Stereoselective Micellar Catalysis. Part 3.† Co-operative Effects of *N*-Decanoyl-L-histidine for the Hydrolysis of Enantiomeric Substrates

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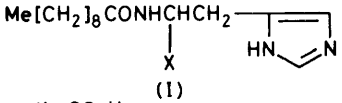
The catalytic activities of *N*-decanoyl-L-histidine (Ia) and its methyl ester (Ib) toward the hydrolyses of enantiomeric substrates (II) have been investigated in the presence of cetyltrimethylammonium bromide micelles. The comparison of catalytic effects (both the rate constants and stereoselectivities) of (Ia) and (Ib) strongly suggests that carboxylate ion of (Ia) intramolecularly enhances the reactivity of the imidazole group. The presence of co-operative effects is also supported by the pH–rate profile and by the thermodynamic parameters of the reaction.

The catalytic mode of α-chymotrypsin and related enzymes depends on a mechanism involving interaction between the imidazole (His-57) and carboxylate (Asp-102) groups.[1] Several studies on stereoselective micellar catalysis have been carried out in order to gain further insight into the stereoselective nature of the enzyme reaction.[2] We have reported previously [3] that the functionalized mixed micelles of *N*-acyl-L-histidine and cationic surfactants are effective stereoselective catalysts for the hydrolyses of enantiomeric substrates, and a mechanism has been suggested for stereoselective catalysis involving acylation of the optically active histidine residue.

Recently, we published a preliminary study of the co-operative effects in the catalytic action of *N*-decanoyl-L-histidine in micelles.[4] This paper describes the detailed results of a comparative analysis of the catalysis of enantiomeric ester hydrolyses by *N*-decanoyl-L-histidine (Ia) and its methyl ester (Ib) in the presence of cetyltrimethylammonium bromide (CTABr) micelles. Both the imidazole and carboxylate functions are involved in the hydrolyses by (Ia) and we sought to elucidate possible co-operative effects between them in aqueous micellar systems. A study of the effects of pH and temperature on the reaction rates was also undertaken to provide further insight into the co-operative effects.

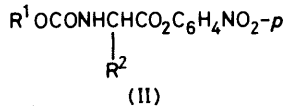
Experimental

Materials.—Cetyltrimethylammonium bromide (CTABr) was purified by established methods.[5] *N*-Decanoyl-L-histidine and its methyl ester were prepared and purified by standard methods.[6,7] The substrates, the *p*-nitrophenyl esters of *N*-benzyloxycarbonyl-D-alanine and *N*-methoxycarbonyl-D- and L-phenylalanine were prepared by standard methods.[8] Other *N*-benzyloxycarbonylamino-acid *p*-nitrophenyl esters were purchased from the Sigma Chemical Co. and were used without further purification. *p*-Nitrophenyl acetate and hexanoate were obtained from Tokyo Kasei Organic Chemicals. *p*-Nitrophenyl acetate was recrystallized from cyclohexane before use.



(I)

a; X = CO_2H
b; X = CO_2Me



(II)

a; $R^1 = R^2 = PhCH_2$
b; $R^1 = PhCH_2$, $R^2 = Me$
c; $R^1 = Me$, $R^2 = PhCH_2$,

† Part 2, Y. Ihara and R. Hosako, *Bull. Chem. Soc., Jpn.*, 1982, **55**, 1979.

Kinetic Measurements.—The general procedure has been described.[2] The formation of *p*-nitrophenol was followed spectrophotometrically at 400 (pH >6) and 317 nm (pH <6). The pseudo-first-order rate constants were obtained from plots of log $(A_\infty - A_t)$ verses time (t) and calculated by the least-squares methods. Correlation coefficients were >0.999.

