Biochimica et Biophysica Acta, 611 (1980) 314—322

BBA 68885

'INVERSE' SUBSTRATES FOR BUTYRYLCHOLINESTERASE

MASAYUKI NOZAWA, KAZUTAKA TANIZAWA and YUICHI KANAOKA

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060 (Japan)

(Received July 26th, 1979)

Key words: Butyrylcholinesterase; Inverse substrate; Choline ester; (Kinetics)

Summary

1. 'Inverse'-type substrates for butyrylcholinesterase (acylcholine acyl-hydrolase, EC 3.1.1.8), i.e., *p*- and *o*-nitrophenyl esters of (3-carboxypropyl)-trimethylammonium iodide, (4-carboxybutyl)trimethylammonium iodide and (5-carboxypentyl)trimethylammonium iodide were prepared, and their kinetic parameters for butyrylcholinesterase-catalyzed hydrolysis were determined.

2. The hydrolysis of these 'inverse'-type substrates were found to proceed through specific binding with the enzyme and efficient production of acyl enzyme intermediates, a pathway essentially identical with that followed by choline esters, normal type substrates.

Introduction

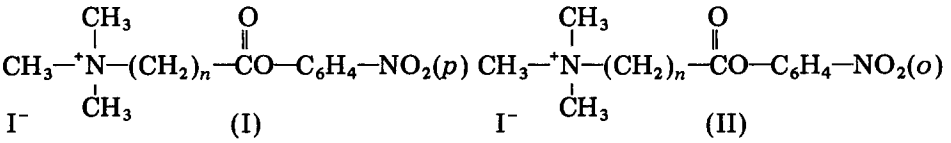
It is a well-definded fact that the active site of butyrylcholinesterase (acylcholine acyl-hydrolase, EC 3.1.1.8) is composed of two subsites, an esteratic site and an anionic site. The specificity of butyrylcholinesterase exhibited for choline esters [1—5] is ascribed to coulombic and hydrophobic attractions between the binding site of the enzyme and the quaternary ammonium head of the esters.

Recently Tanizawa et al. [6] have proposed a new concept of 'inverse substrates' for trypsin, certain ester substrates in which the specific group for the enzyme, a cationic center, is included not in their carbonyl portion as in the normal substrates, but in their leaving group. Based on the application of this entirely new type of substrates, several reporters including fluorogenic, chromogenic and spin-labeling groups have been specifically introduced into the active site of trypsin.

It seems possible that this 'inverse' method may be extensively applied also to other enzyme systems than trypsin and the related proteases. To test this

possibility further, in the present work, esters (I and II) of (trimethylammonio)alkanoic acid were examined whether they are substrates for butyrylcholinesterase or not. Compounds I and II possess the site-specific trimethylammonium group in their acyl portion, and therefore considered to be an 'inverse'-type compared with the normal substrates (III), choline esters.

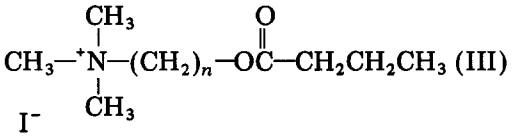
inverse type



(I) (II)

a, $n = 3$; b, $n = 4$; c, $n = 5$

normal type



(III)

a, $n = 2$; b, $n = 3$; c, $n = 4$

Materials and Methods

Enzymes

Horse serum butyrylcholinesterase (acylcholine acyl-hydrolase, EC 3.1.1.8) was purchased from Worthington Biochemical Corp. (lot, ECHP) Freehold, NJ, U.S.A. Its specific activity was experimentally determined (temperature, 25°C; medium, 0.1 M KCl, 5 mM $MgCl_2$, pH 7.4) to be $1.7 \cdot 10^{-5}$ mol butyrylcholine iodide hydrolyzed/min per mg protein. Acetylcholinesterase was also purchased from Worthington Biochemical Corp. (lot, CHE). Its specific activity was determined (temperature, 25°C; medium, 0.05 M phosphate buffer, 5 mM $MgCl_2$, pH 7.4) to be $8.68 \cdot 10^{-4}$ mol acetylthiocholine hydrolyzed/min per mg protein.

