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Investigation of enantioselectivity and enantiomeric elution order of propranolol and its ester derivatives on an ovomucoid-bonded column

Jun Haginaka*, Chikako Seyama, Hiroyuki Yasuda and Koichi Takahashi

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68, Koshien Kyuban-cho, Nishinomiya, Hyogo 663 (Japan)

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ABSTRACT

The enantioselectivity and enantiomeric elution order of racemic propranolol (PP) and its ester derivatives (O-acetyl, -propionyl, -butyryl and -valeryl) on an ovomucoid (OVM)-bonded silica column were investigated with respect to eluent pH (from 3 to 7) and organic modifier (2-propanol, ethanol, methanol and acetonitrile). The enantioselectivity was dependent on the eluent pH and organic modifier used. Reversal of the enantiomeric elution order of racemic PP and its ester derivatives occurred around eluent pH 5–7 and/or by variation of the organic modifier used. The results reveal that chiral recognition or binding properties may be altered by a change in eluent pH and/or addition of organic solvents. Reversal of the enantiomeric elution order suggests that there may be more than one binding site on the OVM-bonded column, and/or that at least two chiral recognition mechanisms may operate on the OVM-bonded column with regard to PP and its ester derivatives. Also, a conformational change of the OVM bonded structure might be caused by a change in eluent pH and/or addition of organic modifier.

INTRODUCTION

Recently, several chiral stationary phases have been developed for the direct resolution of enantiomers by high-performance liquid chromatography (HPLC) [1]. Protein-bonded stationary phases have been widely used for the chiral resolution of racemic compounds, especially racemic drugs, by HPLC. These proteins include albumins such as bovine serum albumin [2] and human serum albumin [3], glycoproteins such as α_1-acid glycoprotein (AGP) [4] and ovomucoid (OVM) [5] and enzymes such as α-chymotrypsin [6] and trypsin [7]. Above all, glycoprotein-bonded phases are promising candidates for the chiral resolution of various racemic solutes owing to their wide chiral recognition properties, their stability against changes in eluent pH and type and content of organic modifier and their relatively high efficiency compared with other protein-bonded phases. An OVM-bonded column, developed by Miwa *et al.* [5], has been utilized for the chiral separation of acidic and basic solutes [8–13].

Reversal of the enantiomeric elution order was reported on Pirkle-type columns, based on (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine [14] and (*S*)- or (*R*)-1-(α-naphthyl)ethylamine with (*S*)- or (*R*)-valine [15]. Also, it was observed on cellulose columns, based on cellulose tribenzoate [16] and cellulose tris(3,5-dimethylphenylcarbamate) [17]. It was reported [18] that the inversion of the enantiomeric elution order for pseudoephedrine occurred on an AGP-based column with the addition of octanoic acid to the eluent. In a previous paper [11], we reported the inversion of the enantiomeric elution order of propranolol (PP) and its ester derivatives on an OVM-bonded column. This paper deals with the investigation of the enantioselectivity and enantiomeric elution order of PP and its ester derivatives on an OVM-bonded column with respect to eluent pH (from 3 to 7) and organic modifier (2-propanol, ethanol, methanol and acetonitrile).

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EXPERIMENTAL

Reagents and materials

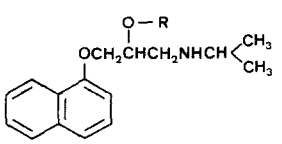
Racemic PP hydrochloride was purchased from Wako (Osaka, Japan) and (*R*)- and (*S*)-PP hydrochlorides from Aldrich (Milwaukee, WI, USA). The racemic O-acetyl (Ac), -propionyl (Pro), -butyryl (Bu) and -valeryl (Val) esters of PP were synthesized according to the procedures reported previously [19]. The (*R*)- and (*S*)-PP derivatives were prepared by the same procedure. The structures of PP and its ester derivatives are shown in Fig. 1. Methanol, ethanol, 2-propanol and acetonitrile of HPLC grade were obtained from Wako. Other reagents were of analytical-reagent grade and were used as received.

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

Chromatography

The HPLC system consisted of a Model 880-PU-pump, a VL-614 injector (both from Japan Spectroscopic, Tokyo, Japan) equipped with a 100-μl loop and an RF-540 spectrofluorimeter (Shimadzu, Kyoto, Japan) with excitation at 285 nm and emission at 340 nm. An OVM-bonded silica column (Ultron ES-OVM, particle diameter 5 μm, 150 × 4.6 mm I.D.) (Shinwa Chemical Industries, Kyoto, Japan) was used with a guard column (10 × 4.0 mm I.D.) (Shinwa Chemical Industries) packed with the same material. The flow-rate was maintained at 1.0 ml/min. Chromatograms were recorded and integrated with a Chromatopac C-R6A integrator (Shimadzu). Capacity factors (k') were calculated using the equation $k' = (t_R - t_0)/t_0$, where t_R and t_0 are elution times of retained and unretained solutes, respectively. The retention time of an unretained solute, t_0, was measured by injecting a solution with an organic modifier content slightly different from that of the eluent used. Enantioseparation factors (α) were calculated using the equation $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the capacity factors of the first- and second-eluted peaks, respectively. All separations were performed at 25°C using a Lauda RMS 6 low-temperature thermostat (Hansen, Kobe, Japan).

