ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 11 (2001) 985–989

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Catalytic specificity of *p*-sulfonatocalix[*n*]arenes on the alcoholysis of *N*-Ac-L-amino acids in methanol solution

Koichi Goto, Yukito Murakami, Ryuichi Ueoka *

Division of Applied Chemistry, Graduate School of Kumamoto Institute of Technology, 4-22-1 Ikeda, Kumamoto 860-0082, Japan

Abstract

The specific acid catalysis of *p*-sulfonatocalix[*n*]arenes ($n = 4$:Calix-S4, $n = 6$:Calix-S6, $n = 8$:Calix-S8) was observed in the alcoholysis of *N*-Ac-L-amino acids in methanol. With respect to the alcoholysis of Ala, His, Phe, Trp and Tyr substrates, the alcoholysis-rate of *N*-Ac-L-His-OH was markedly enhanced in the presence of Calix-SN as compared with that in the presence of *p*-hydroxybenzenesulfonic acid (pHBS), which is a noncyclic analogue of Calix-SN. Furthermore, in relation to ^{1}H NMR results of *N*-Ac-L-amino acids and Calix-SN in CD_3OD, it was found that the spectrum of the mixture of *N*-Ac-L-His-OH and Calix-S4 was significantly different from the combined spectra of the respective compounds. These changes in spectra support the formation of inclusion complex of Calix-S4 and *N*-Ac-L-His-OH. The above results seem to indicate that the catalytic specificity of Calix-SN is originated from the complexation with *N*-Ac-L-His-OH

Keywords: Calixarene; Acid catalysis; Alcoholysis; Amino acid; Inclusion complex

1. Introduction

One of the most important subjects in biomimetic chemistry has been the creation of artificial enzymes with high catalytic specificity that is comparable to that of native enzymes. In the course of our study on esterase models, remarkably stereospecific catalysis was observed in the hydrolysis of amino acid and/or dipeptide esters carried out functional molecular assemblies composed of surfactants and catalytic species or cyclodextrins [1–4].

Even though calix[*n*]arenes [5–8] are macrocyclic compounds, which are useful enzyme mimics and have been studied on conformational characteristics and inclusion properties as host molecules toward various guest molecules in solution, there have been few studies on their enzymatic catalysis.

In this study, we report on the specific acid catalysis demonstrated by *p*-sulfonatocalix[*n*]arenes (Calix-SN), which were originally synthesized as "water-soluble" calix[*n*]arenes by Shinkai et al. [9–

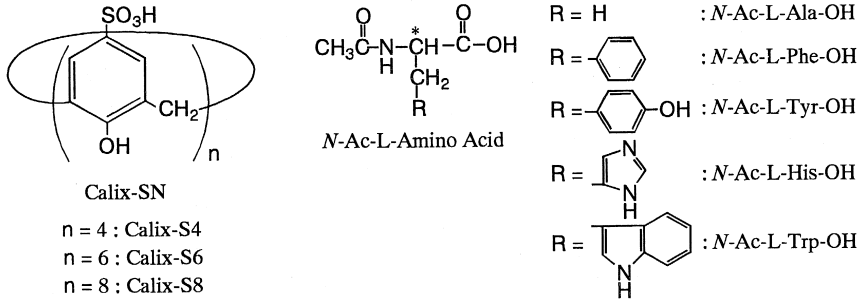
* Corresponding author. Tel.: +81-96-326-3111; fax: +81-96-326-3000.

E-mail address: ueoka@chem.kumamoto-it.ac.jp (R. Ueoka).

1381-1177/01/$ - see front matter
PII: S1381-1177(00)00176-4

12], in the alcoholysis of *N*-Ac-L-amino acids in methanol on the basis of kinetic and ^{1}H NMR measurements. The calix[*n*]arenes and amino acid substrates employed in this study are as follows.

Chemical Structure 1



2. Experimental

2.1. Materials

Calix-SN were purchased from SUGAI Chemical Industry and used after purification by cation-exchange column choromatography (Dow Chemical, DOWEX50W-X8). All of the *N*-Ac-L-amino acids were obtained commercially (Bachem) and used without further purification.