Results and Discussion

Stereoselective Catalytic Effects.—The catalytic activities of (Ia and b) toward the hydrolyses of two enantiomeric substrates are examined at pH 7.30, 0.02M-phosphate buffer, and 25 °C in the presence of CTABr micelles. With the condition [CTABr] > [catalyst] > [substrate], the catalytic second-order rate constants (k_c) were obtained from the slope of a linear plot of the observed pseudo-first-order rate constants (k_ψ) against catalyst concentration at fixed [CTABr]. The k_c values at several CTABr concentrations are shown in Table 1. The catalytic rate is sensitive to the CTABr concentration. The catalytic effects of both (Ia and b) decrease with increasing CTABr concentration, but the ratios of the rates for (Ia and b) are essentially unchanged, suggesting that there is no significant structural difference between the mixed micelles of (Ia and b). The kinetic results show that (Ia) is more reactive

Table 1. Variation of apparent catalytic rate constants k_c with CTABr concentrations [a]

10^3[CTABr]/ M	Catalyst	k_c/l mol^{-1} s^{-1} (IIa) L	(IIa) D	(IIa) L/D	(IIc) L	(IIc) D	(IIc) L/D
1.00	(Ia)	3 220	1 380	2.33	1 390	655	2.12
	(Ib)	1 400	830	1.69	710	426	1.67
2.00	(Ia)	1 730	690	2.51	885	404	2.19
	(Ib)	835	496	1.68	495	327	1.51
4.00	(Ia)	875	350	2.50	471	222	2.12
	(Ib)	458	251	1.82	269	170	1.58
6.00	(Ia)	572	231	2.51	314	145	2.17
	(Ib)	283	154	1.84	184	114	1.61
8.00	(Ia)	408	174	2.34	218	103	2.12
	(Ib)	195	116	1.68	137	84	1.63
10.0	(Ib)	157	91	1.73	101	64	1.58

[a] At pH 7.30, 0.02M-phosphate buffer, and 25 °C. [(II)] 1.0 × 10^{-5}M, [(I)] 0.4—2.0 × 10^{-4}M.

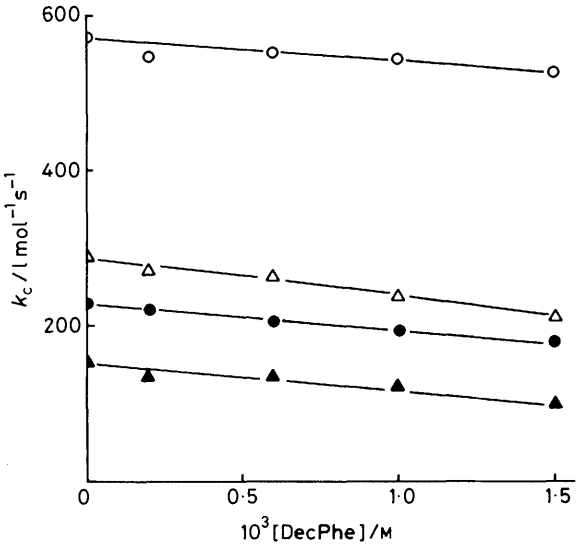


Figure 1. Effect of *N*-decanoyl-L-phenylalanine (DecPhe) on the catalytic rate for the reactions of (IIa) with (Ia and b) in the presence of CTABr: pH 7.30, 0.02M-phosphate buffer, and 25 °C, [CTABr] 6.00 × 10^{-3}M, [(IIa)] 1.0 × 10^{-5}M. ●, ○ (Ia), ● with D-(IIa), ○ with L-(IIa); ▼, △ (Ib), ▲ with D-(IIa), △ with L-(IIa)