The eluents used are specified in the legends of the figures and tables.



R	Abbreviation
H	PP
$COCH_3$	Ac-PP
$COCH_2CH_3$	Pro-PP
$COCH_2CH_2CH_3$	Bu-PP
$COCH_2CH_2CH_2CH_3$	Val-PP

Fig. 1. Structures of propranolol and its ester derivatives used in this study.

Sample preparations

A known amount of PP or its ester derivatives was dissolved in water and the solution was diluted with the eluent to the desired concentration. A 20-μl aliquot of the sample solution was loaded on to the column. The on-column amount was less than 0.2 nmol.

RESULTS AND DISCUSSION

In a previous paper [11], we reported that an increase in the organic modifier content and/or a decrease in the eluent pH resulted in a decreased retention of PP and its ester derivatives. Also, the enantioselectivity and enantiomeric elution order of PP and its ester derivatives on an OVM-based column are dependent on the organic modifier used. In this study, the effects of eluent pH and organic modifier on the enantioselectivity and enantiomeric elution order of PP and its ester derivates were investigated. The eluent pH was varied over the range 3–7 and the organic modifiers used were 2-propanol, acetonitrile, ethanol and methanol at various concentrations.

Fig. 2 shows the effect of eluent pH on the retention, enantioselectivity and enantiomeric elution order of PP and its ester derivatives, with 17.5% of 2-propanol as organic modifier. Almost the same retentions of PP and its ester derivatives were obtained with 20%, 30% and 50% acetonitrile, ethanol and methanol organic modifier contents, respectively. However, the enantioselectivity and enantiomeric elution order of PP and its ester deriv-

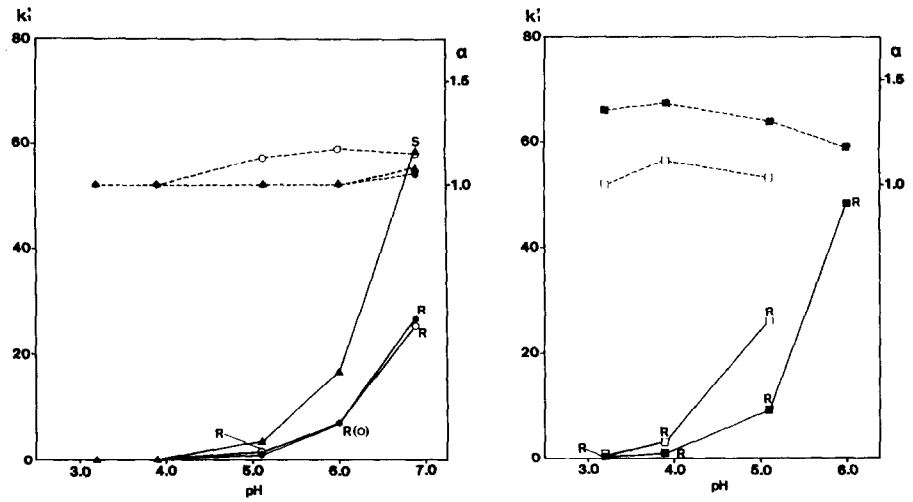


Fig. 2. Effect of eluent pH on retention and enantioselectivity of PP and its ester derivatives. Solid lines, k'; dashed lines, α. Eluent: 20 mM phosphate buffer (pH 3.2, 3.9, 5.1, 6.0, 6.9) containing 17.5% of 2-propanol. R and S indicate the first-eluted enantiomer. ● = PP; ○ = Ac-PP; ▲ = Pro-PP; ■ = Bu-PP; □ = Val-PP.

atives are dependent on the organic modifier in addition to the eluent pH.

