UNIVERSITI PUTRA MALAYSIA

CHEMICAL MODIFICATION OP LIPASE AND ITS IMMOBILIZATION ON POLYMER BEADS FOR USE IN ORGANIC SYNTHESIS

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By

MAHIRAN BASRI

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DEDICATIONS

To my beloved grandmother....

who has taught me all there is about beauty,

love and human grace.

To abah and mak....

who have made me feel very special.

To adik-adik....

who have always given me the challenge to work harder.

To Jamal....

who has given me all the patience, love and support.

To Gaja and Jordie....

who make living a joyous happening.

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LIST OF ABBREVIATIONS

PEG	monomethoxypolyethylene glycol
PEG1900	monomethoxypolyethylene glycol (MW=1900)
PEG5000	monomethoxypolyethylene glycol (MW=5000)
APEG	activated PEG
TNBS	trinitrobenzene sulfonate
RCOOH	polycarboxylic acid
RCN	polyacrylonitrile
DVB	divinyl benzene
FPLC	Fast performance liquid chromatography
IR	infra red
NMR	nuclear magnetic resonance
NL	native lipase
Imidoester I	methyl acetimidate
Imidoester II	methyl benzimidate
Imidoester III	methyl 4-biphenyl benzimidate
Imidoester IV	methyl n-dodecanimidate
Imidoester V	methyl 3-phenylpropionimidate
Imidoester VI	methyl 4-phenylbutyrimidate
PL1900	lipase modified with PEG1900
PL5000	lipase modified with PEG5000
IL(I)	lipase modified with imidoester I
IL(II)	lipase modified with imidoester II
IL(III)	lipase modified with imidoester III
IL(IV)	lipase modified with imidoester IV
IL(V)	lipase modified with imidoester V
IL(VI)	lipase modified with imidoester VI
AL	lipase modified with acetaldehyde (ethyl lipase)
DL	lipase modified with dodecyldehyde
XAD2NL	native lipase immobilized on XAD2
XAD2PL	PEG-lipase immobilized on XAD2
XAD7NL	native lipase immobilized on XAD7
XAD7PL	PEG-lipase immobilized on XAD7
XAD7IL	imidoester lipase immobilized on XAD7
XAD7AL	ethyl lipase immobilized on XAD7
RCOOHNL	native lipase immobilized on RCOOH
RCOOHPL	PEG-lipase immobilized on RCOOH

Abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

CHEMICAL MODIFICATION OF LIPASE AND ITS IMMOBILIZATION ON POLYMER BEADS FOR USE IN ORGANIC SYNTHESIS

By

Mahiran Basri

April 1993

Chairman: Associate Professor Dr. Kamaruzaman Ampon

Faculty: Science & Environmental Studies

A simple and effective method to produce a more active, stable and practical lipase preparation was identified. Soluble lipase from *Candida rugosa* was modified with different types of hydrophobic chemical modifying reagents.

The esterification activities of the modified lipases were enhanced following their modification. The degree of activity enhancement depends on the type and molecular weight of the modifiers used and the degree of modification of the enzyme. A lower degree of enzyme derivatization was required for modification with the high molecular weight modifiers to attain maximal activities. In the case of monomethoxypolyethylene glycol (PEG), however, maximal activity was attained only after exhaustive modification.

The optimum esterification temperature and preference of fatty acids as acyl donors of the modified lipases were very similar to those of the native enzyme. Both were more active in non-polar solvents than in polar solvents. The modified lipases showed higher thermostability, solvent stability and storage stability compared to the native lipase. The lipase modified with PEG1900 was the most thermostable, and that modified with methyl 4-phenylbutyrimidate (imidoester VI) was the most stable when incubated in benzene for ten days. The best storage condition was at low temperature and in the lyophilized form.

When porous polymer beads were added to a solution of the modified lipase at room temperature and stirred gently for 0.5 to 2 hours, the enzyme was strongly adsorbed onto them. Native lipase was only weakly adsorbed onto the supports.

