International Journal of Pharmaceutics, 29 (1986) 137–146
Elsevier

IJP 00973

Degradation of gabexate mesilate catalyzed by human serum albumin

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(Received October 19th, 1984)
(Modified version received September 15th, 1985)
(Accepted October 31st, 1985)

Key words: gabexate mesilate – human serum albumin – degradation – stability of drug – kinetics – HPLC – stability in blood plasma

Summary

The degradation of gabexate mesilate (GM) was accelerated in the presence of human serum albumin (HSA), and was investigated based on Michaelis-Menten-type kinetics involving the formation of HSA–drug complex. The dissociation constant of the complex was 1.19×10^{-4} M and the degradation rate constant of the complex was 1530-fold larger than the spontaneous rate constant (2.23×10^{-6} s^{-1}) at pH 7.4 (25°C). Potential active sites appeared to be histidine residues since the pH profiles indicated the involvement of a group with a pK_a of about 6 and HSA modified with diethylpyrocarbonate (DEP) showed diminished activity. From the relationship between the number of histidine residues modified by DEP and the activity remaining in the partially modified HSA, only one histidine residue was involved in the degradation of GM. The lack of deuterium solvent isotope effect and the accumulation of acylated-HSA intermediate indicated that the enhanced rate of degradation is due to nucleophilic catalysis perhaps by the imidazole group. The degradation rate of GM was faster in human plasma than in HSA where the albumin concentration was adjusted to that in the plasma. The contribution of HSA mediated degradation to the overall degradation in human plasma was estimated about 40%. The results suggest that further attention should be paid to the non-specific esterase-like activity of HSA toward ester drugs especially with a good leaving group as far as drug disposition in plasma is concerned.

Introduction

Gabexate mesilate (*p*-hydroxybenzoic acid ethyl ester 6-guanidinohexanoate monomethanesulfonate, GM) has inhibitory effects on biological activities of plasma kallikrein, thrombin, plasmin and trypsin (Nakahara, 1983; Muramatsu and Fujii, 1972) and is used for the treatment of acute pancreatitis.

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GM is known to be hydrolyzed, yielding ε-guanidinocaproic acid (GCA) and ethyl *p*-hydroxybenzoate (EPB), and is most stable at about pH 3 (Shinkuma et al., 1979). GM is decomposed much more rapidly in plasma than in buffer solution at physiological pH. Consequently, GM is administered clinically by continuous i.v. infusion to maintain therapeutic blood levels (Miyamoto and Hirata, 1978). Ohno et al. (1981) have suggested that the rapid inactivation of GM in plasma is due to the action of plasma esterases.

Human serum albumin (HSA) or bovine serum albumin is known to have a non-specific esterase-

like activity for a variety of esters (Kurono et al., 1982), disulfiram (Agarwal et al., 1983), organophosphorus insecticides (Sultatos et al., 1984) and eicosanoids (Fitzpatrick et al., 1984).

The purpose of this study was to describe kinetics of the degradation of GM in the presence of HSA, exploring the potential active site involved and to elucidate the role of albumin in the degradation of the drug in human plasma.

Materials and Methods

Materials

Human serum albumin (HSA) (Fraction V, lot no. 12F-0051) was obtained from Sigma Chemicals, St. Louis, and was used after purification by the method of Chen (1967). The molecular weight was assumed to be 69,000 daltons and its concentration in aqueous solution was determined using an extinction coefficient $E_{1cm}^{0.1\%}$ of 0.531 at 278 nm (Means and Bender, 1975). Gabexate mesilate (GM) was a gift from Ono Pharmaceuticals, Osaka and used as received. Diethylpyrocarbonate (DEP) and diisopropylfluorophosphate (DFP) were purchased from Aldrich Chemicals, Milwaukee. Stock solutions of DEP in anhydrous ethanol were freshly prepared. Hydroxylamine hydrochloride was purchased from Wako Chemicals, Osaka. N^{α}-Acetyl-L-histidine and bromcresol green were obtained from Tokyo Kasei Kogyo, Tokyo. Pooled plasma was prepared by mixing an equal volume of each plasma obtained from three healthy volunteers (Red Cross Blood Center, Nagoya). All other chemicals were of reagent grade.

Measurement of GM degradation

The reaction of GM with HSA was followed spectrophotometrically by monitoring the appearance of ethyl-*p*-hydroxybenzoate (EPB) at 295 nm. The HSA concentration was always kept more than 5-fold in excess of GM (4.0×10^{-6} M), so that GM should preferentially react with a primary reactive site or sites on HSA (Means and Bender, 1975; Kurono et al., 1979). For the pH-profiles, Michaelis buffer (0.05 M acetate, pH 4.5–5.5) and Sörensen buffer (0.05 M phosphate, pH 6.5–8.3) were used as the reaction media (I.S. 0.2 with NaCl). Temperature was 25 ± 0.2°C.

In the acidic region below pH 6.5, the decrease of GM was followed by high-performance liquid chromatography (HPLC) (Nishijima et al., 1983). After various time intervals aliquots of the reaction mixture were withdrawn, diluted with pH 4.0 acetate buffer and 50 μl was injected into the chromatograph. Chromatographic conditions were as follows: a Jasco Tri-Rotor apparatus equipped with a UVIDEC 100-II UV detector and a reversed-phase column (Deverosil-C_{18}, 5 μm, 200 × 4 mm i.d.) was used. The mobile phase was a mixture of acetonitrile and pH 5.0 acetate buffer (0.05 M) containing 29 mM ammonium sulfate (55 : 45, v/v), operated at a flow-rate of 0.6 ml/min. The column eluate was monitored at 255 nm. Peak areas were obtained with a Shimadzu C-R1B reporting integrator.

