利用酵素與奈米微粒之生物分子交互作用調控催化活性

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摘　　要

蛋白質表面的修飾與辨識在生物科學領域裡常用來探討蛋白質與蛋白質交互作用及酵素催化活性，在本篇研究中，我們利用奈米微粒本身對生物分子的作用力將酵素修飾於粒子的表面並探討其酵素特性，我們發現酵素固定於奈米微粒上其活性會有顯著性的增加，利用動力學及熱力學實驗分析此具有酵素催化功能的奈米粒子，我們能夠解釋和預測其反應行為並了解此增強活性之機制。由於奈米微粒與生物分子的尺寸互相匹配，因此奈米粒子常用於酵素固定化之基材，在接下來的研究中，我們嘗試將酵素修飾於不同大小之奈米微粒，我們發現當固定於較小的粒子時，其反應速率明顯增強，經由進一步的動力學實驗發現，此奈米微粒的粒徑大小會影響此酵素與反應基質之親和力以至於改變其催化活性與行為。為了解釋奈米微粒在反應過程所扮演之角色，我們提出一套理論解釋此現象，由於不同大小之奈米微粒有不同的立體障礙效應，其碰撞機率會隨著粒徑變小而增加，因此對催化速率產生影響。本項研究包含了實驗數據以及理論探討，並詳細地解釋奈米粒子與酵素之間的交互作用，說明奈米微粒對於酵素催化行為所扮演的角色。在生物體中酵素活性的調控與生長、合成和代謝等反應直接相關，利用奈米微粒調控催化活性的能力對於日後在奈米生物領域上的發展有所貢獻。

Regulation of Catalytic Activity *via* the Biomolecular Interactions Based on Enzyme–Nanoparticle Conjugates

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Abstract

Protein surface recognition provides an appealing tool to regulate protein–protein interactions and enzymatic activities in the field of biological science. We are interested in using nanoparticle (NP) to bind enzyme surfaces through multivalent interactions and then engineer the protein properties. In this study, we have investigated the activity of the enzyme–NP conjugates and demonstrated that adsorbing enzyme onto NPs significantly increased its enzymatic activity. We ascribe this event of the enzymatic reactions by kinetic and thermodynamic studies, which provide a way of understanding and predicting the catalytic behaviors of the enzyme-functionalized NPs. In addition, NPs are excellent systems for modeling enzymes' surfaces because they can be readily fabricated on size scales comparable with those of their biomolecular targets. Therefore, we were curious to study whether varying the dimensions of the NPs would affect their catalytic reactions. We have developed a series of kinetic experiments to systematically analyze the NP size–dependent enzymatic activities, and have developed a model to explain the phenomenon. Kinetic studies revealed that association of enzyme with NPs did not influence the turnover number, but smaller NPs did promote the catalytic efficiency of enzyme by increasing its kinetic affinity. A shielding model, based on diffusion–collision theory, explains the correlation between the size effects and the kinetic responses of the enzyme–NP conjugates. This size-effect model provides chemical and physical meaning, leading to the observed substrate specificities and catalytic constants. From the combined kinetic and theoretical investigation of enzyme bound to NPs, we found that these conjugates acted as a controllable and efficient factor for modulating the activity of the enzyme. In nature, controllable modulation of enzyme activity is a potent means of regulating several cellular processes (e.g., signal transduction, biosynthesis, metabolism). The modulation of biocatalytic behavior is an attractive feature for exploitation in the field of nanobiotechnology.

Acknowledgment

碩士班口試時我被問的第一個問題：『中書，怎麼致謝內容都沒有提到人名，你怎麼能夠保證十年、二十年之後你還會記得?』。其實當下我想回答：『會被我忘記的人，怎麼還需要感謝?』。正如同碩士論文致謝裡的一段話：『該感謝的人一個都不能少，不該感謝的人一個都不能多，不然就失去了這一頁的意義~』。然而，現在，三年過去了，值得慶幸的是我依舊記得他們，此頁獻給那些曾經真正在乎我、了解我，而我該感謝的人。