Synthesis of substrates and inhibitors

4-Aminobutyric acid (5.15 g; 50 mmol) was heated with CH_3I (42.6 g 0.3 mol) and K_2CO_3 (18 g; 0.15 mol) in 100 ml CH_3OH at 40°C with stirring for 72 h. KI was filtered and washed with acetone. After the solvent was evaporated in vacuo the resulting residue was recrystallized from acetone to give 11.4 g of colorless prisms of (3-carboxypropyl)trimethylammonium iodide methyl ester, m.p. 144—145°C (79%). Analysis (%): calculated for $C_8H_{18}NO_2I$. C, 33.46; H, 6.32; N, 4.88; I, 44.20; found: C, 33.29; H, 6.44; N, 4.62, I, 44.00. The ester (2.87 g; 10 mmol) was heated with KOH (1.12 g; 20 mmol) in CH_3OH/H_2O (1 : 5, v/v) 70°C for 3 h. The reaction mixture was cooled in an ice-water bath and neutralized with 10% HCl. The residue obtained after evaporation of the solvent was extracted with ethanol. Crystals obtained by evaporation of the ethanol solution in vacuo were recrystallized from acetonitrile to give 2.02 g of colorless prisms of (3-carboxypropyl)trimethyl-

ammonium iodide (IV-a), m.p. 167—168°C (decomp.) (74%). Analysis (%): calculated for $C_7H_{16}NO_2I$: C, 30.78, H, 5.91; N, 5.13; I, 46.67; found: C, 30.83; H, 5.97; N, 5.15; I, 46.47.

(3-Carboxypropyl)trimethylammonium iodide p-nitrophenyl ester (I-a). Dicyclohexylcarbodiimide (1.03 g; 5 mmol) was added to a solution of IV-a (1.11 g; 5 mmol) and *p*-nitrophenol (0.7 g; 5 mmol) in dimethylformamide (5 ml) and acetonitrile (25 ml), and the mixture was stirred for 3 h at room temperature. Precipitated dicyclohexylurea was filtered and washed with acetonitrile. The filtrate was concentrated in vacuo and the residue was recrystallized from acetone to give 1.14 g (72%) of colorless leaflets of (3-carboxypropyl)trimethylammonium iodide *p*-nitrophenyl ester, m.p. 167—167.5°C (decomp.). Analysis (%): calculated for $C_{13}H_{19}N_2O_4I$: C, 39.61; H, 4.85; N, 7.11; I, 32.19; found: C, 39.61; H, 4.89; N, 7.08; I, 32.39.

(3-Carboxypropyl)trimethylammonium iodide o-nitrophenyl ester (II-a). The compound was prepared from IV-a and *o*-nitrophenol in a similar manner. Recrystallization from acetone gave colorless prisms, m.p. 148—149°C (decomp.) (72%). Analysis (%): calculated for $C_{13}H_{19}N_2O_4I$. C, 39.61, H, 4.86; N, 7.11; I, 32.19; found: C, 39.76; H, 4.98; N, 6.84; I, 32.05.

(4-Carboxylbutyl)trimethylammonium iodide methyl ester was prepared from 5-amino-*n*-valeric acid hydrochloride in a similar manner as above. Recrystallization from acetone gave colorless needles, m.p., 136—137°C (81%). Analysis (%): calculated for $C_9H_{20}NO_2I$: C, 35.89; H, 6.69; N, 4.65; I, 42.14; found: C, 35.76; H, 6.83; N, 4.33; I, 42.55. (4-Carboxybutyl)trimethylammonium iodide (IV-b) was obtained after treatment of methyl ester with KOH. Recrystallization from acetone gave colorless needles, m.p. 156—158°C (decomp.) (74%). Analysis (%): calculated for $C_8H_{18}NO_2I$: C, 33.46; H, 6.32; N, 4.88; I, 44.20; found: C, 33.39; H, 6.36; N, 4.83; I, 44.14.

(4-Carboxybutyl)trimethylammonium iodide p-nitrophenyl ester (I-b). The compound was prepared from IV-b and *p*-nitrophenol in a similar manner. Recrystallization from acetone gave colorless needles, m.p. 144.5—146°C (decomp.) (79%). Analysis (%): calculated for $C_{14}H_{21}N_2O_4I$: C, 41.19; H, 5.19; N, 6.68; I, 31.09; found: C, 41.30; H, 5.22; N, 6.76; I, 31.35.