In deuterium oxide (pD 7.5), the pD value was estimated from pD = pH meter reading ± 0.4 (Brubacher et al., 1966), and the concentrations of HSA and GM were 5.0×10^{-5} M and 1.0×10^{-5} M, respectively. The degradation rate of GM was not affected in the presence of EPB or hydrolytic products mixture of GM up to 2-fold of GM (GM 1.0×10^{-5} M; HSA 5.0×10^{-5} M at pH 7.4), indicating no product inhibition under this condition.

Degradation of GM in human plasma

GM (1.0×10^{-4} M) was incubated with plasma dialyzed against isotonic pH 7.4 phosphate buffer. The albumin concentration was maintained at 5.8×10^{-4} M. The albumin concentration was determined by the method of Doumas et al. (1971). 50 μl of the reaction mixture was withdrawn at an interval and was mixed with 1 ml of 99.5% ethanol, and was centrifuged at $1100 \times g$ for 10 min (Nishijima et al., 1983). The remaining GM in the supernatant was determined by HPLC as mentioned above.

Modification of HSA with diethylpyrocarbonate (DEP)

The calibration of the number of histidine residues modified by DEP was first established using

N^{α}-acetyl-L-histidine (Melchior and Fahrney, 1970; Soon et al., 1977). DEP was dissolved in anhydrous ethanol before use. Various concentrations (0.03–0.25 mM) of N^{α}-acetyl-L-histidine were treated with DEP (5 mM) in pH 7.4 phosphate buffer 3 ml (25°C). The final concentration of ethanol in the reaction mixture was 0.5%. This modification was completed within 10 min and the increase of absorbance for resulting N^{α}-acetyl-N-ethoxycarbonylhistidine at 242 nm was measured. A molar absorption coefficient obtained was $3.3 \times 10^3\ M^{-1} \cdot cm^{-1}$.

HSA (5.0×10^{-5} M in pH 7.4 phosphate buffer) was treated with various concentrations (0.8–5 mM) of DEP for 30 min. Likewise, the increase in the absorbance at 242 nm was measured and the number of modified histidine residues was determined using the molar absorption coefficient described above. The final concentration of ethanol was kept to 0.5%. Using the reaction mixture where the number of the modified histidine residues of HSA was determined, the remaining activity toward GM (1.0×10^{-5} M) was estimated by following spectral changes of EPB (25°C). Excess DEP was not removed because it is readily hydrolyzed in aqueous solutions (Berger, 1975), and the half-life of DEP is 6 min at pH 7.4, 25°C (Meyer and Cromartie, 1980). No effect of DEP on GM degradation was observed in the aqueous DEP solution (5 mM) after allowed to stand for 30 min at 25°C.

Recovery of DEP-inactivated HSA with hydroxylamine

HSA (1.8×10^{-4} M) was incubated with DEP (1.1 mM) at pH 7.4 and 25°C for 30 min. Then hydroxylamine (pH 7.4) was added to the final concentration of 0.5 M (Meyer and Cromartie, 1980). After incubation for various times at 25°C, the mixture was applied to a column of Sephadex G-25 (1.6 cm i.d. × 43 cm) equilibrated with pH 7.4 phosphate buffer to remove excess reagent. The concentration of HSA was determined with the absorbance at 278 nm. The HSA activity was assayed in the mixture containing 5.0×10^{-5} M DEP-modified HSA and 1.0×10^{-5} M GM in pH 7.4 and 25°C. The same manipulation of HSA was done without DEP treatment as a control.

Modification of HSA with diisopropylfluorophosphate (DFP)

HSA (1.8×10^{-4} M, pH 7.4 phosphate buffer) was incubated with DFP (3.7 mM and 37 mM) for 14 h at 25°C. The modified HSA was separated through a Sephadex G-25 column (1.6 cm i.d. × 43 cm) equilibrated with pH 7.4 phosphate buffer. The residual activity of the modified HSA (5.0×10^{-5} M) against GM (1.0×10^{-5} M) was determined at pH 7.4 and 25°C.

Fluorescence measurement of DEP-modified HSA

Measurements were made using a Shimadzu RF-520 spectrofluorophotometer (Shimadzu, Kyoto, Japan). HSA (5.0×10^{-5} M) was treated with 0.3 mM DEP at pH 7.4. After the completion of the modification, the solution was excited at a wavelength of 300 nm for tryptophan at 25°C.

Deacylation of ε-guanidinocaproyl-HSA

HSA (5.0×10^{-5} M) was first treated with GM (5.0×10^{-4} M) in buffer solution (pH 7.4) for 3 h at 25°C. The reaction mixture was then dialyzed against the same buffer to remove small molecules, i.e. excess GM and reaction products, for 3 h with renewing the buffer every 10 min. After allowing to stand for various times up to 50 h at 25°C, the activity of the acylated HSA against GM (1.0×10^{-5}M) was determined.