2.2. Kinetic measurements

Rates of methanolysis of *N*-Ac-L-amino acids were monitored by measuring the yield of ester products on a column of HPLC (Hitachi L-6000) at room temperature. The typical conditions are as follows: column, Hitachi gel packed column #3013-N (4 mm (ϕ) $\times$ 250 mm); eluent, CH_3CN/H_2O (2:8 (v/v)); flow rate, 0.50 ml/min; detector, UV (212 nm). Under the conditions of [Calix-SN (or *p*-hydroxybenzenesulfonic acid (pHBS))] $\gg$ [*N*-Ac-L-amino acid], the reaction followed a usual pseudo-first order rate law, and rate constant (k_1) was calculated by Eq. (1),

$$\log(S_\infty - S_t) = -k_1 t/2.303 + \log S_\infty \quad (1)$$

where S_t and S_α denote peak areas of an ester at time t and infinite time, respectively. In the absence of Calix-SN or pHBS, the rate of alcoholysis of *N*-Ac-L-amino acids were extremely slow, so we evaluated the apparent second-order rate constant (k_2) in the concentration unit of sulfonate group of Calix-SN or pHBS by Eq. (2),

$$k_2 = k_1/[\text{sulfonate unit}] \quad (2)$$

2.3. ^{1}H NMR measurements

^{1}H NMR spectra in CD_3OD were measured at room temperature (internal standard TMS) with a 270-MHz NMR apparatus (JEOL JNM-EX270). The concentration of *N*-Ac-L-His-OH was maintained constant (5.0×10^{-2} M) while that of Calix-S4 or pHBS was varied.

3. Results and discussion

3.1. Kinetic studies

The kinetic results for the alcoholysis of *N*-Ac-L-amino acids (Ala, Phe, Tyr, His and Trp) in the presence of Calix-S6 or pHBS, which is a noncyclic

Table 1
Rate constants for the alcoholysis of *N*-Ac-L-amino acids in the presence of pHBS and Calix-S6 in methanol[a]

Substrate	k_1 (h^{-1})		k_2 (M^{-1} h^{-1})		$k_2^{Calix\text{-}S6}/k_2^{pHBS}$ (–)
	pHBS	Calix-S6	pHBS	Calix-S6	
N-Ac-L-His-OH	0.041	1.0	1.4	33	24
N-Ac-L-Ala-OH	–	0.61	–	20	–
N-Ac-L-Trp-OH	0.59	0.51	20	17	0.85
N-Ac-L-Tyr-OH	0.51	0.50	17	17	1.0
N-Ac-L-Phe-OH	0.46	0.41	15	14	0.93

[a] 25°C, [*N*-Ac-L-Amino Acids] = 1.0×10^{-4} M, [pHBS] = 3.0×10^{-2} M, [Calix-S6] = 5.0×10^{-3} M. $k_2 = k_1$/[sulfonate unit].

analogue of Calix-SN, in methanol at 25°C are summarized in Table 1. Except for the reaction of *N*-Ac-L-His-OH, each second-order rate constant (k_2) for the alcoholysis of *N*-Ac-L-amino acids in the presence of Calix-S6 is almost identical with that in the presence of pHBS ($k_2^{Calix\text{-}S6}/k_2^{pHBS}$ values are nearly 1). On the other hand, the alcoholysis of *N*-Ac-L-His-OH was most accelerated in the presence of Calix-S6, though the rate in the presence of pHBS was even slower than those for other substrates.

Furthermore, we examined the catalytic effect of Calix-SN ($n = 4$, 6 and 8) on the alcoholysis of *N*-Ac-L-His-OH in methanol and the results are summarized in Table 2. The remarkable rate enhancements were observed in all of the reactions in the presence of Calix-SN and the rate constants were much greater than that in the presence of pHBS ($k_2^{Calix\text{-}SN}/k_2^{pHBS}$ = 24–86). The rate-enhancement increases in the following sequence, pHBS ≪ Calix-S6 < Calix-S8 < Calix-S4 and these results show that Calix-SN act as efficient catalysts in the alcoholysis of *N*-Ac-L-His-OH substrate.

Table 2
Rate constants for the alcoholysis of *N*-Ac-L-His-OH in the presence of Calix-SN and pHBS in methanol[a]

Catalyst	k_1 (h^{-1})	k_2 (M^{-1} h^{-1})	$k_2^{Calix\text{-}SN}/k_2^{pHBS}$ (–)
pHBS	0.041	1.4	–
Calix-S4	3.6	120	86
Calix-S6	1.0	40	24
Calix-S8	1.7	60	41

[a] 25°C, [*N*-Ac-L-His-OH] = 1.0×0^{-4} M, [pHBS] = 3.0×10^{-2} M, [Calix-S4] = 7.5×10^{-3} M, [Calix-S6] = 5.0×10^{-3} M, [Calix-S8] = 3.75×10^{-3} M. $k_2 = k_1$/[sulfonate unit].

