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The Catalytic Activities of IgG and IgM Monoclonal Antibodies for the Hydrolysis of *p*-Nitrophenyl Acetate

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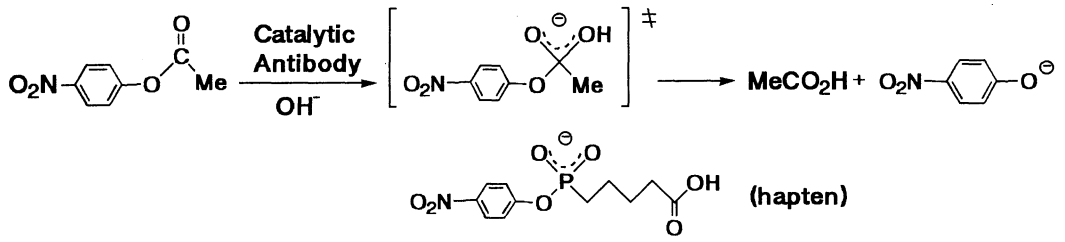
The catalytic activities of IgG and IgM monoclonal antibodies (Antibody KD2-1 - KD2-260) derived from a hapten of *p*-nitrophenyl hydrogen 4-(hydroxycarbonyl)butyl-phosphonate were examined in the hydrolysis of *p*-nitrophenyl acetate in 2.4 - 4.0% (v/v) MeCN-H_2O in Tris-HCl buffer (pH 5.0 - 8.0) at 293 - 308 K. The most efficient Antibody KD2-260 resulted in k_{cat} / k_{uncat} = 2.1 x 10^3 (308 K) - 3.1 x 10^3 (293 K), K_m = 4.9 x 10^{-6} (293 K) - 1.5 x 10^{-5} mol dm^{-3} (308 K), and K_m / K_i = 80 (303 K) at pH 6.0.

Since monoclonal antibodies complementary to transtion state analogues (TSA) have been found to catalyze the hydrolysis of *p*-nitrophenyl N-trimetyl carbonate in 1986,[1] the catalytic efficiency of IgG monoclonal antibodies prepared with TSA have hitherto been tested as catalytic antibodies, in the hydrolyses of carboxylic acis esters,[2-6] carbonate esters,[5-7] and amides.[8] The acivities of catalytic antibodies have been appraised by the rate ratio (k_{cat} / k_{uncat}) of the catalyzed and uncatalyzed reactons, and the reported k_{cat} / k_{uncat} values for the hydrolyses of the carbonate and carboxylic acid esters with the catalytic antibodies prepared by the haptens of the phosphonate derivatives fall in the range of 770 (*p*-nitrophenyl N-trimethyl carbonate)[1] - 6.5 x 10^5 (caroboxylic aryl ester).[3] In the present paper, we wish to report the esterolytic activities of catalytic IgG and IgM antibodies obtained with a hapen of *p*-nitrophenyl hydrogen 4-(hydroxycarbonyl)butylphosphonate for the hydrolysis of *p*-nitrophenyl acetate by taking notice of the analogy between the transition state (TS) and TSA (or the hapten) in their electronic structures and of the evaluated kinetic parameters including activation ones.



The IgG and IgM antibodies (purities > 98%) were obtained respectively by the immunization of BALB / c mice injected with *p*-nitrophenyl hydrogen 4-(hydroxycarbonyl)butylphosphonate (hapten)-KLH conjugate as follows: the immunized mice spleen cells-myeloma ones (P3-X63-Ag8-U1) hybridoma supernatants possessing high anti-hapten activities, which were screened by an ELISA technique and were grown in RPMI supplemented with 10% fetal calf serum, were concentrated on FILTORON Mini-Ultrasette™ by using a 10000 MW cut-off membrane to apply the concentrated ones to γ Protein A-agarose (Repligen) column and were then dialyzed against 20 mmol dm^{-3} Tris-HCl buffer (pH 8.0) to obtain purified IgG monoclonal antibodies. The culture supernatants containing IgM monoclonal antibodies were also concentrated on FILTORON Mini-Ultrasette™ by using a 50000 MW cut-off membrane and were treated with 50% ammonium sulfate to precipitate antibodies which were then dissolved in phosphate buffered saline (PBS) and purified by TSKgel G3000SW column (21.5 mmID x 60 cm) with PBS.