Results and Discussion

Kinetics of GM degradation accelerated by HSA

Fig. 1 shows that the degradation of GM followed pseudo-first-order kinetics at least up to two half-lives in the presence of HSA (0.05 M phosphate buffer, pH 7.4, 25°C, and accelerated with increasing HSA concentration. The half-life of the spontaneous degradation was about 3.6 days. Significant acceleration occurred at the concentrations of HSA much lower than its normal plasma level of $5.5–7.7 \times 10^{-4}$ M. For instance, the half-lives were 18.6 and 6.3 min with protein concentrations of 2.7×10^{-5} M and 1.5×10^{-4} M, respectively.

The pseudo-first-order rate constants were plotted as a function of the HSA concentration, as

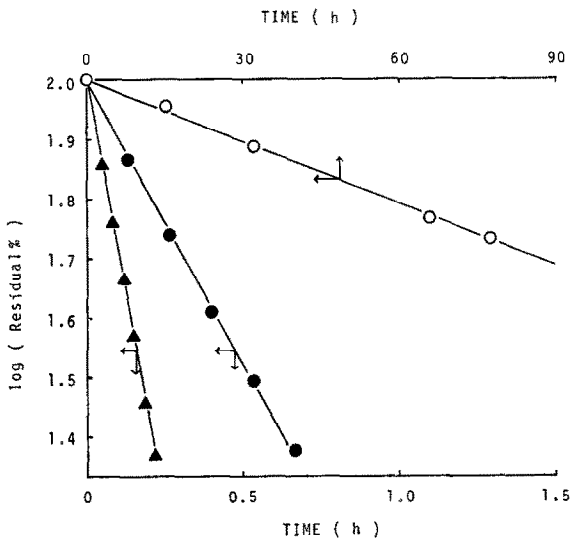
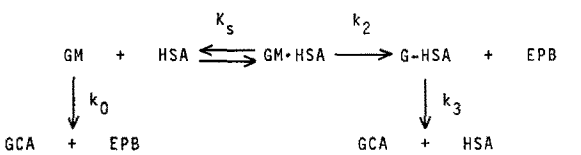


Fig. 1. First-order plots for the degradation of GM in the absence and presence of HSA: ○, 0 M; ●, 2.7×10^{-5} M; ▲, 1.5×10^{-4} M. GM, 4.0×10^{-6} M, pH 7.4, I.S. 0.2 and 25°C.

shown in Fig. 2, which showed a tendency to reach a plateau. These results indicate that the degradation of GM with HSA proceeds through the formation of GM–HSA complex and the whole reaction was assumed as follows (Scheme I) (Kurono et al., 1979):



Scheme 1

Where GM–HSA is a Michaelis-Menten-type complex, G-HSA is ε-guanidinocaproyl-HSA, k_0 and k_2 are the pseudo-first-order rate constants for the degradation of GM and the complex, respectively. K_s denotes the dissociation constant of the complex. k_3 is a pseudo-first-order rate constant for the deacylation of G-HSA.

When the concentration of HSA is in excess of the substrate, the pseudo-first-order rate constant, k_{obs}, may be represented by:

$$k_{obs} = \frac{k_0 K_s + k_2 [HSA]_0}{K_s + [HSA]_0} \qquad (1)$$

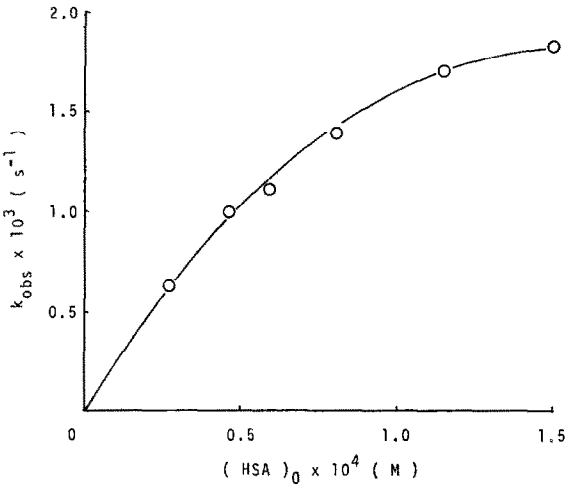


Fig. 2. Effect of HSA concentration on the degradation of GM, pH 7.4, I.S. 0.2, and 25°C. GM, 4.0×10^{-6} M.

where $[HSA]_0$ is the initial concentration of HSA. Eqn. 1 can be rearranged to the following double-reciprocal form (Kurono et al., 1979):

$$\frac{1}{k_{obs} - k_0} = \frac{K_s}{k_2 - k_0} \cdot \frac{1}{[HSA]_0} + \frac{1}{k_2 - k_0} \qquad (2)$$

Fig 3 shows a typical plot according to Eqn. 2 for the dependency of k_{obs} on the concentration of HSA observed at pH 7.4, giving an intercept of 2.94×10^2 and a slope of 3.50×10^{-2}. The values of k_0, k_2 and K_s were estimated to be 2.23×10^{-6} s^{-1}, 3.41×10^{-3} s^{-1} and 1.19×10^{-4} M, respectively, indicating that the GM–HSA complex decomposes almost 1530 times faster than the free form of GM. The values of these constants are calculated based on the scheme with subsequent mathematical expression, and may be therefore considered as apparent values.

