Influence of Blood Proteins on Biomedical Analysis. VII.[1] Electrophoretic Analysis of the Interaction of Pseudocholinesterase with Fatty Acid and/or Human Serum Albumin

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In order to characterize the fatty acid (FA)-induced inhibition of pseudocholinesterase (pchE), the interaction of pchE with FA (*n*-capric acid and *n*-lauric acid) and/or human serum albumin (HSA) was studied by electrophoreses on a cellulose acetate membrane and a polyacrylamide gel thin-layer. It was demonstrated that pchE binds with n-[1-^{14}C]lauric acid, suggesting that the FA-induced inhibition of pchE activity is based on the interaction of the enzyme with FA. The binding of pchE with n-[1-^{14}C]lauric acid was depressed by HSA. Consequently, the present results suggest that the reduction of FA-induced inhibition of pchE activity by HSA is associated closely with the depression of pchE–FA binding by HSA. Moreover, no selectivity in the *n*-capric acid-induced inhibition of pchE isozymes was found in the present study, *i.e.*, all isozymes (C_1–C_4) of pchE were equally inhibited by *n*-capric acid.

Keywords——fatty acid-induced pseudocholinesterase inhibition; cellulose acetate membrane electrophoresis; polyacrylamide gel thin-layer electrophoresis; *n*-capric acid; *n*-lauric acid; human serum albumin; pseudocholinesterase

The role of acetylcholinesterase (EC 3.1.1.7) in the nervous system is established, but the role of pseudocholinesterase (pchE, EC 3.1.1.8) in serum is not well understood. It has been proposed that pchE is associated with fatty acid metabolism,[2] regulation of choline level in serum[3] and lipoprotein metabolism.[4] Although an inhibitory effect of fatty acid (FA) on esterases such as pancreatic lipase has been reported,[5] the FA-induced inhibition of pchE has only been described in our previous report.[6] In that report, we showed that the pchE was inhibited by some FA's containing 10—12 carbon atoms, and the FA-inhibited esterase activity was restored by the addition of human serum albumin (HSA) to the reaction mixture. Moreover, it was suggested that the mechanism of the FA-induced inhibition of pchE was different from that of eserine- or L-histidine-induced inhibition of pchE.[6]

In the present study, in order to characterize the FA-induced inhibition of pchE, the interaction of n-[1-^{14}C]lauric acid with pchE and/or HSA as well as the selectivity of *n*-capric acid-induced inhibition of pchE isozymes was examined by electrophoreses on a cellulose acetate membrane and a polyacryamide gel thin-layer.

Materials and Methods

Chemicals——Sodium *n*-caprate and sodium *n*-laurate used for experiments were products of Tokyo Kasei Kogyo Co., Ltd., Japan. Human serum albumin (HSA, fatty acid free) and pseudocholinesterase (EC 3.1.1.8, type IV, 12.7 U/mg, from horse serum) were purchased from Sigma Chemical Co., U.S.A. Commercial pchE was purified by gel filtration on a Sephadex G-150 column (1.5 × 90 cm; solvent, 0.02 M phosphate buffer, pH 7.6) before use.

Pooled human serum (SDR-97128; pchE activity, 1500 U/1) prepared in our laboratory was used as material containing pchE. n-[1-^{14}C]Lauric acid (^{14}C-LA, 32 mCi/mmol) was purchased from the Radiochemical Centre, England. NCS tissue solubilizer was purchased from Amersham Co., U.S.A. POPOP [1,4-bis-2-(5-phenyloxazoyl)benzene] and DPO (2,5-diphenyloxazole) were products of Dojindo Laboratories Ltd., Japan. All other chemicals were products of Wako Pure Chemical Industries Ltd., Japan.

Cellulose Acetate Membrane Electrophoresis——Electrophoresis was carried out on a cellulose acetate membrane (Separax, Joko Sangyo Ltd., Japan) in 0.06 M barbital buffer (pH 8.6, $\mu = 0.06$) at 0.8 mA/cm for 40 min at 0—10 °C.[7] The banding pattern of proteins on the membrane was obtained by staining with an aqueous trichloroacetic acid solution (6%) containing 0.8% Ponceau 3R dye. The esterase activity on the membrane was determined by the following procedure: the membrane was cut into 0.5 cm strips, and each strip was soaked in 1 ml of the color reagent of a cholinesterase assay kit (enzymatic method, "cholinesterase B-test," Wako Pure Chemical Industries Ltd., Japan) for 20 min at 37 °C. The esterase activity in the surpernatant was determined colorimetrically.[8] For the determination of ^{14}C-LA content, each strip of the membrane was soaked in 10 ml of scintillation cocktail (0.1 g of POPOP, 4 g of DPO and 100 ml of NCS tissue solubilizer, dissolved in 1 1 of toluene) at room temperature (25—26 °C). After 1 h, the radioactivity was measured using a liquid scintillation counter (Packard 3385 Tri-Carb scintillation spectrophotometer, Packard Instruments, U.S.A.).