(4-Carboxybutyl)trimethylammonium iodide o-nitrophenyl ester (II-b). The compound was prepared from IV-b and *o*-nitrophenol in a similar manner. Recrystallization from acetone/ether mixture gave colorless needles, m.p. 182—183°C (decomp.) (66%). Analysis (%): calculated for $C_{14}H_{21}N_2O_4$: C, 41.19; H, 5.19; N. 6.68; I, 31.09; found: C, 41.42; H, 5.20; N, 6.76; I, 31.33.

(5-Carboxypentyl)trimethylammonium iodide methyl ester was similarly prepared from 6-amino-*n*-caproic acid. Recrystallization from acetone gave colorless prisms, m.p. 123.5—124.5°C (decomp.) (87%). Analysis (%): calculated for $C_{10}H_{22}NO_2I$: C, 38.11; H, 7.04; N, 4.44; I, 40.26; found: C, 37.99; H, 7.07; N, 4.48; I, 40.23. (5-Carboxypentyl)trimethylammonium iodide (IV-c) was prepared from the above ester in a similar manner. Recrystallization from acetone gave colorless prisms, m.p. 140—141°C (81%). Analysis (%): calculated for $C_9H_{20}NO_2I$: C, 35.89; H, 6.69; N, 4.65; I, 42.14; found: C, 35.69; H, 6.72; N, 4.47; I, 42.15.

(5-Carboxypentyl)trimethylammonium iodide p-nitrophenyl ester (I-c). The compound was prepared from IV-c and *p*-nitrophenol in a similar manner.

Recrystallization from ethanol/ether mixture gave pale-yellow needles, m.p. 111—112°C (decomp.) (77%). Analysis (%): calculated for $C_{15}H_{23}N_2O_4I$: C, 42.67; H, 5.49; N, 6.63; I, 30.05; found: C, 42.91; H, 5.50; N, 6.52; I, 30.14.

(5-Carboxypentyl)trimethylammonium iodide o-nitrophenyl ester (II-c). The compound was prepared from IV-c and *o*-nitrophenol in a similar manner. Recrystallization from acetone/ether mixture gave pale-yellow needles, m.p. 168.5—169.5°C (decomp.) (68%). Analysis (%): calculated for $C_{15}H_{23}N_2O_4I$: C, 42.67; H, 5.49; N, 6.63; I, 30.05; found: C. 42.80; H, 5.60; N, 6.73; I, 30.36.

N-Methylacridinium iodide. Acridine 3.2 g (20 mmol) was reacted with 12 ml of methyl iodide in 18 ml of acetone under stirring at room temperature for 2 days. Resulted precipitate was recrystallized from methanol to give 3.4 g (53%) of deep-red needles, m.p. 226—226.5°C (decomp.). Analysis (%): calculated for $C_{14}H_{12}NI$: C, 52.36; H, 3.77; N, 4.36; I, 39.51; found: C, 52.21; H, 3.68; N, 4.39; I, 39.47.

Neostigmine iodide (m-hydroxyphenyl)trimethylammonium iodide dimethylcarbamate). Dimethylcarbamyl chloride (1.1 g; 10 mmol) was added dropwise to a solution of *m*-dimethylaminophenol (1.52 g; 10 mmol) and triethylamine (1.01 g; 10 mmol) in 20 ml tetrahydrofuran at 0°C with stirring. The reaction mixture was kept at room temperature for 6 h. Then methyl iodide (5 ml) was added to the reaction mixture, and stirred for 3 days at room temperature. Crystals which separated were filtered and recrystallized from acetonitrile/ether mixture to give 3.25 g (83%) of colorless leaflets, m.p. 158—158.5°C (decomp.). Analysis (%): calculated for $C_{12}H_{19}N_2O_2I$: C, 41.14, H, 5.47; N, 8.00; I, 40.78; found: C, 40.78; H, 5.43; N, 7.86; I, 36.80.

Other chemicals

Butyrylcholine iodide and acetylthiocholine iodide were purchased from Daiichi Chemical, and diisopropylphosphorofluoridate was purchased from Sigma Chemical.

All other chemicals used were products of Koso Chemical and Wako Pure Chemical, analytical grade.