3.2. ^{1}H NMR spectroscopy

We carried out ^{1}H NMR measurements to explore the origin of the catalytic activity of Calix-SN in the alcoholysis of *N*-Ac-L-His-OH. Figs. 1 and 2 show the ^{1}H NMR spectra of *N*-Ac-L-His-OH in the presence of pHBS and Calix-S4 in CD_3CD at room temperature, respectively. With respect to the spectra of *N*-Ac-L-His-OH, chemical shifts of C2 and C4 protons of the imidazole (Im) ring were observed to shift to lower magnetic fields in the presence of pHBS or Calix-S4. The shifts seem to be caused by protonation of the imidazole nitrogen with sulfonic acid groups of pHBS or Calix-S4. Fig. 3 shows the chemical shifts for the C2 and C4 protons as a function of the [sulfonate unit]/[*N*-Ac-L-His-OH]

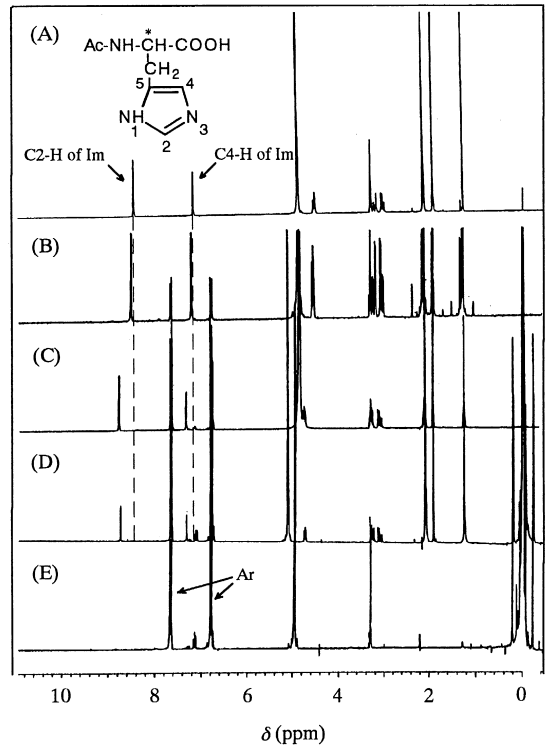


Fig. 1. ^{1}H NMR spectra of *N*-Ac-L-His-OH alone (A), mixture with pHBS (B–D), and pHBS alone (E) in CD_3OD at room temperature. [*N*-Ac-L-His-OH]:[pHBS] = 1:0 (A), 1:0.1 (B), 1:1 (C), 1:4 (D), 0:1 (E).

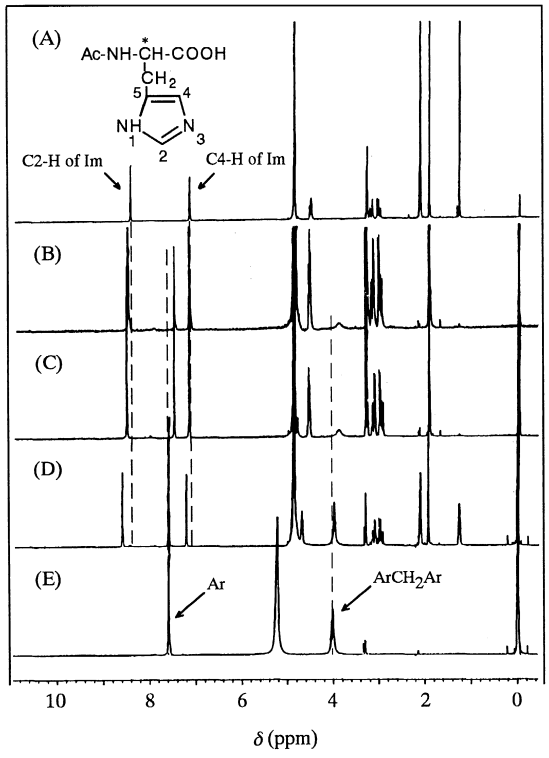


Fig. 2. ^{1}H NMR spectra of *N*-Ac-L-His-OH alone (A), mixture with Calix-S4 (B–D), and Calix-S4 alone (E) in CD_3OD at room temperature. [*N*-Ac-L-His-OH]:[Calix-S4] = 1:0 (A), 1:0.025 (B), 1:0.05 (C), 1:0.25 (D), 0:1 (E).

ratio. The $\Delta\delta$ values increase along with increase of the ratio for both systems, but the values are smaller

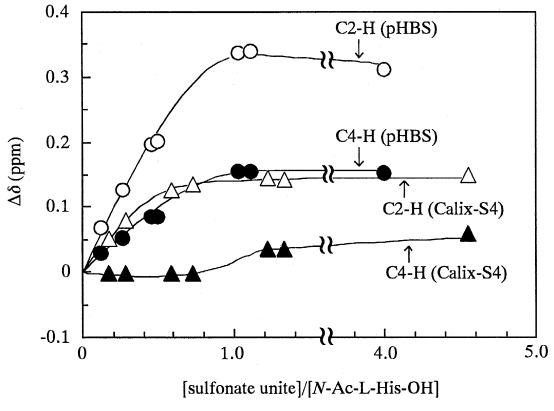


Fig. 3. Chemical shifts of C2 and C4 protons of imidazole ring of *N*-Ac-L-His-OH as a function of [sulfonate unit]/[*N*-Ac-L-His-OH] ration in CD_3OD at room temperature. $\Delta\delta = \delta$ (*N*-Ac-L-His-OH + Calix-S4 (or pHBS)) - δ (*N*-Ac-L-His-OH).