Polyacrylamide Gel Thin-Layer Electrophoresis——Electrophoresis was carried out on a polyacrylamide gel thin-layer (P.A.-gel plate, 2 mm, Eiken Kizai Co., Japan) in 0.3 M borate buffer (pH 8.2) at 3 mA/cm for 70 min at 0—10 °C. The detection of esterase activity was performed by Ogita's method using α-naphthylacetic acid as the substrate for coloration.[9] The intensity of staining was measured at 525 nm using a densitometer (Flur-vis autoscanner, Helena Laboratories, U.S.A.).

Preparations of n-Capric Acid and n-Lauric Acid Solutions——n-Capric acid (sodium n-caprate) or n-lauric acid (sodium n-laurate) was dissolved at various concentrations in 0.02 M phosphate buffer (pH 7.6); if necessary, it was heated at 37 °C. For the experiments, a suitable amount of ^{14}C-LA, HSA or pchE was added to the fatty acid solution.

Results and Discussion

Figure 1 shows electrophoretic patterns of pooled human serum (a), HSA (b), pchE with HSA (c) or with n-lauric acid (d) and pchE alone (e) on the cellulose acetate membrane. Two active bands of Ponceau 3R-stained pchE were found at positions corresponding to α_1 and α_2-β globulin. The electrophoretic mobilities of pchE bands were not changed in the presence of HSA or n-lauric acid. Chu et al. proposed that there are two pools of pchE activity in human serum; one is free pchE and the other is bound to β-lipoprotein.[10] The present result suggests that the esterase activity in α_2-β globulin fraction is due to β-lipoprotein-bound pchE.

The distribution of ^{14}C-LA in three Ponceau 3R-stained protein fractions (albumin, α_1 and α_2-β) was studied after electrophoresis on the cellulose acetate membrane (Table I). Over 96% of ^{14}C-LA was distributed in the albumin fraction, suggesting that ^{14}C-LA binds with HSA [samples 1, 2 and 3]. The distribution of ^{14}C-LA was not changed by addition of HSA in a different order (see caption in Table I). In the case of samples containing HSA, ^{14}C-LA was distributed in both the albumin and α_1 globulin fractions, but it was not found in the α_2-β

γ-globulin

α₂—β-globulin

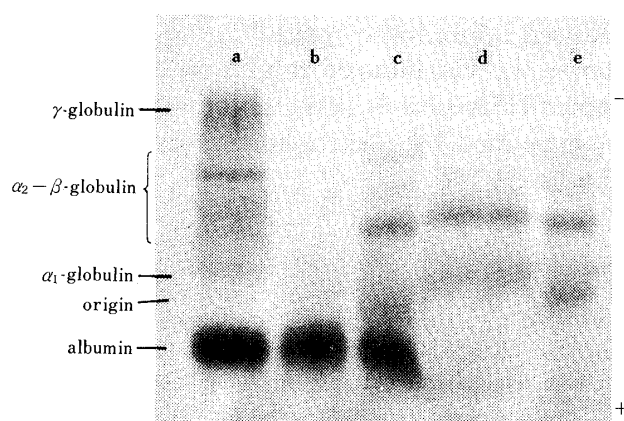α₁-globulin

origin

albumin

a   b   c   d   e

Fig. 1. Electrophoretic Banding Patterns of Serum Proteins and pchE on Cellulose Acetate Membrane

Each protein band was detected by staining with Ponceau 3R dye. (a), Pooled human serum; (b), HSA; (c), pchE and HSA; (d), pchE and n-lauric acid; (e), pchE alone. Samples of (b), (c), (d) and (e) for electrophoresis were prepared in 0.02 M phosphate buffer (pH 7.6). The final concentrations of HSA, pchE and n-lauric acid were 1 mM, 8 mg (100 U)/ml and 6 mM, respectively, and 2 μl of sample was applied to the membrane for electrophoresis.

TABLE I.   Distribution of Radioactive n-Lauric Acid (^{14}C-LA) on Electrophoresis

Sample[b]	Radioactivity, cpm (%)[a]		
	Albumin	Globulin	
		α_1[e]	α_2-β[e]
1[c] pchE + HSA, ^{14}C-LA	26298 (96.1)	1068 (3.9)	0 (0)
2[d] pchE, ^{14}C-LA + HSA	28855 (96.4)	1072 (3.6)	0 (0)
3 HSA + ^{14}C-LA	25546 (96.5)	933 (3.5)	0 (0)
4 pchE + ^{14}C-LA	1521 (5.6)	25271 (93.6)	201 (0.7)
5 ^{14}C-LA	0 (0)	29467 (100)	0 (0)

Samples for electrophoresis were prepared by dissolving HSA, pchE, n-lauric acid and ^{14}C-LA in 0.02 M phosphate buffer (pH 7.6), and 2 μl (33000 cpm) of the sample was subjected to electrophoresis.

a)   Each value is the mean of 4—5 experiments. The mean recovery of radioactivity was 84%.

b)   The final concentrations of pchE, HSA and ^{14}C-LA were each the same as in Fig. 1.

c)   Sample 1 was prepared by addition of pchE solution to a mixture of HSA and ^{14}C-LA solutions.