Kinetic parameter measurements

The determination of K_m and V values for the nitrophenyl esters were carried out by measuring *p*- and *o*-nitrophenol liberation at 400 nm and at 414 nm, respectively, using a Simazu UV 200 double-beam spectrophotometer; pH 7.4, 0.05 M phosphate buffer containing 5 mM $MgCl_2$, at 25°C. The substrate concentrations were in the range of 0.25—0.008 mM and the enzyme concentrations were 2 μg/ml for butyrylcholinesterase and 23 μg/ml for acetylcholinesterase.

K_m and V values for *p*-nitrobenzoylcholine (V) were determined by measurement of absorbance change at 290 nm in the similar manner. The substrate concentrations were in the range of 0.25—0.025 mM.

Hydrolysis of butyryl esters (III) and *p*-nitrophenyl butyrate (VI) was measured potentiometrically using a Radiometer titrater TTT-2 and titrigraph type SBR-3/AUB-12: pH 7.4, 0.1 M KCl containing 5 mM $MgCl_2$, at 25°C. The substrate concentrations were in the range of 0.15—2.8 mM.

The determination of K_m and V values for acetylthiocholine was determined by measurement of absorbance change at 410 nm in the presence of 5,5'-dithiobis(2-nitrobenzoic acid) (2 mM), pH 7.4, 0.05 M phosphate buffer containing 5 mM $MgCl_2$, at 25°C. The substrate concentration was $5.69 \cdot 10^{-4}$—$5.70 \cdot 10^{-5}$ M [7].

Determination of K_i values

The K_i values for *N*-methylacridinium iodide, trimethylamine hydrochloride and choline in the hydrolysis of butyrylcholinesterase-catalyzed reaction were determined using *p*-nitrobenzoylcholine and I-a as a substrate: pH 7.4, 0.05 M phosphate buffer containing 5 mM $MgCl_2$ at 25°C.

Irreversible inhibition of butyrylcholinesterase

Irreversible inhibition of butyrylcholinesterase by diisopropylphosphorofluoridate and neostigmine iodide were carried out as follows. To 4 ml of an enzyme solution (0.03 mg protein in 5 mM $MgCl_2$, 0.05 M phosphate buffer, pH 7.4), 0.05 ml of 10^{-6}—10^{-7} M diisopropylphosphorofluoridate or 10^{-6} M neostigmine in acetonitrile was added, and the mixture was incubated at 25°C for 5—10 min.

Results

Kinetic parameters for butyrylcholinesterase-catalyzed hydrolysis of the inverse-type substrates

The kinetic parameters for the inverse-type substrates (I and II) were listed in Table I. All of these esters were hydrolyzed by horse serum butyrylcholinesterase, and the hydrolysis obeyed the Michaelis-Menten kinetics as shown in Fig. 1. For comparison, the parameters for normal-type substrates (III), *p*-nitrobenzoylcholine (V) and *p*-nitrophenyl butyrate (VI) were listed in Table II.

It must be noted that, as seen in Table II, butyrylcholinesterase-catalyzed hydrolysis of the normal-type substrates, even when the methylene length between the quarternary nitrogen and the carbonyl carbon is longer than that

TABLE I

KINETIC PARAMETERS FOR BUTYRYLCHOLINESTERASE-CATALYZED HYDROLYSIS OF 'INVERSE' SUBSTRATES

Enzyme concentration: 5.7 μg/ml. Substrate concentration: $2.5 \cdot 10^{-4}$—$7.9 \cdot 10^{-6}$ M. pH 7.4, at 25°C, 0.05 M phosphate buffer containing 5 mM $MgCl_2$. Values (K_m and V) were computed by the method of Wilkinson [8].

Substrates	K_m (μM)	V (μmol/min per mg protein)	k_{spont} (min^{-1})
I-a	20.7 ± 1.2	3.93 ± 0.07	$4.19 \pm 0.33 \cdot 10^{-3}$
II-a	21.4 ± 1.2	3.41 ± 0.06	$2.72 \pm 0.21 \cdot 10^{-3}$
I-b	40.4 ± 1.5	5.54 ± 0.09	$8.71 \pm 0.68 \cdot 10^{-4}$
II-b	13.4 ± 1.3	0.65 ± 0.02	$1.14 \pm 0.06 \cdot 10^{-3}$
I-c	10.7 ± 0.8	2.23 ± 0.05	$8.74 \pm 0.27 \cdot 10^{-4}$
II-c	8.8 ± 0.6	1.41 ± 0.02	$4.42 \pm 0.26 \cdot 10^{-4}$