Fig. 4 shows the pH profiles of k_0, k_2 and K_s. The k_2 value became larger with increasing pH with two inflection points around pH 6 and pH 9 and was nearly independent of pH from pH of 6.5 to 8.3. A possible residue known to exist in proteins with pK_a 6–7 is the imidazole ring of histidine. The apparent pK_a about 6 suggests the involvement of the imidazole group in HSA as similar as the imidazole group at the esteratic site in cholinesterase and related enzymes (Brown et

al., 1981; Davies et al., 1958). Therefore, the following experiments described in the next section were conducted to assure a possible involvement of histidine residues in the catalytic degradation of GM by HSA. The second inflection may arise from the contribution of anionic imidazole group or imidazolide ion (pK_a 13) with large activity (Brown et al., 1980), although we cannot exclude a possible effect of the N–B transition of HSA molecules.

Modification of HSA with diethylpyrocarbonate (DEP)

HSA was modified by DEP which was been widely used as a histidine-acylating reagent for proteins (Miles, 1977; Soon et al., 1977; Horiike et al., 1979; Gomi and Fujioka, 1983).

Fig. 5 shows the relationship between the residual activity of HSA (A/A_0) and the number of histidine residues modified. A is k_{obs} of DEP-modified HSA and A_0 is k_{obs} of intact HSA. The activity was linearly diminished when the number of modified residues (m) was less than 2 and approached zero activity with increasing m. Accordingly, the number of modified histidines to give ultimate inactivation was assumed to be 16, i.e. all 16 histidine residues existing in one HSA molecule (Rosenoer et al., 1977) were assumed to

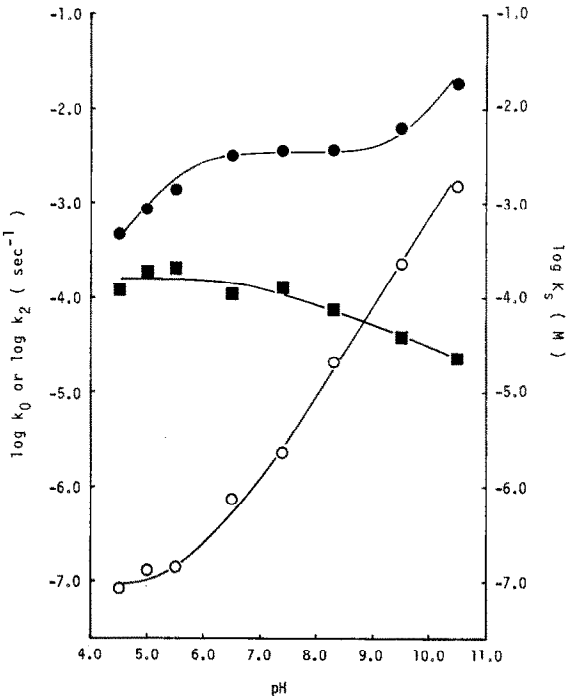


Fig. 4. pH Dependence of log k_0, log k_2 and log K_s for the degradation of GM at 25°C. ○, log k_0; ●, log k_2; ■, log K_s.

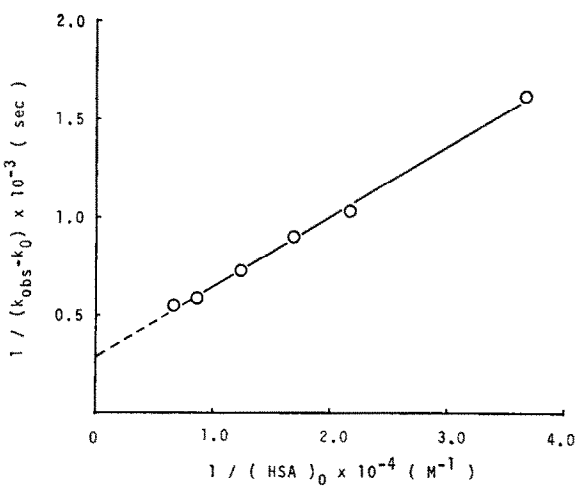


Fig. 3. Double-reciprocal plot according to Eqn. 2; pH 7.4 and 25°C.

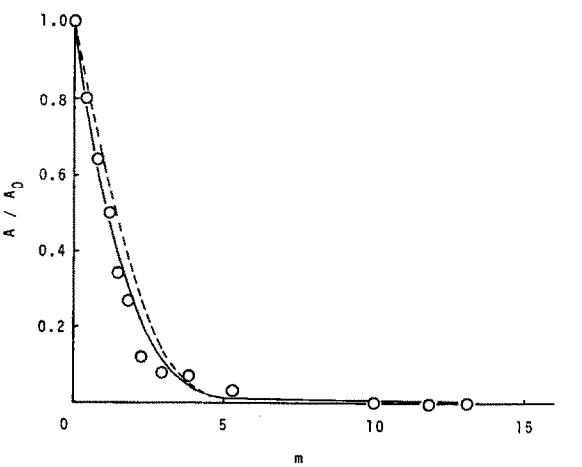


Fig. 5. The relationship between the residual HSA activity (A/A_0) and the number of histidine residues modified by DEP (m). HSA, 5.0×10^{-5} M; GM, 1.0×10^{-5} M; pH 7.4, I.S. 0.2, ethanol 0.5% and 25°C. ○, experimental data; solid line, calculated by Eqn. 3 with $n=16$, $p=i=1$ and $\alpha=0.068$; dotted line, calculated with $n=16$, $p=2$, $i=1$ and $\alpha=0.049$.

be modifiable. However, the number of 16 obtained by such a method does not always correspond to that of histidine residues essential for activity (Tsou, 1962; Horiike et al., 1979).