最近常常聽到的一句話：『哇!中書，你今年就可以畢業了喔!』。接下來就是一些，你很優秀、很厲害、很順利之類的客氣話~然而每當這些話語過後，我都會試想這個人到底了解我多少。我想，如果是真的認識我的人，應該不會對我說這些客套話吧!這三年，我只是很認真的去做每件事，我不優秀但我願意花時間去學；我不厲害但我可以犧牲睡眠時間把事情盡快完成；投稿不順利但我會在最短的時間內修改完回覆給教授。真正認識我的人，我做的一切他們都看在眼中，我的付出我的努力，他們不會用優秀、厲害、順利，這樣的字眼帶過，而這樣的人，才有資格放在這頁裡，如果把他們的名字也跟別人列在一起，那是否對我該感謝的人也太不尊重了些?

『肩膀，要夠大』，這是一位學長曾經跟我說的話，他是我在研究領域上的啟蒙者，很多好(或不好)的觀念，都是他影響了我，而我也繼續傳承下去。這句話涵義很深，在博士班生涯中我已體會箇中滋味，在未來的旅程中隨著年齡的增長，角色和責任會越來越多，學習如何『扛』下，我還需要更多的學習和歷練；『馬步站穩，眼光放遠』，這是另一位影響我很深的學長在信件往返中所送我的話，這句話代表他做人做事的準則，講求把『基本功』練好，著眼未來就會有好的發展空間。他是我人生道路上的典範，也許是因為人生經驗相差太多，往往跟他討論時我能夠得到不同角度分析事情的看法，雖然很遺憾跟他學習的時間並不多，但對我卻很受用；對指導教授的感謝，相信這三年來我已用行動表達了我的感謝之意，有幸在教授的門下盡情發揮，著實讓我學習、體驗到不少事物，也因為有你，我們大家才有相處在一起的機會。

在這頁的最後，獻上最大的感謝之意給我的家人，包括：媽媽、哥哥以及女朋友，你們是我的避風港，當我情緒低落、失意的時候提供一個溫暖、無私的空間，因為是我的家人，任性的一面才能在你們面前表露，所以這三年給予了你們莫大的壓力，但也正因為你們，我才能很優秀、很厲害、很順利的拿到這該屬於我們一家人的—博士學位。

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K^*_m of immobilized CRL has converged to 22.09 μM with four significant figures after five successive approximation (r = 1–5). (c) The predicted K^*_m values, the slopes of regression lines fitting lines in eq (5), of immobilized CRL are the result of three set of independent experiment data (n = 1–3).

List of Abbreviations

ATP	adenosine triphosphate
AuNPs	gold nanoparticles
CD	circular dichroism
ChT	α-chymotrypsin
CRL	*Candida rugosa* lipase
Cy5	cyanine dye 5
DI water	deionized water
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
Fab	antigen-binding fragment
FTIR	fourier transform infrared spectroscopy
IPA	propan-2-ol
IPP	image-pro plus
KCN	potassium cyanide
MMPCs	mixed monolayer protected gold clusters
MPTMS	3-mercaptopropyl-trimethoxy-silane
MRI	magnetic resonance imaging
*p*NP	*p*-nitrophenol
*p*NPP	*p*-nitrophenyl palmitate
NPs	nanoparticles
SAMs	self-assembled monolayers
SEM	scanning electron microscopy
SPR	surface plasmon resonance
XPS	X-ray photoelectron spectroscopy

List of Symbols

ν	reaction rate
V_{max}	maximum velocity
k_{cat}	turnover number
K_m	Michaelis constant
k_{cat}/K_m	specificity constant
E_{NP}	enzyme/NP conjugate
$k^{coll}_{E–NP}$	bimolecular rate constant
C_E	enzyme concentration
C_S	substrate concentration
Z	frequency of collisions
p	fraction of substrates with correct orientation
E_{act}	activation energy
R	gas constant
T	absolute temperature
Ω	open solid-angle fraction
η	shielding factor
r_{NP}	NP radii
r_E	enzyme radii
r_S	substrate radii
D_E	enzyme diffusion coefficient
D_S	substrate diffusion coefficient
N_E	enzyme number concentration
N_S	substrate number concentration
N_{Avo}	Avogadro's constant
k_B	Boltzmann constant
μ	viscosity of solution
k^{eff}_{max}	maximum frequency of effective collisions


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