As PP, Ac-PP and Pro-PP were not retained with acidic eluents and Bu-PP and Val-PP were not eluted with neutral eluents, by changing the organic modifier content the enantiomeric elution order was investigated as described below. Tables I–IV show the effects of eluent pH and type and content of organic modifier on the enantioselectivity and enantiomeric elution order of PP and its ester derivatives when the organic modifier content was altered. Although a decrease or almost no change in enantioselectivity for PP and its ester derivatives was observed with increase in organic modifier content, the enantiomeric elution order was not affected by a change in the organic modifier content studied, except that both enantiomers were co-eluted. When an eluent of pH <5.1 was used, PP was eluted with the enantiomeric elution order of S followed by R (S/R) with all organic modifiers. Reversal of the elution order occured with an eluent of pH 6.9 or pH $>$ 6.0. The enantiomeric elution order of Ac-PP was R/S except when the eluent of pH >6.0 was used with addition of methanol. When acetonitrile was used as an organic modifier, Pro-PP was eluted with the enantiomeric elution order S/R for all eluent pH values. When an alkanol was used as the organic modifier, the elution order of Pro-PP was R/S with an eluent of acidic pH and S/R with the eluent of neutral pH. When ethanol, methanol or acetonitrile was used as the organic modifier, the enantiomeric elution order of Bu-PP was R/S with an eluent of acidic pH and S/R with an eluent of neutral pH. When 2-propanol was used, the enantiomeric elution order of Bu-PP was R/S for all eluent pH values. The enantiomeric elution order of Val-PP, depending on the eluent pH and organic modifier used, was complicated. When acetonitrile was used as the organic modifier, the enantiomeric elution order was R/S over the range of eluent pH studied, whereas with methanol the elution order was S/R. When 2-propanol was used, the elution order was R/S for eluent pH <5.1 and S/R for pH >6.0. With ethanol as the organic modifier the elution order was S/R except for the eluent of pH 3.9.

The above results indicate that chiral recognition or binding properties may be altered by a change in eluent pH and/or addition of organic solvents. Reversal of the enantiomeric elution order suggests that there may be more than one binding site on the OVM-bonded column, and/or that at least two chiral recognition mechanisms may operate on the OVM-bonded column with regard to PP and its es-

TABLE I

ENANTIOMERIC ELUTION ORDER OF PP AND ITS ESTER DERIVATIVES ON AN OVM COLUMN WITH 2-PROPANOL AS ORGANIC MODIFIER[a]

pH[b]	Parameter	PP	AC-PP	Pro-PP	Bu-PP	Val-PP
3.2	k'_1 (modifier, %)[c]	1.35 (0.5)	1.20 (2.5)	3.75 (2.5)	2.27 (8)	7.76 (8)
	α[d]	1.00	1.33	1.33	1.53	1.09
	Elution order	–	*R/S*	*R/S*	*R/S*	*R/S*
3.9	k'_1 (modifier, %)	3.10 (2.5)	3.19 (5)	10.8 (5)	6.92 (10)	33.5 (10)
	α	1.14	1.26	1.25	1.55	1.16
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*R/S*
5.1	k'_1 (modifier, %)	17.4 (5)	12.0 (7.5)	10.5 (15)	9.02 (20)	24.4 (20)
	α	1.08	1.34	1.10	1.38	1.20
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*R/S*
6.0	k'_1 (modifier, %)	49.7 (7.5)	5.95 (20)	41.1 (15)	35.0 (20)	33.9 (25)
	α	1.04	1.15	1.00	1.28	1.13
	Elution order	*S/R*	*R/S*	–	*R/S*	*S/R*
6.9	k'_1 (modifier, %)	11.5 (25)	8.64 (25)	15.9 (25)	38.5 (25)	22.4 (30)
	α	1.06	1.11	1.06	1.05	1.31
	Elution order	*R/S*	*R/S*	*S/R*	*R/S*	*S/R*

[a] The eluent used was a mixture of 20 m*M* phosphate buffer and various concentrations of 2-propanol.
[b] Eluent pH before addition of organic modifier.
[c] The k'_1 value indicates the capacity factor of the first-eluted peak and the percentage of organic modifier used is given in parentheses.
[d] Separation factor of the enantiomers.

TABLE II

ENANTIOMERIC ELUTION ORDER OF PP AND ITS ESTER DERIVATIVES ON AN OVM COLUMN WITH ACETONITRILE AS ORGANIC MODIFIER[a]

pH[b]	Parameter	PP	AC-PP	Pro-PP	Bu-PP	Val-PP
3.2	k'_1 (modifier, %)[c]	2.53 (0.5)	4.65 (2.5)	14.5 (2.5)	23.6 (5)	10.2 (10)
	α[d]	1.00	1.00	1.12	1.11	1.58
	Elution order	–	–	*S/R*	*R/S*	*R/S*
3.9	k'_1 (modifier, %)	14.5 (2.5)	8.96 (7)	6.00 (10)	24.9 (10)	9.76 (15)
	α	1.06	1.04	1.16	1.17	1.93
	Elution order	*S/R*	*R/S*	*S/R*	*R/S*	*R/S*
5.1	k'_1 (modifier, %)	13.5 (10)	5.97 (15)	3.65 (20)	8.87 (20)	16.6 (20)
	α	1.00	1.21	1.11	1.20	2.17
	Elution order	–	*R/S*	*S/R*	*R/S*	*R/S*
6.0	k'_1 (modifier, %)	6.31 (20)	30.7 (15)	16.6 (20)	14.1 (25)	8.97 (30)
	α	1.10	1.21	1.20	1.17	2.13
	Elution order	*R/S*	*R/S*	*S/R*	*R/S*	*R/S*
6.9	k'_1 (modifier, %)	21.3 (20)	4.74 (30)	7.14 (30)	14.1 (30)	23.9 (30)
	α	1.21	1.05	1.23	1.11	1.46
	Elution order	*R/S*	*R/S*	*S/R*	*S/R*	*R/S*

[a] The eluent used was a mixture of 20 m*M* phosphate buffer and various concentrations of acetonitrile.
[b–d] See footnotes to Table I.