Based on the method of Tsou (1962) in which the number of essential residues modified by a specific reagent can be determined from the relationship between the number of such residues modified and the activity remaining in the partially modified enzyme, we analyzed the data in Fig. 5, in which the assumption that the stepwise modification of essential residues must bring about complete inactivation is satisfied.

Assuming that n modifiable residues among which p residues including i essential residues react with the reagent at a pseudo-first-order rate constant (k_1) and n-p residues which are not essential react at αk_1, the relationship between the number of residue modified (m) and the activity remaining (A/A_0) will be given by (Horiike et al., 1979):

$$m = n(1-x) = n - p(A/A_0)^{1/i} - (n-p)(A/A_0)^{\alpha/i} \quad (3)$$

where x is the fraction of the unmodified residues. Eqn. 3 can be rewritten as:

$$\log\left[\frac{nx}{(A/A_0)^{1/i}} - p\right] = \log(n-p) + \left(\frac{\alpha-1}{i}\right)\log(A/A_0) \quad (4)$$

A plot of log $[nx/(A/A_0)^{1/i} - p]$ against log (A/A_0) should give a straight line having a slope of $(\alpha - 1)/i$. The plot of experimental data gave a satisfactorily linear line when $n = 16$ and $p = i = 1$, as shown in Fig. 6. The value of α calculated from the slope was 0.068. The solid line in Fig. 5 is the result that was calculated by Eqn. 3 using the above parameters, which was in good agreement with the experimental data. Other combinations of p and i values yielded some deviations from the experimental data: $p = 2$ and $i = 1$ gave a dotted line (Fig. 5), and $p = 2$ and $i = 2$ yielded $\alpha < 0$. Thus, only one histidine residue is most likely to be involved in activity, and this residue has about 15 times higher reactivity with DEP compared with the non-essential residues.

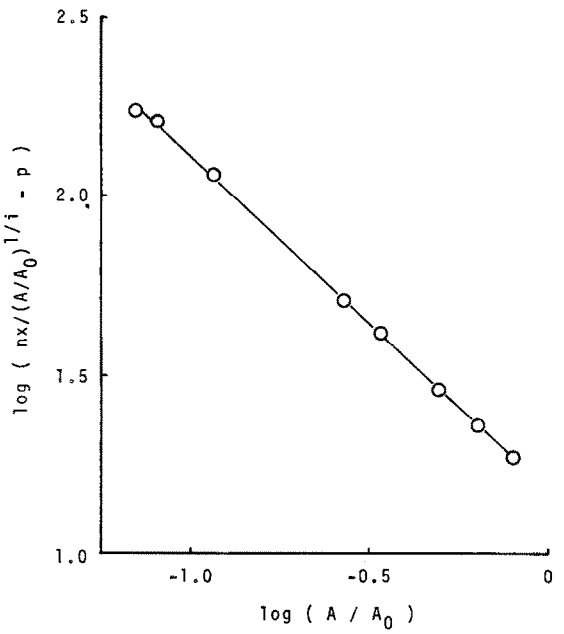


Fig. 6. Analysis of the residual activity of HSA modified by DEP (A/A_0) by the method of Horiike et al. (1979). The experimental data in Fig. 5 were plotted according to Eqn. 4 with $n = 16$ and $p = i = 1$.

DEP, however, reacts with other nucleophilic residues in proteins (Miles, 1977). Therefore, the behaviors of other possible residues which may react with DEP or GM were investigated. The difference spectrum between intact HSA and DEP-treated HSA showed a peak with an absorption maximum near 240 nm which is characterized of N-carbethoxyhistidine residue in protein (Ovádi et al., 1967; Gomi and Fujioka, 1983) (Fig. 7). The spectrum also showed a slight decrease in absorbance above 270 nm. The negative difference spectrum at 280 nm results from the modification of tryptophan (Rosén and Fedorcsák, 1966) or from O-carbethoxylation of tyrosine (Mühlrád et al., 1967). Meantime, fluorescence emission studies showed that no quenching was observed at the emission maximum for DEP-treated HSA (remaining activity to GM, 12%) excited at 300 nm for tryptophan residue (Steinhardt et al., 1971), indi-

