ปีการศึกษา 2546 - 2547	ประเภทของผลงานทางวิชาการ : บทความวิจัย
ชื่อเรื่อง	**Improvement in Hydrolytic Antibody Activity by Change in Haptenic Structrure from Phosphate to Phosphonate with Retention of a Common Leaving-group determinant: Evidence for the "Flexibility" Hypothesis**
วารสารที่ตีพิมพ์	**Biochemical Journal 376,813 – 821, 2003**

Improvement in Hydrolytic Antibody Activity by Change in Haptenic Structrure from Phosphate to Phosphonate with Retention of a Common Leaving-group determinant: Evidence for the "Flexibility" Hypothesis

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To investigate the hypothesis that decreased hapten flexibility may lead to increased catalytic antibody activity, we used two closely related immunogens differing only in the flexibility of the atomic framework around the structural motif of the haptens, analogous to the reaction centre of the corresponding substrates. Identical leaving-group determinants in the haptens and identical leaving groups in the substrates removed the ambiguity inherent in some data reported in the literature. Anti-phosphate and anti-phosphonate kinetically homogeneous polyclonal catalytic antibody preparations were compared by using carbonate and ester substrates respectively, each containing a 4-nitrophenolate leaving group. Synthetic routes to a new phosphonate hapten and new ester substrate were developed. The kinetic advantage of the more rigid anti-phosphonate/ester system was demonstrated at pH8.0 by a 13-fold advantage in $k_{cat}/k_{non\text{-}cat}$

and a 100-fold advantage in the proficiency constant, $k_{cat}/k_{non\text{-}cat} \cdot K_{m}$. Despite these differences, the pH-dependences of the kinetic and binding characteristics and the results of chemical modification studies suggest closely similar catalytic mechanisms. The possible origin of the kinetic advantage of the more rigid happten/substrate system is discussed.

Key words : flexibility of hapten, hydrolytic catalytic antibody, kinetic characterization, leaving-group determinant, phosphonate synthesis.

Abbreviations used : EDC, 1-(dimethylaminopropyl) –3-ethylcarbodi-imide; KLH, keyhole-limpet haemocyanin; NSS, normal sheep serum; PCA, polyclonal catalytic antibody preparation; PCA 271-22(etc.), PCA isolated from the antiserum of sheep no. 271 in week 22 of the immunization programme (etc.).

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