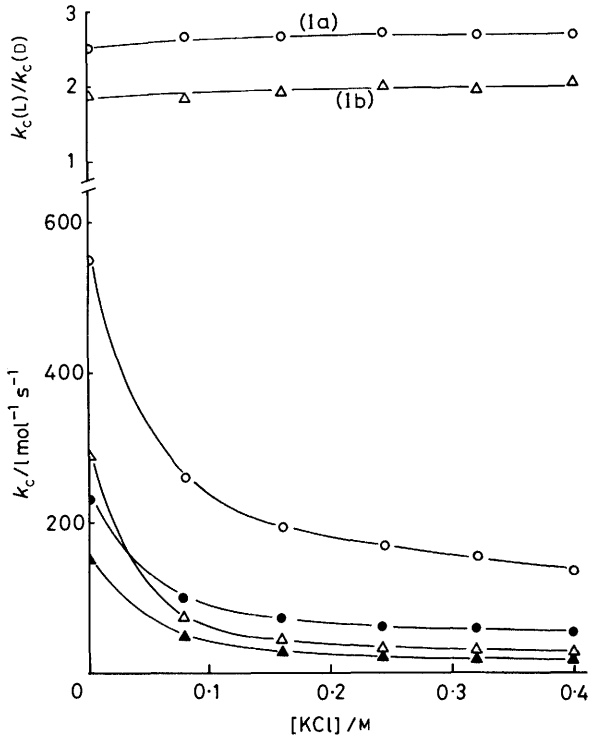


Figure 2. Effect of salt on the catalytic rate for the reaction of (IIa) with (Ia and b) in the presence of CTABr: pH 7.30, 0.02M-phosphate buffer, and 25 °C, [CTABr] 6.00 × 10^{-3}M, [(IIa)] 1.0 × 10^{-5}M. ●, ○ (Ia), ● with D-(IIa), ○ with L-(IIa); ▲, △ (Ib), ▲ with D-(IIa), △ with L-(IIa)

than (Ib) and that greater stereoselectivity is also observed for (Ia than b). In general, ionization of the carboxy-group of (Ia) suppresses ionization of the imidazole group, and also partially neutralizes the positively charged CTABr head groups. Both factors reduce the catalytic potential in micellar systems. Thus, as shown in Figure 1, addition of *N*-decanoyl-

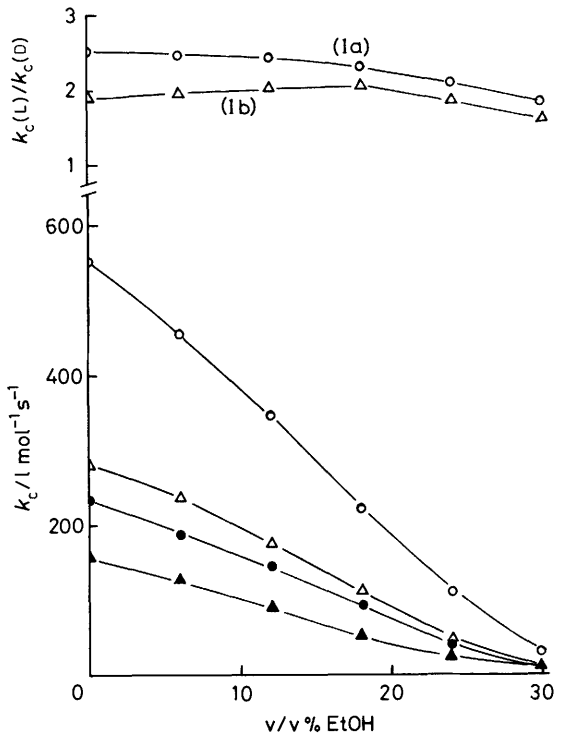


Figure 3. Effect of organic solvent on the catalytic rate for the reaction of (IIa) with (Ia and b) in the presence of CTABr: pH 7.30, 0.02M-phosphate buffer, and 25 °C, [CTABr] 6.00 × 10^{-3}M, [(IIa)] 1.0 × 10^{-5}M. ●, ○ (Ia), ● with D-(IIa), ○ with L-(IIa); ▲, △ (Ib), ▲ with D-(IIa), △ with L-(IIa)

Table 2. Effect of salt on rate constant [a]

	k_c/l mol^{-1} s^{-1}					
	(IIa)			(IIc)		
Catalyst	L	D	L/D	L	D	L/D
(Ia)	130	50.5	2.57	82.0	37.5	2.19
(Ib)	29.0	13.9	2.09	18.4	10.9	1.69

[a] At pH 7.30, 0.02M-phosphate, and 25 °C in the presence of 6.00 × 10^{-3}M-CTABr and 0.40M-KCl, [(II)] 1.0 × 10^{-5}M.

L-phenylalanine to the reaction medium decreases the rates under comparable conditions. Therefore, the enhanced reactivity and stereoselectivity of (Ia) suggest that some of the co-operative effects are due to the active site of the catalyst.

