Tetrahedron Letters No. 8, pp 643 - 646, 1974. Pergamon Press. Printed in Great Britain.

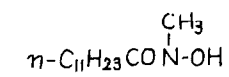
ORGANIC CATALYST OF ENZYME ACTIVITY, N-METHYL-N-LAUROYLHYDROXAMIC ACID IN CTAB MICELLE

Iwao Tabushi*, Yasuhisa Kuroda and Shigeki Kita
Department of Pharmaceutical Science,
Kyushu University, Fukuoka, 812 Japan

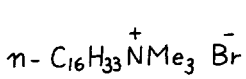
(Received in Japan 8 December 1973; received in UK for publication 17 January 1974)

Preparation of a simple organic catalyst of enzyme *activity* has been attracted physical organic, organic and bioorganic chemists'attentions. Some organic catalysts whose behavior were similar to chymotrypsin, one of the best understood enzymes, have been found[1] and among them are especially important some derivatives of hydroxamic acid[2], cycloamyloses[3] and their derivatives[4,5] in the sense that the catalysis consisted of the acyl transfer to the C ·ion of the catalyst and the (usually rate determining) hydrolysis of O-ester thus formed, although the catalytic activities (rate constants of the catalytic hydrolyses) of these catalysts were still considerably lower than that of chymotrypsin itself[6] (see Table 1).

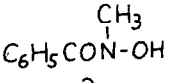
Now the authors wish to report that N-methyl-N-lauroylhydroxamic acid, 1, when used in a CTAB (2) micelle in an alkaline condition, displayed a very large catalytic acitivty, the largest catalytic constant among the chymotrypsin-type catalysts (see Table 1) and greater than or close to the activity of chymotrypsin itself. The present system was found to fit the Michaelis-Menten kinetics (see Fig 1 for the modified Michaelis-Menten treatment[10]). In Fig. 2 are shown the



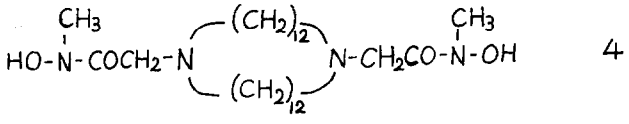
1



2



3



4

log k-pH relationship observed, demonstrating from pKa that the active species to which the acyl

*To whom correspondence should be addressed.

Fig. 1 Modified Michaelis-Menten Plot. [p-$NO_2\phi$OAc] = 2 × 10^{-5}M, [1]/[Micelle] = 2

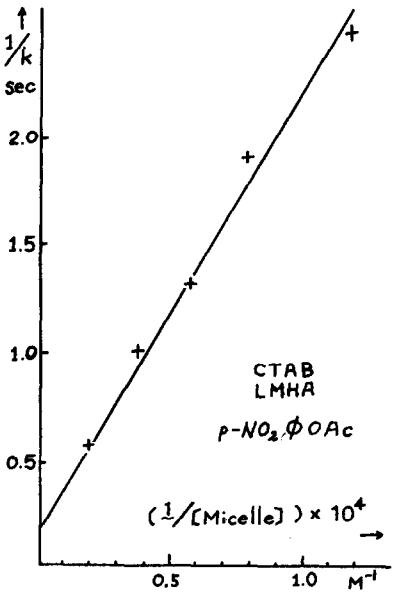
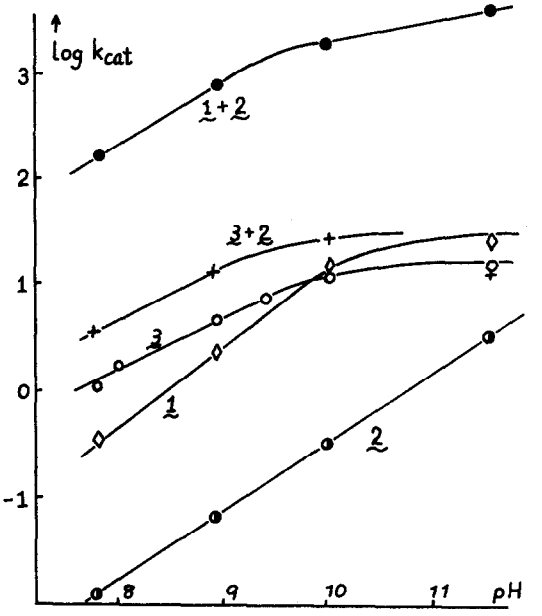


Fig. 2 Log k-pH Plot for p-$NO_2\phi$OAc



transfer took place from p-nitrophenyl carboxylate was the hydroxamate ion. In Table 1 are listed the catalytic constants of the catalysts. Interesting to note was that 1 or 3 alone showed only a small catalytic effect even in alkaline condition. The catalytic constant of a mixed micelle, 2 + 3, $k_{cat}(2 + 3)$ was only ca. twice as large as $k_{cat}(3)$ (k_{cat} at pH 9.99), but $k_{cat}(1 + 2) / k_{cat}(1)$ at pH 8.96 was observed remarkably larger to be 333/1. The marked difference in the acceleration demonstrated that only the *hydrophobic* hydroxamate ion afforded the extraordinarily great catalytic effect when it was bound in the ammonium micelle. K_{diss}, k, and $k_{cat} = k/K_{diss}$ for p-nitrophenyl acetate are ;1.38 × 10^{-3}M, 6.67 sec^{-1} and 4,830 $sec^{-1}M^{-1}$, respectively.