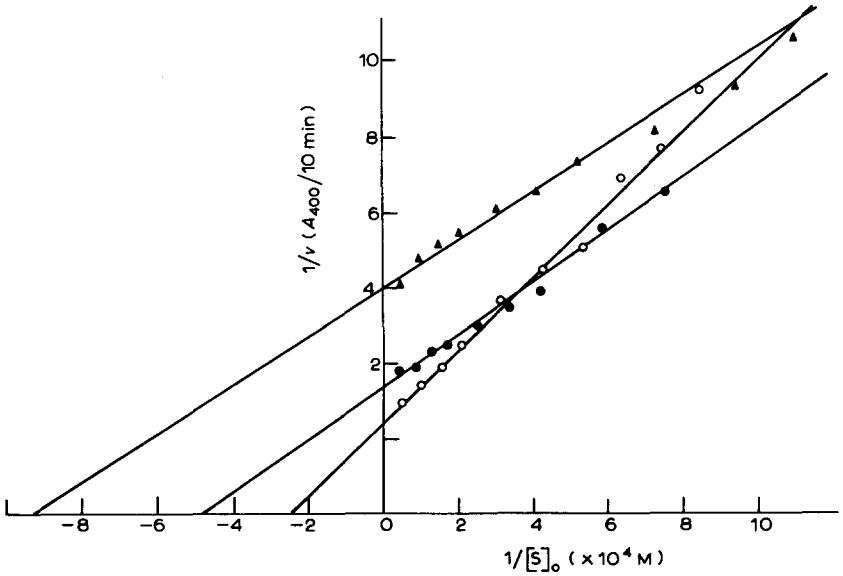


Fig. 1. Lineweaver-Burk plot of butyrylcholinesterase-catalyzed hydrolysis of 'inverse'-type substrate. •, I-a; ○, I-b; ▲, I-c; 0.05 M phosphate buffer containing 5 mM $MgCl_2$, pH 7.4, at 25°C.

of choline esters (n = 2 in III). However, in general, cholinesterases hydrolyze series of non-choline esters at a rate which decreases with the extent to which they deviate from the structure of choline esters [9,10]. Thus the hydrolytic rate, V, of the normal-type substrates (III) decreased as the increase of chain length between quarternary ammonium group and acyl residue. By contrast, the V, values for the inverse-type substrates (I and II), in which the chain length (n) ranges from 3 to 5, were relatively independent of the distance between the quarternary ammonium group and the carbonyl carbon. The values K_m for the inverse-type substrates, not so much different from each other, were about 10—20 times smaller than that for butyrylcholine and about ten times smaller than that for benzoylcholine [11,12]. When compared with the p-nitrophenyl butyrate (VI), in which a site-specific group is not involved,

TABLE II

KINETIC PARAMETERS FOR BUTYRYLCHOLINESTERASE-CATALYZED HYDROLYSIS OF NORMAL-TYPE SUBSTRATES

The parameters for III-a—c were obtained from measurements in pH-stat: 0.1 M KCl, 5 mM $MgCl_2$, pH 7.4, at 25°C. The parameters for V and VI were obtained by spectrophotometric measurements: 0.05 M phosphate buffer containing 5 mM $MgCl_2$, pH 7.4, at 25°C. Substrate concentration: $3.10 \cdot 10^{-3}$—$2.58 \cdot 10^{-4}$ M for IIIa, $2.43 \cdot 10^{-3}$—$1.74 \cdot 10^{-4}$ M for III-b, $2.81 \cdot 10^{-3}$—$2.81 \cdot 10^{-4}$ M for IIIc, $2.47 \cdot 10^{-4}$—$\cdot 2.47 \cdot 10^{-5}$ M for IV, and $1.83 \cdot 10^{-3}$—$1.51 \cdot 10^{-4}$ M for V. Enzyme concentration: 5.4—13.4 μg/ml.