Effects of Salt and Organic Solvent.—Figures 2 and 3 show the effects of added salt and organic solvent on the reaction rates for the hydrolyses of (IIa) by (Ia and b). Additions of salt (KCl) and organic solvent (ethanol) to the reaction media markedly decrease the rates. We have also observed the same phenomena in the presence of CTABr alone. Tables 2 and 3 list the results of added salt (0.4M-KCl) and organic solvent (30% ethanol) for reactions of several substrates with (Ia and b). Micellar catalysis is usually inhibited by additions of salts or organic solvents.[8,9] These additives affect the catalytic rates by changing the dissociation behaviour of the catalyst and/or the structure of the mixed micelles. However, the influence of these additives in our systems is smaller for (Ia) than for (Ib). Greater stereoselectivity is also observed for (Ia) than (Ib) in all the conditions studied. We also find that

Table 3. Effect of organic solvent of rate constant [a]

	k_c/l mol^{-1} s^{-1} (IIa)			(IIb)			(IIc)		
Catalyst	L	D	L/D	L	D	L/D	L	D	L/D
(Ia)	36.0	18.8	1.91	8.00	6.08	1.32	10.6	6.28	1.69
(Ib)	10.1	6.38	1.58	2.95	2.46	1.20	3.38	2.32	1.46
(Ia) [b]	1.64	1.15	1.43				0.996	0.773	1.29
(Ib) [b]	0.604	0.529	1.14				0.425	0.373	1.14

[a] At pH 7.30, 0.02M-phosphonate buffer, and 25 °C in the presence of 6.00×10^{-3}M-CTABr and 30% v/v ethanol unless specified otherwise. [b] In the absence of CTABr.

Table 4. Structural effect of substrates [a]

Substrate [b]	k_c/l mol^{-1} s^{-1} (Ia)	(Ib)	Rel. k_c
PNPA	10.0	4.43	2.26
PNPH	19.5	8.35	2.34
PNPL	20.3	7.90	2.57
ZGlyONp	162	85.0	1.91
L-Z AlaONp (IIb)	280	141	1.99
L-ZValONp	58.1	29.6	1.96
L-ZLeuONp	415	203	2.04
L-ZIleONp	36.1	20.4	1.77
L-ZPheONp (IIa)	572	283	2.02
L-ZTyrONp	74.1	49.3	1.50
L-ZTryONp	42.3	31.4	1.35
L-ZCys(Bzl)ONp	972	518	1.88

[a] At pH 7.30, 0.02M-phosphate buffer, and 25 °C in the presence of $6.00 \times 10B^3$M-CTABr, [Substrate] 1.0×10^{-5}M. [b] Abbreviations used are: PNPA, *p*-nitrophenyl acetate; PNPH, *p*-nitrophenyl hexanate; PNPL, *p*-nitrophenyl laurate; for amino-acid ester derivatives, Z, benzyloxycarbonyl; ONp, *p*-nitrophenyl ester; Bzl, benzyl.

(Ia) is better catalyst than (Ib) in 30% ethanol at pH 7.30 and 0.02M-phosphate buffer in the absence of surfactant (Table 3).

Structural Effects of Substrates.—The results shown in Table 4 indicate the effect of the amino-acid side chain of the substrates on catalysis by (Ia and b). For comparison, the results of non-specific substrates such as *p*-nitrophenyl acetate (PNPA), hexanoate (PNPH), and laurate (PNPL) are also listed in Table 4. The catalytic rate depends on the structure of the substrates. Thus (Ia) is *ca.* 1.3—2.5 times as reactive as (Ib) for all substrates used.

Temperature Dependence of Reaction Rates.—The effect of temperature change (16.4—34.3 °C) on the reaction rates for the hydrolyses of (IIa and c) by (Ia and b) in the presence of 6.00×10^{-3}M-CTABr was examined at pH 7.3 in 0.02M-phosphate buffer and the results are listed in Table 5. The stereoselectivity slightly increases as the temperature is lowered. Similar results have been observed by Yamada *et al.*[2e,f]

The calculated thermodynamic parameters are listed in Table 6. The $\Delta G^{\ddagger}$ values for the two enantiomeric substrates are practically equal for (Ia and b), while the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of (Ia) are *ca.* 0.2—1.4 kcal mol^{-1}, and 0.3—3.6 cal mol^{-1} K^{-1} smaller than those of (Ib). The effect of the free carboxy-group is primarily to lower $\Delta H^{\ddagger}$ for activation. This is partly balanced by a small unfavourable change in the entropy of activation.

pH Dependence of Reaction Rates.—In order to gain further insight in the catalytic action of (Ia), pH–rate profiles for