The enhanced catalytic effect of the hydrophobic hydroxamate suggests that hydroxamate ion in a micelle becomes a strong nucleophile probably because of reasonable separation from a gegen cation, poor hydration and (still) polar atomosphere[11] (DK ~ 36) as in an aprotic polar solvent. The abnormally great nucleophilicity of Ser^{195}-O^- in the active site of chymotrypsin may be partly due to the above type of strengthening of nucleophilicity of oxy anion in a polar non-

aqueous region.

Acknowledgement The auther wish to acknowledge partial support of this research from Takeda Pharmaceutical Co. Research Fund.

REFERENCES

1. R. Breslow "Studies on Enzyme Model" in "Bioinorganic Chemistry" R.F. Gould, Ed., Amer. Chem. Soc., Washington (1971).
2.a) W.B. Gruhn and M.L. Bender, J. Amer. Chem. Soc., 91, 5883 (1969).
b) R. Hershfield and M.L. Bender, ibid., 94, 5009 (1972).
3.a) M.L. Bender, R.L. Van Etten, G.A. Clowes and J.F. Sebastian, ibid., 88, 2318, 2319 (1966).
b) R.C. Van Etten, J.F. Sebastian, G.A. Clowes and M.L. Bender, ibid., 89, 3242, 3253 (1967).
4.a) F. Cramer, and G. Mackersen, Angew. Chem., 78, 641 (1966).
b) F. Cramer, and G. Mackersen, Chem. Ber., 103, 2138 (1970).
5. R. Breslow, and L.E. Overman, J. Amer. Chem. Soc., 92, 1075 (1970).
6. Most important characteristics of enzyme catalysis on the viewpoint of chemistry seem to be enormous acceleration, specificity (or selectivity) toward substrates and large turn-over number.
7. C.Gitler, and A. Ochoa-Solano, J. Amer. Chem. Soc., 90, 5004 (1968).
8. M.L. Bender, and K. Nakamura, J. Amer. Chem. Soc., 84, 2577 (1962).
9. C.J. Golbow, and A.E. Axelrod, J. Biol. Chem., 234, 294 (1959).
10. F.M. Menger, and C.E. Portnoy, J. Amer. Chem. Soc., 89, 4698 (1967).
11. P. Mukerjee, and A. Ray, J. Phys. Chem., 70, 2144 (1966).

Table 1 Rate constants for p-nitrophenol release from p-nitrophenyl acetate by 1,2,3 and other chymotrypsin type catalysts

Cat.	$k_{obs} \times 10^3$ (sec^{-1})[a]	k_{cat} ($sec^{-1}M^{-1}$)[b]	pH
None	0.241	——	8.96
1	0.640	2.84	8.96
2	0.279	0.065	8.96
3	1.11	6.0	8.96
1 + 2	142	945 [c]	8.96
1 + 2	312	2060 [c]	9.99
2 + 3	2.54	15.1 [c]	8.96
α-CD [d),3]	17.9	1.10	10.60
α-CD [d),3]	46.6	3.97	10.60
β-CD-Imd [e),4]	4.00	1.18	8.50
α-CD-PCA-Ni^{2+} [f),5]	3.22	0.322	5.71
MirHis-CTAB [g),7]	—	6.28	7.2
MirHis-CTAB [g),7]	—	122 [h]	7.2
(5) [2]	—	0.693	6.80
(4) [2]	—	1.18	6.80
(4) [2]	—	152 [i]	6.80
α-Chymotrypsin [j),8]	—	563	7.94
α-Chymotrypsin [k),9]	—	1.5×10^7	8.0

$((CH_3)_2CHCH_2)_2NCH_2\overset{O}{\overset{\|}{C}}\underset{CH_3}{\underset{|}{N}}OH$ (5)

a) 22°c, borate buffer, 5% aqueous acetonitrile, N-methylhydroxamic acid 1.5×10^{-4}M, CTAB 1.0×10^{-3}M and p-nitrophenyl acetate 0.5×10^{-4}M.

b) $(k_{obs}^{cat}-k_{obs}^{none})$/[N-methylhydroxamic acid].

c) $(k_{obs}^{cat}-k_{obs}^{2})$/[N-methylhydroxamic acid].

d) cyclodextrin

e) imidazolylmethyl ether of CD.

f) a complex of PCA, Ni^{2+} and CD-pyridinedicarboxylic acid monoester.

g) N^{α}-myristoyl-L-histidine.

h) p-Nitrophenyl hexanoate

i) p-Nitrophenyl dodecanoate.

j) The acylation of chymotrypsin.

k) p-Nitrophenyl ester of N^{α}-acetyl-L-tryptophan.