Substrate	K_m (mM)	V (μmol/min per mg protein)
III-a	0.337 ± 0.004	18.05 ± 0.75
III-b	0.713 ± 0.042	3.56 ± 0.10
III-c	0.278 ± 0.027	0.23 ± 0.01
p-Nitrobenzoylcholine (V)	0.176 ± 0.012	12.71 ± 0.52
p-Nitrophenyl butyrate (VI)	1.14 ± 0.05	2.60 ± 0.23

TABLE III

KINETIC PARAMETERS FOR ACETYLCHOLINESTERASE-CATALYZED HYDROLYSIS OF 'INVERSE'-TYPE SUBSTRATES

pH 7.4, at 25°C, 0.05 M phosphate buffer containing 5 mM $MgCl_2$.

Substrate	K_m (M)	V (mol/min per mg protein)
I-a *	11.2 ± 0.9	0.196 ± 0.09
II-a **	6.3 ± 0.1	0.066 ± 0.03
Acetylthiocholine ***	7.18 ± 0.43	868 ± 15

* Enzyme: 69 μg/ml, substrate: $1.62 \cdot 10^{-4}$—$3.42 \cdot 10^{-5}$ M.
** Enzyme: 69 μg/ml, substrate: $1.18 \cdot 10^{-4}$—$3.63 \cdot 10^{-5}$ M.
*** Enzyme: 0.14 μg/ml, substrate: $5.69 \cdot 10^{-4}$—$5.70 \cdot 10^{-5}$ M.

the K_m values for the inverse-type substrates were about 50—200 times smaller [12,13].

The effects of 'inverse'-type substrates for acetylcholinesterase

Acetylcholinesterase-catalyzed hydrolysis of the 'inverse'-type substrates was very slow. Kinetic analysis at very high concentrations of acetylcholinesterase revealed that the rate was about 1/5000—1/1000 of that of acetylthiocholine as shown in Table III. These results are in agreement with previous studies on the requirements of active site of acetylcholinesterase [10,14]. Acetylcholinesterase does not hydrolyze choline esters with a large acyl group, such as benzoylcholine.

Inhibition of butyrylcholinesterase-catalyzed hydrolysis of 'inverse'-type substrates

Anionic site inhibitors such as trimethylamine hydrochloride, choline and *N*-methylacridine [3] competitively inhibited butyrylcholinesterase-catalyzed hydrolysis of the 'inverse'-type substrates. As compared in Table IV, the K_i values obtained from competition with the inverse-type substrates and with the normal-type substrates were about the same.

TABLE IV

INHIBITION CONSTANTS FOR ANIONIC SITE INHIBITORS MEASURED IN THE BUTYRYLCHOLINESTERASE-CATALYZED HYDROLYSIS OF I-a (INVERSE TYPE) AND *p*-NITROBENZOYLCHOLINE (NORMAL TYPE)

All assays were carried out in 0.05 M phosphate buffer containing 5 mM $MgCl_2$ and 1.6% acetonitrile. Substrate concentration range: $3.38 \cdot 10^{-4}$—$3.68 \cdot 10^{-5}$ M for I-a and $4.16 \cdot 10^{-4}$—$3.76 \cdot 10^{-5}$ M for *p*-nitrobenzoylcholine. Inhibitor concentration: $7.85 \cdot 10^{-8}$—$7.85 \cdot 10^{-7}$ M for *N*-methylacridine, $9.88 \cdot 10^{-4}$—$1.36 \cdot 10^{-2}$ M for trimethylamine hydrochloride and $1.13 \cdot 10^{-3}$—$1.2 \cdot 10^{-2}$ M for choline.

Inhibitor	Substrate	K_i (M)
N-Methylacridinium iodide	I-a	$1.56 \cdot 10^{-7}$
	p-nitrobenzoylcholine	$1.05 \cdot 10^{-7}$
Trimethylamine hydrochloride	I-a	$5.90 \cdot 10^{-3}$
	p-nitrobenzoylcholine	$6.63 \cdot 10^{-3}$
Choline	I-a	$2.13 \cdot 10^{-3}$
	p-nitrobenzoylcholine	$1.07 \cdot 10^{-3}$

Diisopropylphosphorofluoridate and neostigmine which irreversibly inhibits the esteratic site of butyrylcholinesterase [15] also prevented butyrylcholinesterase activity toward 'inverse'-type substrates.