d)   Sample 2 was prepared by the addition of a mixture of pchE and ^{14}C-LA solutions to HSA solution.

e) α_1 and α_2-β globulin fractions are abbreviated as α_1 and α_2-β, respectively.
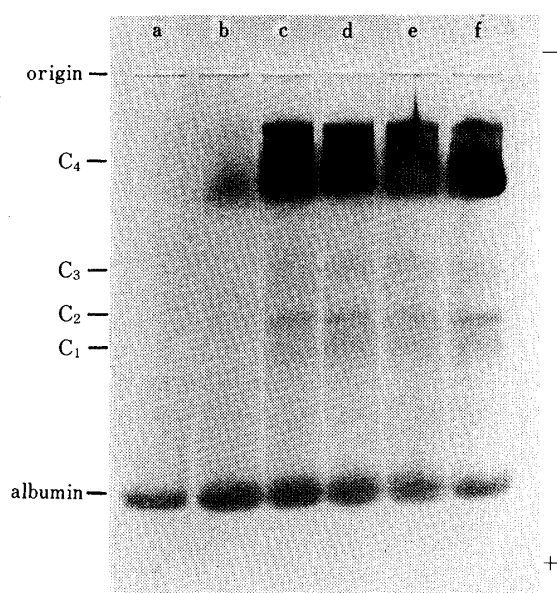

Fig. 2.   Zymograms of the Esterase Activity of pchE Isozymes on Polyacrylamide Gel Thin-Layer Electrophoresis and Inhibition by n-Capric Acid

Samples for electrophoresis were prepared by the addition of n-capric acid solution at various concentrations to the pooled serum. The reaction mixture was allowed to stand at room temperature (25—27 °C) for at least 10 min, then 6 μl of the sample was subjected to electrophoresis. Concentrations of n-capric acid in serum were: a), 100 mM; b), 50 mM; c), 25 mM; d), 12.5 mM; e), 6.25 mM; f), 0 mM (serum alone).
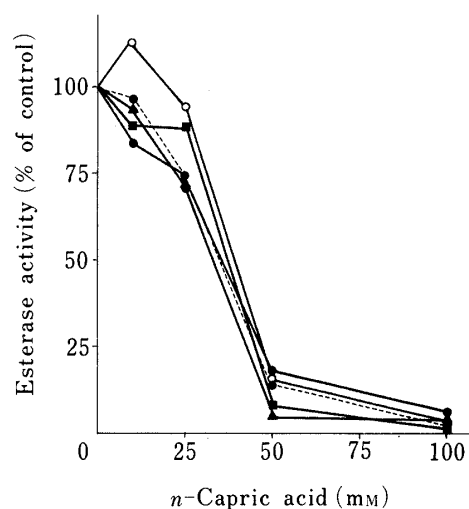

Fig. 3.   Inhibition Curves for pchE Isozymes by n-Capric Acid

Each point is the mean value of three separate experiments. C_1, —●—; C_2, —○—; C_3, —■—; C_4, —▲—; original pooled serum, ---●---. The relative esterase activity was derived densitometrically from the zymograms in Fig. 2. The esterase activity for the original pooled serum (total esterase activity) was determined by using the pchE assay kit, "cholinesterase B-test".

position. Even in sample 3, containing no pchE, radioactivity (933 cpm, 3.5%) was found in the α_1 globulin fraction, showing that radioactivity (1068 and 1072 cpm) in the α_1 globulin fractions in samples 1 and 2 is not due to pchE-bound ^{14}C-LA. On the other hand, in the case

of sample 4, the ^{14}C-LA contents in the albumin, α_1 and α_2-β globulin fractions were 5.6, 93.6 and 0.7%, respectively. ^{14}C-LA in the albumin fraction was probably due to the presence of contaminating albumin in the commercial sample of pchE. The radioactivity (201 cpm, 0.7%) in the α_2-β globulin fraction may be due to ^{14}C-LA-bound pchE, and this ^{14}C-LA binding with pchE seems to be inhibited by HSA. The present result suggests that the inhibition of pchE activity by FA is based on FA-pchE binding[11] and the inhibition of FA-pchE binding by HSA is closely associated with the reduction of FA-induced inhibition of esterase activity by HSA.

Isozyme analyses can be of significant value in differential diagnosis. Because of its clinical importance, the development of a convenient and selective determination technique for isozymes in serum is a field of intense investigative activity. If n-capric acid selectively inhibits the esterase activity of pchE isozymes, it may be useful for the selective determination of pchE isozymes in human serum. Zymograms of pchE isozymes were obtained and inhibitory effects of n-capric acid on the pchE isozymes were clearly demonstrated by polyacrylamide gel thin-layer electrophoresis (Fig. 2). The inhibitory effects of n-capric acid on the isozymes (C_1–C_4) are shown as percentages of the control in Fig. 3. Each inhibition curve showed a similar concentration (30—40 mM) for 50% inhibition of esterase activity. The inhibition curves for total pchE and its isozymes showed similar profiles. Thus, the inhibition by n-capric acid of the esterase activity of pchE isozymes was not specific.

References and Notes

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