Table 5. Temperature dependence of reaction rate

t/°C	Catalyst	k_c/l mol^{-1} s^{-1} (IIa) L	D	L/D
16.4	(Ia)	432	147	2.94
	(Ib)	184	91.0	2.02
21.2	(Ia)	484	182	2.66
	(Ib)	254	136	1.78
29.6	(Ia)	766	323	2.37
	(Ib)	374	217	1.72
34.1	(Ia)	908	399	2.28
	(Ib)	452	289	1.56

[a] In 6.00×10^{-3}M-CTABr at pH 7.30, 0.02M-phosphate buffer, [(IIa)] 1.0×10^{-5}M.

Table 6. Thermodynamic parameters for reactions of (IIa and b) with (Ia and b) in the presence of CTABr [a]

	Catalyst	(IIa) L	D	(IIc) L	D
$\Delta G^{\ddagger}$ [b]/kcal mol^{-1}	(Ia)	13.7	14.2	14.0	14.5
	(Ib)	14.1	14.4	14.4	14.6
$\Delta H^{\ddagger}$/kcal mol^{-1}	(Ia)	7.25	9.79	7.98	10.4
	(Ib)	8.42	10.5	9.42	10.6
$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1}	(Ia)	−21.5	−14.8	−20.2	−13.8
	(Ib)	−19.0	−13.1	−16.6	−13.5

[a] In 6.00×10^{-3}M-CTABr at pH 7.30, 0.02M-phosphate buffer. The parameters were calculated by plotting the data of Table 5 in the form of $\ln(k_c/T)$ *versus* $1/RT$. [b] For the values at 298.2 K.

the reaction of (IIa) with (Ia and b) in the presence of CTABr were examined at 25 °C and shown in Figure 4. The log k_c–pH profiles indicate that there are two kinds of catalytic species in the present reactions, neutral and anionic imidazole groups. However, (Ia) is more reactive than (Ib) for both D- and L-enantiomers over the pH range studied. This result indicates that catalysis by (Ia) involves co-operative interaction between the imidazole and carboxylate groups. More importantly, for catalyst (Ia) in the neutral region (pH 4—7), the stereoselectivity increases with increasing pH (paralleling the increasing concentrations of both carboxylate anion and free imidazole base). In contrast, the stereoselective behaviour of (Ib) is quite different from that of (Ia); the stereoselectivity remains unchanged from pH 4 to 6 and then decreases with increasing pH. Thus, these stereoselective differences between (Ia and b) cannot be explained by an increase only in the free imidazole groups because (Ib) gives almost completely the same stereoselectivity. We suggest therefore that the carboxylate ion of (Ia) enhances the reactivity of the imidazole group to produce an increase in stereoselectivity. In other words, this enhanced reactivity by (Ia) translates into enhanced reaction stereoselectivity. The differences between (Ia and b)