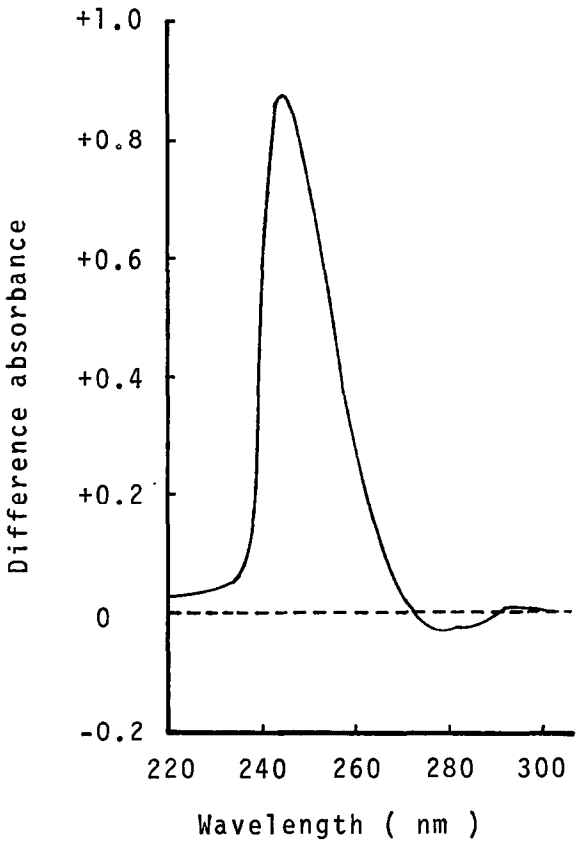


Fig. 7. The difference absorption spectrum between intact HSA (— — —) and DEP-treated HSA (———). HSA, 5.0×10^{-5} M; DEP, 1.0×10^{-3} M. The spectrum was recorded at 25°C after 10 min.

cating that no changes occur at the tryptophan residue.

Sanger (1963) reported that one tyrosine residue in HSA out of 18 tyrosine residues reacts with diisopropylfluorophosphate (DFP) to form O-diisopropylphosphoryl tyrosine and HSA does not have active serine(s) in the molecule, although DFP generally reacts with serine residue(s). Furthermore, when HSA was incubated with a large excess of DFP (20- and 200-fold in molar ratio), fully-modified HSA still retained its activity to GM degradation 92 and 89%, respectively.

It is reported that hydroxylamine can remove the carbethoxy groups from modified histidine, tyrosine and serine, but not from lysine, arginine, cysteine and the NH_2-terminal amino group (Melchior and Fahrney, 1970; Miles, 1977). When the activity of HSA to GM was reduced to 8% with DEP, the treatment with 0.5 M hydroxylamine (pH 7.4, 25°C) resulted in a complete recovery of the activity within 3 h (Table 1).

These results are largely in favor of the involvement of histidine residues, dismissing the involvement of lysyl, arginyl and cysteinyl residues, and other NH_2-terminal amino groups. Thus, it is reasonable to consider that the inactivation of HSA by DEP is due to the modification of histidine residues. Since DEP reacts only with the unprotonated species of imidazole (pK_a = ca. 6) in proteins (Holbrook and Ingram, 1973) and the pK_a value about 6 found in the log k_2–pH profile is within the range expected for a histidine residue, an essential site for GM degradation very likely contains a histidine.

HSA has not been generally categorized in the esterase family, but it can function as an esterase for a variety of esters, especially for those substances with a good leaving group (weak conjugate base) at the ester linkage (Kurono et al., 1979; Ohta et al., 1983; Ozeki et al., 1980). The degradation of GM was accelerated by three orders of magnitude compared with the spontaneous reaction at physiological pH conditions. However, the rate enhancement with HSA was much slower than that of so-called enzyme-catalyzed reactions in which several types of catalytic residues operate simultaneously and yield such efficient catalytic action. So it is conceivable that only a single residue (i.e. a histidine residue) is responsible for the catalytic function of HSA, even if some three-dimensional structure is essential for the GM and HSA interaction.

TABLE 1

RESTORATION OF DEP-INACTIVATED HSA WITH HYDROXYLAMINE

Incubation time (h)	Activity * (%)
0	8
0.5	82
1.0	94
3.0	99

* k_{obs}, treated with NH_2OH/k_{obs}, intact $\times 100$.

Mechanistic function of histidine against GM

The criteria that the mechanistic function of the imidazole ring is due to either nucleophilic catalysis or general-base catalysis would include: (1) accumulation of an acyl-intermediate (ε-guanidinocaproyl-HSA) formed; and (2) deuterium solvent isotope effects (Bender et al., 1964; Dugas and Penny, 1981). As discussed later in the section "Deacylation of ε-guanidinocaproyl-HSA intermediate", evidence was found that the acyl-intermediate was formed and had accumulated. Furthermore, the observed rate constants for degradation at pH and pD 7.5 were 9.53×10^{-4} s^{-1} and 1.03×10^{-3} s^{-1}, respectively, and little difference was found. These results therefore indicate that the degradation which is possibly catalyzed by the imidazole ring is mainly due to nucleophilic catalysis.

Deacylation of ε-guanidinocaproyl-HSA intermediate

In Scheme I, the formation of ε-guanidinocaproyl-HSA was assumed following the precedents that a variety of substrates were degraded leaving behind respectively acyl-HSA intermediates (Ohta et al., 1983; Kurono et al., 1979). A characteristic of the acyl-HSA intermediate was that the deacylation rate is generally much slower than that of usual enzymes. Thus the catalytic activity of HSA in hydrolysis is referred to "esterase-like" activity.

Fig. 8 shows the relationship between the activity of ε-guanidinocaproyl-HSA and the standing time to allow deacylation of the acyl-HSA. The results indicate that the restoration of the activity is very slow, taking about 50 h for the 50% restoration; k_3 could be estimated as 4×10^{-6} s^{-1} at pH 7.4. Such a slow deacylation of the acyl-HSA might be analogous to a mechanism that GM acts as an inhibitor against proteolytic enzymes, preventing the enzymes from restoring their activities.

Degradation of GM in human plasma

In order to elucidate the role of HSA in the degradation of GM in plasma, the apparent rates of degradation in intact plasma and in plasma dialyzed against pH 7.4 phosphate buffer were compared with that in a purified HSA solution where the albumin concentration of HSA was adjusted so as to be equivalent to that of the plasma (5.8×10^{-4} M).