When a number of Ig G and Ig M monoclonal antibodies complementary to *p*-nitrophenyl hydrogen methyl phosphonate (TSA) were supplied for the present hydrolysis of *p*-nitrophenyl acetate, seven catalytic antibodies indicating the high binding activities resulted in the relatively large k_{cat}^{app} (apparent second-order catalytic rate constant) values for the hydrolysis reaction at 303 K (Table 1). Among the

Table 1. Apparent second-order catalytic rate constants for the hydrolysis of *p*-nitrophenyl acetate with catalytic antibodies[a)]

Clone number	Type H, L (clone)	Relative binding activity[b)]	$10^{-5}\ k_{cat}^{app}$[c)] / $mol^{-1}\ dm^3\ min^{-1}$	(pH)
KD2-3	γ2b, κ (IgG)	0.34	1.09	(8.0)
KD2-103	γ1, κ (IgG)	0.26	0.97	(8.0)
KD2-260	γ2b, κ (IgG)	2.73	1.45	(8.0)
			0.61[d)]	(8.0)
			1.16[d)]	(7.0)
			3.82	(6.0)
			4.42[d)]	(6.0)
			1.88[d)]	(5.0)
KD2-56	μ, κ (IgM)	2.78	1.38	(8.0)
KD2-173	μ, κ (IgM)	2.59	1.54	(8.0)
KD2-182	μ, κ (IgM)	3.04	3.10	(8.0)
KD2-201	μ, κ (IgM)	2.74	4.37	(6.0)
			3.42	(8.0)

a) [substrate] = 3.0 x 10^{-6} mol dm^{-3} and [antibody] = 3.8 x 10^{-8} mol dm^{-3} in 2.4% (v/v) MeCN/H_2O (Tris-HCl buffer, pH 5.0 - 8.0) at 303 K. b) Measured as the optical density at 450 nm by the ELISA method with the immunoglobulin-horseradish peroxidase conjugate. c) Evaluated from (k_{obs} - k_{uncat}) / [antibody] (k_{obs} or k_{uncat} = pseudo-first-order rate constant obtained with or without an antibody). d) Measured in 4% (v/v) MeCN/H_2O (Tris-HCl buffer, pH 5.0 - 8.0) at 303 K.

monoclonal antibodies tested, an antibody (Antibody KD2-260) catalyzed the present hydrolysis reaction most efficiently. The catalytic activity of Antibody KD2-260 for the ester hydrolysis in the cavity through the incoporation of the ester substrate by the antibody was then investigated by the Michaelis-Menten analysis of the present reaction (Table 2). When the evaluated kinetic parameters of K_m = 4.9 x 10^{-6} (293

Table 2. Kinetic parameters for the hydrolysis of *p*-nitrophenyl acetate catalyzed by Antibody KD2-260[a)]

Temp	10^7 V_{max}	10^5 K_m	k_{cat}	k_{cat}/k_{uncat}
K	mol dm^{-3} min^{-1}	mol dm^{-3}	min^{-1}	
293	0.83	0.49	2.53	3.12×10^3
298	1.09	0.59	3.34	2.71×10^3
303	1.80	0.96	5.52	2.33×10^3
308	2.58	1.50	7.91	2.09×10^3

a) [antibody] = 3.26×10^{-8} mol dm^{-3} and [substrate] = 3.0×10^{-6} - 1.2×10^{-5} mol dm^{-3} in 4.0% (v/v) $MeCN/H_2O$ (Tris-HCl buffer, pH 6.0).

K) - 1.5×10^{-5} mol dm^{-3} (308 K) and k_{cat} = 2.53 (293 K) - 7.91 min^{-1} (308 K) are compared with those (K_m (303 K) = 4.30×10^{-4} mol dm^{-3} and k_{cat} (303 K) = 7.4 min^{-1}) obtained by Schultz *et al.*[5)] for the hydrolysis of *p*-nitrophenyl acetate with the most efficient catalytic antibody (Antibody 48G7-4A1) complementary to the same TSA, the present catalytic antibody (Antibody KD2-260) was found to be also efficient in comparison with the Schultz's antibody (Antibody 48G7-4A1) in terms of the antibody-substrate complex formation (reflected by K_m) and the reaction of the complex with OH^- in the antibody cavity (reflected by k_{cat}). The efficient activity of Antibody KD2-260 was also supported by the large ratio of k_{cat}/k_{uncat} = 2.1×10^3 (308 K) - 3.1×10^3 (293 K), the values of which are appreciably larger than k_{cat}/k_{uncat} = 1.6×10^3 (303 K) reported by Schultz *et al.*[5)] for the same hydrolysis reaction with Antibody 48G7-4A1.