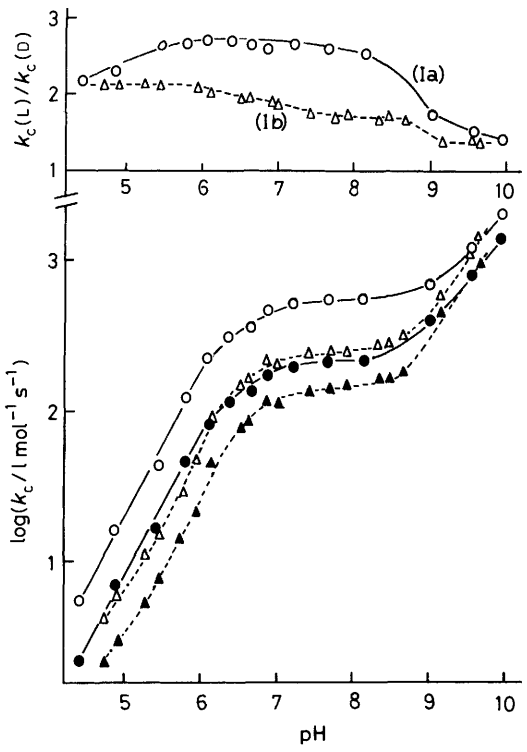


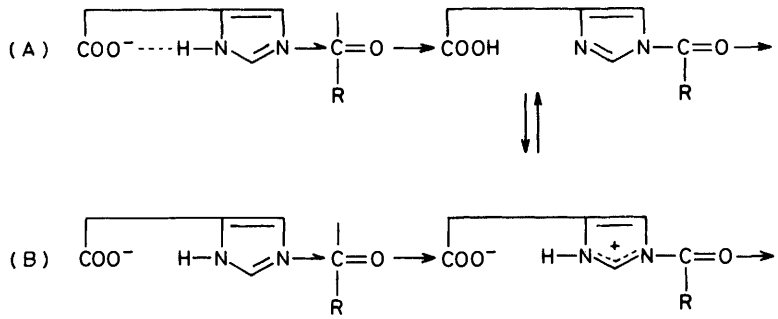
Figure 4. pH–Rate profiles for the reactions of (IIa) with (Ia and b) in CTABr at 25 °C, μ 0.05M (KCl), 0.04M-acetate (pH ≤ 6), 0.02M-phosphate (9 > pH > 6), and 0.02M-carbonate (pH < 9); [CTABr] 6.00×10^{-3}M; [(IIa)] $(0.5—1.0) \times 10^{-5}$M. —— (Ia), ● with D-(IIa), ○ with L-(IIa); - - - - (Ib), ▼ with D-(IIa), △ with L-(IIa)

suggest that the enhanced reactivity of (Ia) is due to co-operative interaction between the carboxy and imidazole groups rather than to the pK_a difference between the imidazole groups. This co-operative effect is also supported by the kinetic results observed in previous sections; catalytic activities and stereoselectivities of (Ia) were always greater than those of (Ib) for several kinetic conditions studied. These results can be reasonably explained in terms of intramolecular hydrogen-bonding between the carboxylate and imidazole groups. It appears that proton transfer accompanies nucleophilic attack by the imidazole which is expected to be much more nucleophilic than a normal imidazole group.

Co-operative Effects in the Action of (Ia).—Gitler [6a] and Tagaki [10] and their co-workers showed that *N*-myristoyl-histidine in CTABr is an effective nucleophilic catalyst for the hydrolyses of *p*-nitrophenyl acetate as confirmed by the accumulation of acylated intermediates during the reaction. Our previous studies have already demonstrated the nucleophilic mechanism for the reaction of enantiomeric substrates with *N*-acyl-L-histidine in the presence of surfactant micelles.[3a] We also observed spectrophotometrically (245 nm) that the catalysis of (Ia and b) of hydrolyses of (II) involves the formation and the decay of an acylated intermediate.*

All our results suggest the mechanism given in the Scheme; the carboxylate ion of (Ia) enhances the reactivity of the imidazole group through intramolecular hydrogen-bonding [step (A)], and/or the carboxylate anion of (Ia) acts to stabilize the cationic acylimidazolium group of the intermediate [step (B)].

Very recently, Murakami [11] and his co-workers showed that the carboxy-group of cationic peptide surfactants bearing both histidyl and aspartyl residues intramolecularly enhances the reactivity of the imidazole group, but the mechanism is



Scheme

in reactivity and stereoselectivity are relatively small but significant. Similar results were also observed for the reaction of (IIc) with (Ia and b). The kinetic pK_a values of the imidazole groups were determined from plots of $[H^+]$ *versus* $1/k_c$ using the data at pH 5.3—7.0 (Figure 4). pK_a Values are 6.35 ± 0.08 and 6.24 ± 0.10 for (Ia and b), respectively. These results different from our present micellar systems since the reaction undergoes general base rather than nucleophilic catalysis.

Acknowledgement

We thank Professor I. M. Klotz for helpful discussions.

* An attempt was made to prepare the acylated intermediate under anhydrous conditions. Thus (Ia) in *NN*-dimethylformamide was treated with the *N*-hydroxysuccinimide ester of *N*-benzyloxycarbonyl-L-phenylalanine and two equivalents of triethylamine. The resulting solution was syringed into buffered CTABr solution at pH 7.3 and the u.v. spectrum was recorded immediately. The acylated intermediate was observed at $\lambda_{max.}$ 260 nm, using the previously hydrolysed solution as a reference (Y. Ihara, Y. Kimura, M. Nango, and N. Kuroki, to be published).

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