in the presence of Calix-S4. On the other hand, as for the spectra of Calix-S4, the Ar proton signals are shifted to a higher magnetic field in the presence of an excess amount of *N*-Ac-L-His-OH (Fig. 2B and C). Such a change in spectrum was not observed with pHBS. Furthermore, the $ArCH_2Ar$ methylene proton signal of Caix-S4 is also shifted and broadened. These observations may be attributable to the inclusion of *N*-Ac-L-His-OH into the Calix-S4 cavity, that is, the ring current effect due to the imidazole moiety tends to shift the signals toward higher magnetic fields and the fixed conformation broadens the methylene peak [9,10]. Plausibly, the imidazole moiety of *N*-Ac-L-His-OH is not only protonated but also undergoes the ring current effect due to Calix-S4, so that the chemical shifts to lower magnetic fields were less pronounced relative to those for pHBS.

It is well known that the acid catalyzed alcoholysis of carboxylic acids proceeds via the formation of several cationic intermediates. In the alcoholysis of Ala, Phe, Trp and Tyr substrates in the presence of Calix-SN or pHBS, they would catalyze the reactions as "simple" acid catalysts. As for the case of alcoholysis of *N*-Ac-L-His-OH, the imidazole seem to be protonated and the positive charge may prevent the formation of cationic intermediates, so that the rate enhancement was even smaller than those for other substrates in the presence of pHBS. On the other hand, the alcoholysis rate of *N*-Ac-L-His-OH was extremely enhanced in the presence of Calix-SN. Calix-SN seems to promote the formation by the complexation with *N*-Ac-L-His-OH, as shown schematically in Fig. 4, and such stereochemical arrangement may induce stabilization of the cationic intermediates with the anionic sulfonate groups.

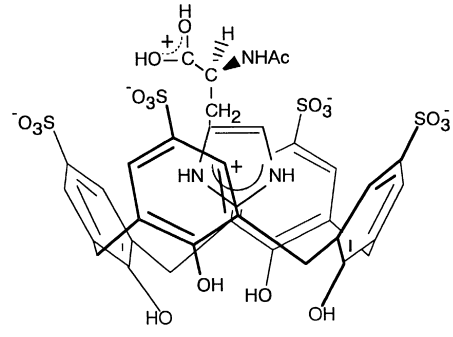


Fig. 4. A plausible mechanism for the complexation of Calix-S4 with *N*-Ac-L-His-OH.

4. Conclusion

In conclusion, the specific acid catalysis of Calix-SN was observed in the alcoholysis of *N*-Ac-L-His-OH in methanol. The ^{1}H NMR measurements seem to indicate that the catalytic specificity is originated from the formation of the inclusion complex of Calix-SN with *N*-Ac-L-His-OH.

References

[1] R. Ueoka, Y. Matsumoto, R.A. Moss, S. Swarup, A. Sugii, K. Harada, J. Kikuchi, Y. Murakami, J. Am. Chem. Soc. 110 (1988) 1588.
[2] R. Ueoka, S. Mori, R.A. Moss, Langmuir 10 (1994) 2892.
[3] K. Goto, R. Ueoka, Y. Matsumoto, J. Org. Chem. 60 (1995) 3342.
[4] R. Ueoka, Y. Matsumoto, K. Harada, H. Akahoshi, Y. Ihara, Y. Kato, J. Am. Chem. Soc. 114 (1992) 8339.
[5] C.D. Gutsche, B. Dhawan, K.H. No, K.R. Muthukrishnan, J. Am. Chem. Soc. 103 (1981) 3782.
[6] C.D. Gutsche, L.J. Bauer, J. Am. Chem. Soc. 107 (1985) 6052.
[7] C.D. Gutsche, L.J. Bauer, J. Am. Chem. Soc. 107 (1985) 6059.
[8] L.J. Bauer, C.D. Gutsche, J. Am. Chem. Soc. 107 (1985) 6063.
[9] S. Shinkai, S. Mori, H. Koreishi, T. Tsubaki, O. Manabe, J. Am. Chem. Soc. 108 (1986) 2409.
[10] S. Shinkai, K. Araki, O. Manabe, J. Am. Chem. Soc. 110 (1988) 7214.
[11] S. Shinkai, K. Araki, T. Matsuda, O. Manabe, Bull. Chem. Soc. Jpn. 62 (1989) 3856.
[12] S. Shinkai, K. Araki, T. Matsuda, N. Nishiyama, H. Ikeda, I. Takasu, M. Iwamoto, J. Am. Chem. Soc. 112 (1990) 9053.