Tetrahedron Letters, Vol.29, No.3, pp 311-312, 1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

CATALYTIC PROPERTIES OF DIMETHYL-β-CYCLODEXTRIN BEARING IMIDAZOLYLETHYL GROUP. pH DEPENDENCE AND REGIOSELECTIVITY OF THE HYDROLYSIS OF NITROPHENYL ACETATES

Hiroshi Ikeda, Ryoichi Kojin, Chul-Joong Yoon,
Tsukasa Ikeda, and Fujio Toda*

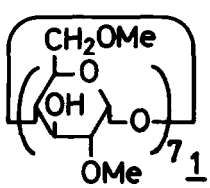
Department of Bioengineering and Bioscience, Faculty of Engineering, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152, Japan

Catalytic properties of dimethyl-β-cyclodextrin bearing imidazolylethyl group (2) were demonstrated. pK_a of 2 was 7.28. $(k_{cat})_{max}$ for the hydrolysis of p-nitrophenyl acetate was 3×10^{-2} s^{-1}. 2 showed para-selectivity for the hydrolysis of nitrophenyl acetates and caused 921-fold acceleration.

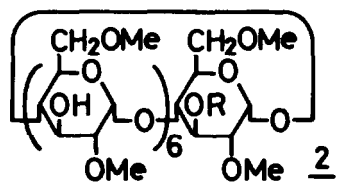
Dimethylcyclodextrins (DMCDs) are a series of cyclic oligomers consisting of α-1,4-linked 2,6-di-O-methyl D-glucopyranose units and have quite unique properties which are different from those of cyclodextrins (CDs).[1,2] Therefore much attention has been paid to DMCDs. Recently, we firstly reported the useful method for the modification of β-DMCD and its reactivity as an enzyme model.[1] β-DMCD bearing imidazolylethyl group (2) caused extensive acceleration of the hydrolysis of p-nitrophenyl acetate (PNPA), whereas β-DMCD (1) depressed the reaction.[1] Then, more detail features of 2 are of great interest. In this report, we describe the important catalytic properties of 2 such as the pH dependence of catalytic activity and the substrate specificity.

The rate of hydrolysis reaction of PNPA was measured under the conditions of large excess of substrate at 25 °C in phosphate buffers of different pH values. Kinetic parameters were calculated by Michaelis-Menten's treatment.[1] log(k_{cat}) vs. pH is shown in Fig. 1. The relationship between k_{cat} and pH can be represented by eq 1.[3] pK_a (=pK_{ES}) and $(k_{cat})_{max}$ of 2 were calculated by the nonlinear-least-squares fitting of kinetic data to eq 1. pKa is 7.28. It indicates that imidazolyl group may play on an important role in the rate determining step and that 2 can cause sufficient catalytic activity around neutral pH condition. $(k_{cat})_{max}$ is 3×10^{-2} s^{-1}. It is larger than that of α-chymotrypsin.[5]

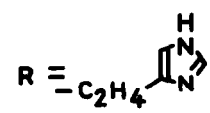
Next, the regioselectivity for the hydrolysis of nitrophenyl acetate isomers by 2 was demonstrated. The reactions were carried out under the con-



1



2



ditions of excess of substrate in pH 8.2 phosphate buffer. Kinetic parameters are shown in Table 1. Both k_{cat} and k_{cat}/K_m for PNPA are larger than others. k_{cat}/K_m for PNPA is 6 times larger than that for o-nitrophenyl acetate (ONPA). The ability of rate acceleration can be estimated by k_{cat}/k_{un} (Table 1). k_{cat}/k_{un} for PNPA is also largest. These are contrast to the case of unmodified cyclodextrins which have meta-selectivity.[4] It may be due to the difference of the geometry of inclusion complex, especially distance between reaction centers.

K_m values indicate that 2 mostly tends to form inclusion complex with m-nitrophenyl acetate (MNPA) and it is also different from unmodified cyclodextrins.[4]

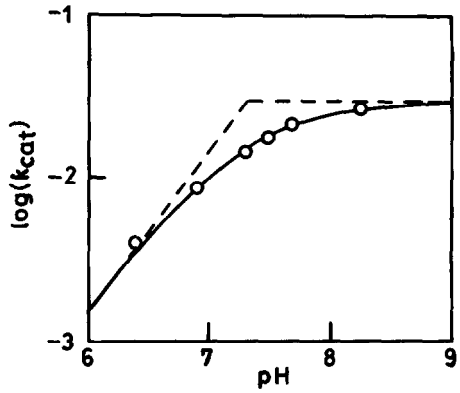


Fig. 1. Plot of the pH dependence of k_{cat} for the hydrolysis of PNPA by 2.

$$k_{cat} = \frac{(k_{cat})_{max} \cdot K_{ES}}{K_{ES} + [H^+]} \quad (1)$$

Where K_{ES} is the ionization constant of the enzyme-substrate complex.

Table 1. Kinetic parameters for the hydrolysis of nitrophenyl acetate by 2

substrate	k_{cat} / 10^{-3} s^{-1}	K_m / 10^{-3} mol dm^{-3}	k_{cat}/K_m / s^{-1} mol^{-1} dm^3	k_{un} / 10^{-5} s^{-1}	k_{cat}/k_{un} / -
ONPA	4.96	3.22	1.54	1.93	257
MNPA	4.48	1.27	3.54	1.40	320
PNPA	26.7	2.90	9.20	2.90	921

at 25 °C in pH 8.2 phosphate buffer, [2] = 1.75×10^{-5} mol dm^{-3}, concentrations of ONPA, MNPA, and PNPA were from 6.3×10^{-5} to 5.3×10^{-3} mol dm^{-3}.

k_{cat} for ONPA is 1.1 times larger than that for MNPA and K_m for the former is 2.5 times larger than that for latter. Therefore k_{cat}/K_m for the latter is 2.3 times larger than that for the former. It shows that the ability to form inclusion complex is important for accelerating the rate.

In the conclusion, pK_a of 2 was 7.28 and $(k_{cat})_{max}$ was 3×10^{-2} s^{-1} for the hydrolysis of PNPA by 2. 2 had para-selectivity for the hydrolysis of nitrophenyl acetate isomers and it is contrast to unmodified cyclodextrins. 2 made 921-fold increase in the rate of hydrolysis of PNPA at pH 8.2.

References

1) H. Ikeda, R. Kojin, C-J. Yoon, T. Ikeda, and F. Toda, Chem. Lett., 1987, 1495.
2) J. Szejtli, J. Inclusion Phenom., 1, 135 (1983).
3) A. Fersht, "Enzyme Structure and Mechanism," 2nd ed, Freeman (1985).
4) R. L. VanEtten, T. F. Sebastian, R. C. Clowes, and M. L. Bender, J. Am. Chem. Soc., 89, 3242 (1967).
5) V. T. D'Souza, K. Hanabusa, T. O'Leary, C. R. Gadwood, and M. L. Bender, Biochem. Biophys. Res. Commun., 129, 727 (1985).

(Received in Japan 21 July 1987)