Fig. 9 shows that the degradation followed first-order kinetics and the rate of drug degradation in plasma (7.7×10^{-3} s^{-1}) was about 2.4 times faster than that in plain HSA (3.2×10^{-3} s^{-1}). This indicates that plasma esteratic compo-

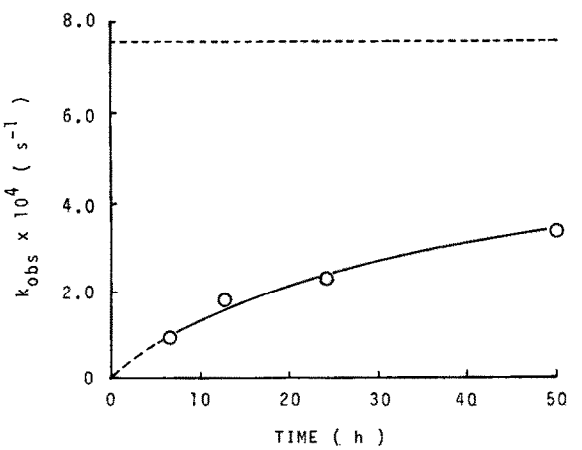


Fig. 8. The recovery of the activity of ε-guanidinocaproyl-HSA with the incubation time. The abscissa indicates the incubation time after 3 h dialysis, and then GM was introduced. — — —, control (assumed k_{obs} for HSA untreated with GM) HSA: 5.0×10^{-5} M, pH 7.4 and 25°C.

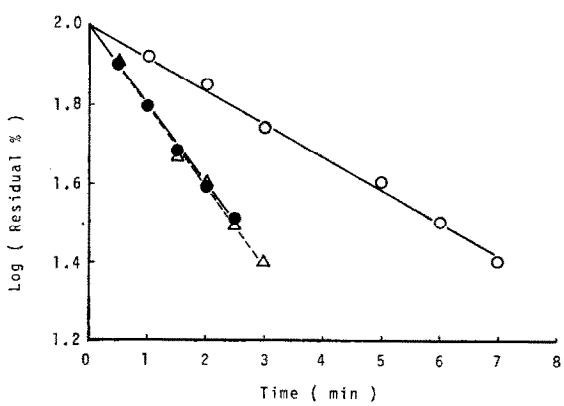


Fig. 9. The degradation of GM in HSA (○), in intact plasma (△) and in dialyzed plasma (●) at 25°C. GM: 1.0×10^{-4} M. The albumin concentrations are all 5.8×10^{-4} M. pH of the plasma was adjusted to that of the HSA solution (pH 7.4).

nents other than HSA are obviously involved in the degradation of GM. However, it should be noticed that the activity of the albumin is considerably high, assuming that purified HSA used was comparable to intact albumin in terms of the esterase-like activity.

The contribution of the HSA mediated degradation to the overall degradation in human plasma was about 40%. Considering a large amount of albumin about 50–65 wt.% in plasma proteins, the esterase-like activity of HSA may play a key role in the overall disposition of ester drugs after administration.

Acknowledgements

The authors are grateful to Mr. Shigeo Isomura for his technical assistance and skill.

References

Agarwal, R.P., McPherson, R.A. and Phillips, M., Rapid degradation of disulfiram by serum albumin. Res. Commun. Chem. Pathol. Pharmacol., 42 (1983) 293–310.

Bender, M.L. and Kedzy, F.J., The current status of the α-chymotrypsin mechanism. J. Am. Chem. Soc., 86 (1964) 3704–3714.

Berger, S.L., Diethyl pyrocarbonate: an examination of its properties in buffered solutions with a new assay technique. Anal. Biochem., 67 (1975) 428–437.

Brown, J.M., Bunton, C.A., Diaz, S. and Ihara, Y., Dephosphorylation in functional micelles. The role of the imidazole group. J. Org. Chem., 45 (1980) 4169–4174.

Brown, S.S., Kalow, W., Pilz, W., Whittaker, M. and Woronick, C.L., The plasma cholinesterases. Adv. Clin. Chem., 22 (1981) 1–123.

Brubacher, L.J. and Bender, M.L., The preparation and properties of *trans*-cinnamoyl-papain. J. Am. Chem. Soc., 88 (1966) 5871–5880.

Chen, R.F., Removal of fatty acids from serum albumin by charcoal treatment. J. Biol. Chem., 242 (1967) 173–181.

Davies, D.R. and Green, A.L., In Nord, F.F. (Ed.), Advances in Enzymology and Related Subjects of Biochemistry, Vol. XX, Interscience Publishers, New York, 1958, pp. 283.

Doumas, B.T., Watson, W.A. and Biggs, H.G., Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31 (1971) 87–96.

Dugas, H. and Penny, C., In Cantor, C.R. (Ed.), Bioorganic Chemistry, A Chemical Approach to Enzyme Action. Springer-Verlag, New York, 1981, pp. 185–188.

Fitzpatrick, F.A., Liggett, W.F. and Wynalda, M.A., Albumin–eicosanoid interactions. J. Biol. Chem., 259 (1984) 2722–2727.

Gomi, T. and Fujioka, M., Evidence for an essential histidine residue in S-adenosylhomocysteinase from rat liver. Biochemistry, 22 (1983) 137–143.