Discussion

The kinetic parameters K_s and k_2 are indices for substrate specificity [6]. The former can provide information on the binding which is a characteristic of the enzymic process, while the latter reflects accessibility of the carbonyl function of the substrate molecule to the catalytic residue(s) of the enzyme in the ES complex. The efficient production of ES complex during the course of butyrylcholinesterase-catalyzed hydrolysis of the 'inverse' substrates is rather stronger than that of normal-type substrates (Tables I and II).

The same acyl enzyme intermediate (butyryl enzyme) is involved in the reaction process for the hydrolysis of the normal-type substrates (compounds III-a—c, Table II). Observed variation in the V values for the series of compounds probably arose from the nature of the reaction course that the rate-determining step is not associated with the deacylation process. Then the observed V value should be nearly equal to the acylation rate. Structural requirements of the enzyme catalytic residue(s) for the catalysis, therefore, is reflected in their V values. It is evident that the chain length between the nitrogen and the carbonyl carbon is significant in enzymic accessibility. For the 'inverse' substrates (I and II, a—c), comparison of the V values within the compounds (I and II) is less meaningful because each substrate affords a different acyl enzyme which will exhibit its inherent deacylation rate. However, comparison of the parameters of compound (I) with those of (II) in the respective series (a—c) is reasonable because they afford the identical acyl enzyme intermediate. It was shown that I-a and II-a gave comparable V values, though V values of I-b and II-b as well as those of I-c and II-c are different. These results indicate that only for the 'inverse' substrate, such as I-a and II-a, in which the nitrogen and the carbonyl carbon is linked by a three-methylene unit, the deacylation is rate determining, i.e., the acylation is relatively fast and efficient. In contrast, the 'inverse substrates' with a larger span are not suited favorably to the enzyme active site and result in decreasing of acylation rate over the deacylation rate because the different V values were observed in I-b and II-b as well as in I-c and II-c.

All these kinetic characteristics support the idea that I-a and II-a act as specific substrates of 'inverse' type for butyrylcholinesterase, and are subjected to the hydrolysis following a similar mechanism with that for the normal-type substrates as shown in Scheme I [15]. The normal substrates for butyrylcholin-

$$E + S \overset{K_s}{\rightleftharpoons} ES \underset{P_1}{\overset{k_2}{\rightleftharpoons}} EA \xrightarrow{k_3} E + P_2$$

Scheme I.

esterase have its specificity-determining group in its alcohol portions, in contrast to those for trypsin which have a specificity-determining group in its carbonyl portion. The finding of 'inverse' substrates not only for trypsin [6]

but also for butyrylcholinesterase, now extends this new concept to more general applicability.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan, the Yamada Science Foundation, and the Foundation for the Promotion of Research on Medicinal Resources.

References

1 Bergmann, F. (1958) Adv. Catal. 10, 130—164
2 Agustisson, K.-B. (1966) Biochim. Biophys. Acta 128, 351—361
3 Chan, L.M., Himel, C.M. and Main, A.R. (1974) Biochemistry 13, 86—90
4 Mayer, R.T. and Himel, C.M. (1972) Biochemistry 11, 2082—2090
5 Eriksson, H. and Augustisson, K.-B. (1979) Biochim. Biophys. Acta 567, 161—173
6 Tanizawa, K., Kasaba, Y. and Kanaoka, Y. (1977) J. Am. Chem. Soc. 99, 4485—4488
7 Ellman, G.L. (1959) Arch. Biochem. Biophys. 82, 70—77
8 Wilkinson, G.N. (1961) Biochem. J. 80, 324—332
9 Sturge, L.M. and Whittaker, V.P. (1950) Biochem. J. 47, 518—525
10 Mounter, L.A. and Whittaker, V.P. (1950) Biochem. J. 47, 525—530
11 Beckett, A.H., Mitchard, M. and Clitherow, J.W. (1968) Biochem. Pharmacol. 17, 1601—1607
12 Lee, J.C. and Harpst, J.A. (1971) Arch. Biochem. Biophys. 145, 55—63
13 Main, A.R., Miles, K.E. and Braid, P.E. (1961) Biochem. J. 78, 769—776
14 Whittaker, V.P. (1951) Physiol. Rev. 31, 312—343
15 Oosterbaan, R.A. and Jansz, H.S. (1965) in Comprehensive Biochemistry (Florkins, M. and Stoltz, E.M., eds.), Vol. 16, pp. 1—47, Elsevier, Amsterdam