In regard to the catalytic activity of Antibody KD2-260 for the recognition of the TS of the present hydrolysis reaction, the inhibitor (I) of *p*-nitrophenyl hydrogen pentylphosphonate brought about K_i = 1.2×10^{-7} mol dm^{-3} in the Dixon's analysis[9)] of Antibody KD2-260 (3.26×10^{-8} mol dm^{-3})-catalyzed hydrolysis of *p*-nitrophenyl acetate (3.0×10^{-4} and 6.0×10^{-4} mol dm^{-3}) at 303 K (pH 6.0). Although the catalytic antibodies capable of recognizing TS entirely establish the correlation of $K_m / K_i = k_{cat} / k_{uncat}$[10)] so long as the inhibitor *per se* is corresponding to TS completely, the present K_m/K_i ratio of 80 was correponding to $(k_{cat}/k_{uncat})/29$, the value of which fell in the usual range of $(10^{-2} - 10^{-1})$ k_{cat}/k_{uncat}.[11)] In this respect, the electronic structures of TS and TSA (or the inhibitor), which were respectively computed as the simplified species of Me-C(=O)(OH^-)-OMe and Me-P(=O)(O^-)-OMe with the *ab initio* STO-3G basis set, are approximately the same each other in their structures, but they are appreciably different in their atomic charges, as indicated in Fig. 1. Anyway, it can be mentioned that Antibody KD2-260 exhibits the catalytic activity efficiently through the stabilization of the OH^- attacked ester (TS) in the hydrolysis reaction.

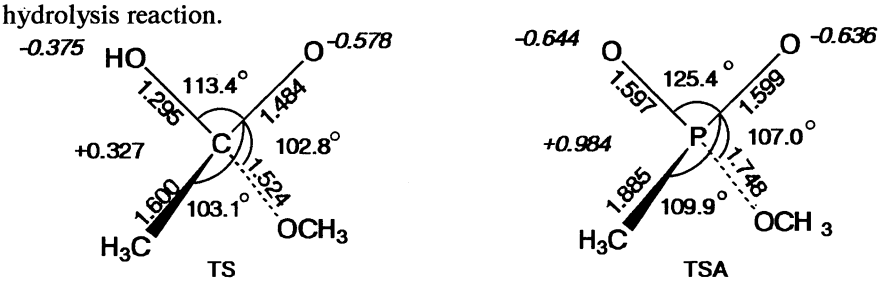


Fig. 1. Electronic structures of simplified TS and TSA (*ab initio* STO-3G calculation).

The catalytic activity of Antibody KD2-260 for the *p*-nitrophenyl acetate hydrolysis will now be discussed on the basis of the activation parameters listed in Table 3. The hydrolysis reaction of the ester

Table 3. Activation parameters for the hydrolysis of *p*-nitrophenyl acetate catalyzed by Antibody KD2-260[a)]

	k_{cat}^{app}	k_{cat}	k_{uncat}
$\Delta H^{\ddagger}$ / kcal mol^{-1}	7.4	13.8	21.1
$\Delta S^{\ddagger}$ / cal K^{-1} mol^{-1}	-7.8	-17.6	-9.2
$\Delta G^{\ddagger}$ / kcal mol^{-1} (at 298-308 K)	9.7-9.8	19.3-19.9	23.8-23.9

a) [antibody] = 3.26 x 10^{-8} mol dm^{-3} and [substrate] = 3.0 x 10^{-6} mol dm^{-3} in 4.0% (v/v) MeCN/H_2O (Tris-HCl buffer, pH 6.0).

substrate and OH^- was favored in the cavity of Antibody KD2-260, as reflected by the smaller $\Delta G^{\neq}$ (or $\Delta H^{\neq}$) values for the catalyzed reaction (k_{cat}^{app} and k_{cat}) than that for the uncatalyzed one (k_{uncat}); the apparent activation barrier (9.7 - 9.8 kcal / mol) for Antibody KD2-260 catalyzed reaction is one half of that (23.8 - 23.9 kcal / mol) for the uncatalyzed one. Therefore, it can be concluded that Antibody KD2-260 worked efficiently as a catalytic antibody for the present hydrolysis reaction.

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