TABLE III

ENANTIOMERIC ELUTION ORDER OF PP AND ITS ESTER DERIVATIVES ON AN OVM COLUMN WITH ETHANOL AS ORGANIC MODIFIER[a]

pH[b]	Parameter	PP	AC-PP	Pro-PP	Bu-PP	Val-PP
3.2	k'_1 (modifier, %)[c]	1.12 (1)	2.50 (1)	7.69 (1)	4.80 (10)	55.3 (5)
	α[d]	1.20	1.45	1.61	1.28	1.07
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*S/R*
3.9	k'_1 (modifier, %)	2.07 (10)	4.21 (10)	14.8 (10)	8.94 (15)	91.3 (10)
	α	1.19	1.22	1.13	1.26	1.08
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*R/S*
5.1	k'_1 (modifier, %)	7.35 (15)	11.7 (15)	37.5 (15)	34.0 (20)	15.1 (30)
	α	1.12	1.19	1.00	1.17	1.18
	Elution order	*S/R*	*R/S*	–	*R/S*	*S/R*
6.0	k'_1 (modifier, %)	16.7 (20)	21.9 (20)	9.55 (30)	22.5 (30)	43.7 (30)
	α	1.03	1.12	1.13	1.14	1.37
	Elution order	*S/R*	*R/S*	*S/R*	*S/R*	*S/R*
6.9	k'_1 (modifier, %)	16.4 (30)	11.2 (35)	15.3 (35)	12.3 (40)	17.9 (40)
	α	1.06	1.07	1.15	1.19	1.46
	Elution order	*R/S*	*R/S*	*S/R*	*S/R*	*S/R*

[a] The eluent used was a mixture of 20 m*M* phosphate buffer and various concentrations of ethanol.
[b–d] See footnotes to Table I.

TABLE IV

ENANTIOMERIC ELUTION ORDER OF PP AND ITS ESTER DERIVATIVES ON AN OVM COLUMN WITH METHANOL AS ORGANIC MODIFIER[a]

pH[b]	Parameter	PP	AC-PP	Pro-PP	Bu-PP	Val-PP
3.2	k'_1 (modifier, %)[c]	1.43 (5)	3.57 (5)	3.29 (15)	11.9 (15)	7.14 (25)
	α[d]	1.14	1.38	1.30	1.38	1.16
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*S/R*
3.9	k'_1 (modifier, %)	8.47 (10)	23.6 (10)	3.41 (25)	7.84 (30)	20.3 (30)
	α	1.15	1.26	1.14	1.22	1.23
	Elution order	*S/R*	*R/S*	*R/S*	*R/S*	*S/R*
5.1	k'_1 (modifier, %)	15.6 (20)	17.7 (25)	7.79 (40)	18.5 (40)	34.5 (40)
	α	1.08	1.07	1.18	1.20	1.65
	Elution order	*S/R*	*R/S*	*S/R*	*S/R*	*S/R*
6.0	k'_1 (modifier, %)	13.2 (40)	17.5 (40)	31.0 (40)	20.1 (50)	
	α	1.08	1.10	1.36	1.65	
	Elution order	*R/S*	*S/R*	*S/R*	*S/R*	
6.9	k'_1 (modifier, %)	13.5 (50)	12.1 (50)	18.8 (50)		
	α	1.16	1.17	1.50		
	Elution order	*R/S*	*S/R*	*S/R*		

[a] The eluent used was a mixture of 20 m*M* phosphate buffer and various concentrations of methanol.
[b–d] See footnotes to Table I.

ter derivatives. Also, a conformational change of the OVM bonded structure might be caused by a change in eluent pH and/or addition of organic modifier [20,12]. Taking into account the pK_a value of PP (9.45) [21], PP and its ester derivatives are protonated over the range of eluent pH studied. At eluent pH 5–7, where reversal of the enantiomeric elution order occurs, OVM is negatively charged because of the isoelectric point of OVM (3.8–4.3). Also, the organic modifier might compete with solutes at chiral recognition or binding site(s). Hence electrostatic interactions and the type of organic modifier might play an important role in the chiral recognition of PP and its ester derivatives on an OVM-bonded column.

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