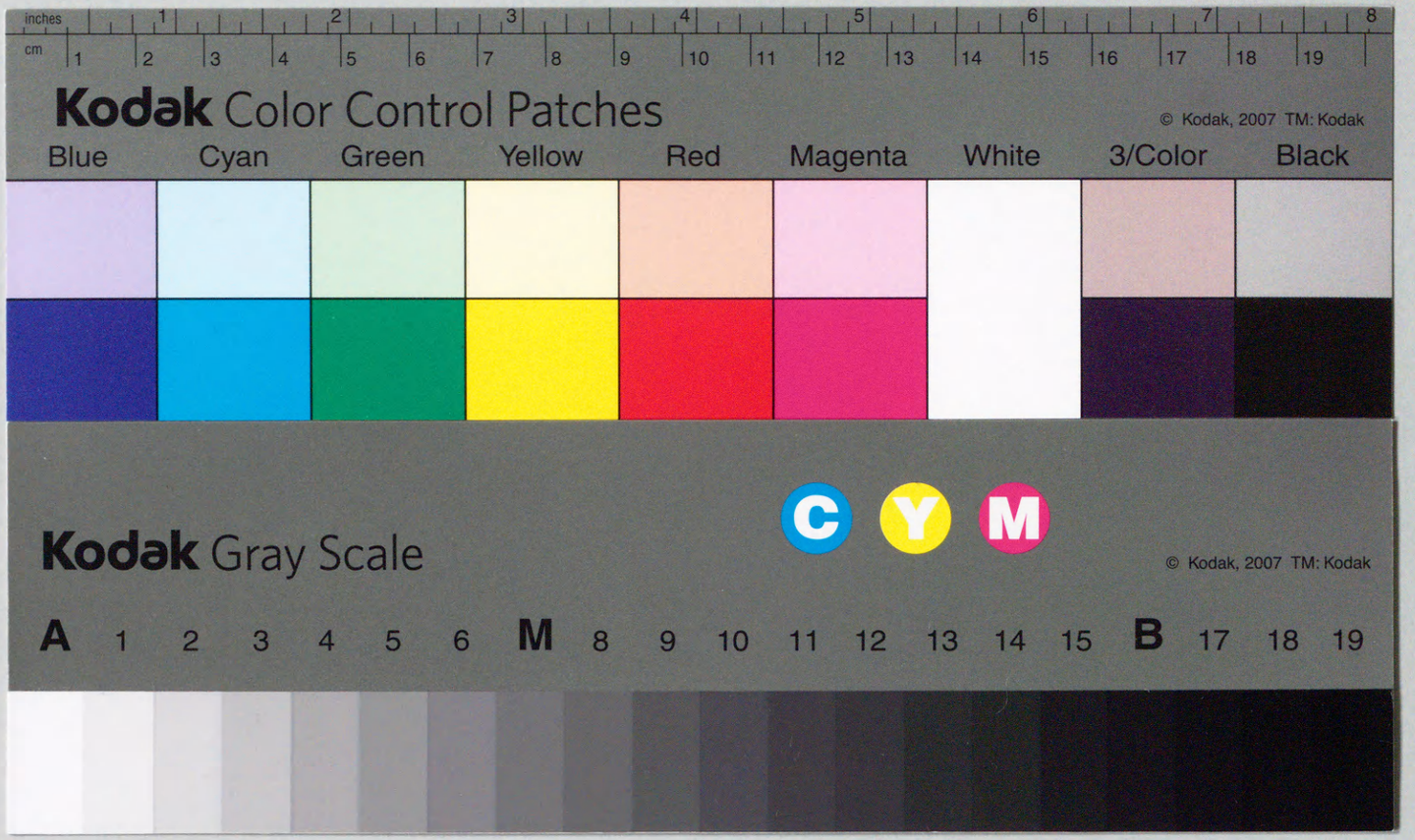

A
B
a
b
c
d
e
f
Fluorescence
100
50
0
Sodium phosphate (mM, ----)
10
20
30
40
Retention time (min)

Fig. 6


inches
cm
Kodak Color Control Patches
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Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19

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