Holbrook, J.J. and Ingram, V.A., Ionic properties of an essential histidine residue in pig heart lactate dehydrogenase. Biochem. J., 131 (1973) 729–738.

Horiike, K., Tsuge, H. and McCormick, D.B., Evidence for an essential histidyl residue at the active site of pyridoxamine (pyridoxine)-5′-phosphate oxidase from rabbit liver. J. Biol. Chem., 254 (1979) 6638–6643.

Kurono, Y., Maki, T., Yotsuyanagi, T. and Ikeda, K., Esterase-like activity of human serum albumin: structure–activity relationships for the reactions with phenyl acetates and *p*-nitrophenyl esters. Chem. Pharm. Bull., 27 (1979) 2781–2786.

Kurono, Y., Yamada, H. and Ikeda, K., Effects of drug binding on the esterase-like activity of human serum albumin. V. Reactive site towards substituted aspirins. Chem. Pharm. Bull., 30 (1982) 296–301.

Means, G.E. and Bender, M.L., Acetylation of human serum albumin by *p*-nitrophenyl acetate. Biochemistry, 14 (1975) 4989–4994.

Melchior, W.B. and Fahrney, D., Ethoxyformylation of proteins. Reaction of ethoxyformic anhydride with α-chymotrypsin, pepsin, and pancreatic ribonuclease at pH 4. Biochemistry, 9 (1970) 251–258.

Meyer, S.E. and Cromartie, T.H., Role of essential histidine residues in L-α-hydroxy acid oxidase from rat kidney. Biochemistry, 19 (1980) 1874–1881.

Miles, E.W., Modification of histidyl residues in proteins by diethylpyrocarbonate. Methods Enzymol., 47 (1977) 431–442.

Miyamoto, T. and Hirata, F., Metabolic fate of ethyl *p*-(6-guanidinohexanoyloxy)benzoate methanesulfonate (FOY) II. Pharmacometrics (Tokyo), 15 (1978) 15–20.

Muramatsu, M. and Fujii, S., Inhibitory effects of ω-guanidino acid esters on trypsin, plasmin, plasma kallikrein and thrombin. Biochim. Biophys. Acta, 268 (1972) 221–224.

Mühlrád, A., Hegyi, G. and Tóth, G., Effect of diethylpyrocarbonate on proteins. Acta Biochim. Biophys. Acad. Sci. Hung., 2 (1967) 19–29.

Nakahara, M., Inhibitory effect of aprotinin and gabexate mesilate of human plasma kallikrein. Arzneim.-Forsch./Drug Res., 33 (1983) 969–971.

Nishijima, M.K., Takezawa, J., Taenaka, N., Shimada, Y. and Yoshiya, I., Application of HPLC measurement of plasma concentration of gabexate mesilate. Thromb. Res., 31 (1983) 279–284.

Ohno, H., Kambayashi, J., Chang, S.W. and Kosaki, G., FOY: [Ethyl *p*-(6-guanidinohexanoyloxy)benzoate] methanesulfonate as a serine proteinase inhibitor. II. Thromb. Res., 24 (1981) 445–452.

Ohta, N., Kurono, Y. and Ikeda, K., Esterase-like activity of human serum albumin II: Reaction with N-*trans*-cinnamoyl-imidazoles. J. Pharm. Sci., 72 (1983) 385–388.

Ovádi, J., Libor, S. and Elödi, P., Spectrophotometric determination of histidine in proteins with diethylpyrocarbonate. Acta Biochim. Biophys. Acad. Sci. Hung., 2 (1967) 455–458.

Ozeki, Y., Kurono, Y., Yotsuyanagi, T. and Ikeda, K., Effects of drug binding on the esterase activity of human serum albumin. Chem. Pharm. Bull., 28 (1980) 535–540.

Rosén, C.-G. and Fedorcsák, I., Studies on the action of diethyl pyrocarbonate on proteins. Biochim. Biophys. Acta, 130 (1966) 401–405.

Rosenoer, V.M., Oratz, M. and Rothschild, M.A., In Brown, J.R. (Eds.), Albumin, Structure, Function and Uses. Pergamon Press, Oxford-London, 1977, pp. 27.

Sanger, F., Amino-acid sequences in the active centers of certain enzymes. Proc. Chem. Soc., (1963) 76–83.

Shinkuma, D., Hamaguchi, T., Muro, C., Yamanaka Y. and Mizuno, N., Stability of gabexate mesilate. Byouin Yakugaku (Tokyo), 5 (1979) 186–192.

Soon, C.Y., Shepherd, M.G. and Sullivan, P.A., Modification of lactacte oxidase with diethyl pyrocarbonate. Biochem. J., 165 (1977) 385–393.

Steinhardt, J., Krijn, J. and Leidy, J.G. Differences between bovine and human serum albumins: binding isotherms, optical rotatory dispersion, viscosity, hydrogen ion titration, and fluorescence effects. Biochemistry, 10 (1971) 4005–4015.

Sultatos, L.G., Basker, K.M., Shao, M. and Murphy, S.D., The interaction of the phosphorothioate insecticides chlorpyrifos and parathion and their oxygen analogue with bovine serum albumin. Mol. Pharmacol., 26 (1984) 99–104.

Tsou, C.-L., Relation between modification of functional groups of proteins and their biological activity. Sci. Sinica, 11 (1962) 1535–1558.