The immobilized activity of modified lipases were higher compared with the native enzyme. The magnitude of the difference depended on the type of modifiers, the degree of modification of the enzyme and the type of polymers used for immobilization. Lipase that was modified with the more hydrophobic modifiers showed higher immobilized activity compared to those modified with the less hydrophobic modifiers (except for PEG5000). The highest immobilized activity was exhibited by lipase modified with PEG1900 (95% modification).

Amberlite XAD7, XAD8 and Polycarboxylic acid beads (RCOOH) were good supports for enzyme immobilization by the above methods.

The optimum esterification temperature of lipase immobilized in this manner was not changed. However, its preference for certain fatty acids as acyl donors was altered. Medium chain length fatty acids were favoured over longer chain fatty acids when the enzymes were immobilized. Their activities were increased even in the presence of polar solvents. The immobilized lipases also showed higher thermostability, solvent stability and storage stability compared with native and modified lipases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi keperluan Ijazah Doktor Falsafah.

PENGUBAHSUAIAN LIPASE SECARA KIMIA DAN SEKATGERAKANYA KEPADA BEBERAPA POLYMER UNTUK KEGUNAAN DALAM SINTESIS ORGANIK.

Oleh

Mahiran Basri

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Pengerusi : Prof. Madya Dr. Kamaruzaman Ampon

Fakulti : Sains & Pengajian Alam Sekitar

Satu kaedah yang mudah dan berkesan telah dikenalpasti untuk menghasilkan lipase yang lebih aktif, stabil dan praktikal. Lipase dari *Candida rugosa* yang terlarut telah diubahsuai dengan beberapa bahan pengubahsuai hidrofobik.

Lipase terubahsuai telah menunjukkan kelebihan aktiviti esterifikasi. Darjah pertambahan aktiviti bergantung kapada jenis dam berat molekul bahan pengubahsuai yang digunakan dan darjah ubahsuai enzim itu sendiri. Darjah yang lebih rendah adalah diperlukan bagi mengubahsuai enzim dengan bahan yang mempunyai berat molekul yang tinggi bagi mendapatkan aktiviti maksimum. Untuk monomethoxypolyethylena glycol (PEG), aktiviti maksimum didapati dengan mengubahsuai lipase habis-habisan.

Suhu optimum esterifikasi dan pemilihan terhadap asid lemak sebagai penderma kumpulan asil untuk lipase terubahsuai adalah sama dengan lipase natif. Selain dari itu, kedua-dua jenis lipase didapati lebih aktif di dalam pelarut organik yang tidak berkutub berbanding dengan di dalam pelarut organik yang lebih berkutub. Lipase terubahsuai menunjukkan kestabilan terma, kestabilan dalam pelarut organik dan kestabilan penyimpanan yang lebih tinggi. Lipase yang diubahsuai dengan PEG1900 menunjukkan kestabilan terma yang tertinggi dan lipase yang dimodifikasi dengan metil 4-fenilbutirimidat (imidoester VI) adalah yang paling stabil apabila dieram dalam benzena selama sepuluh hari. Keadaan penyimpanan terbaik adalah pada suhu rendah dan keadaan beku-kering.

Apabila butir-butir polimer yang berliang telah dimasukkan ke dalam larutan lipase terubahsuai pada suhu bilik dan digoncang selama 0.5-2 jam, enzim didapati terjerap lebih kuat. Lipase natif hanya terjerap dengan lemah kapada butir-butir polimer.

Lipase terubahsuai menunjukkan aktiviti tersekatgerak yang lebih tinggi berbanding dengan lipase natif. Penambahan aktiviti bergantung kepada jenis bahan pengubahsuai, darjah ubahsuai dan jenis polimer yang digunakan. Lipase yang diubahsuai dengan bahan yang lebih hidrofobik menunjukkan aktiviti tersekatgerak yang lebih tinggi berbanding dengan

lipase diubahsuai dengan bahan yang kurang hidrofobik (melainkan PEG5000). Lipase diubahsuai dengan PEG1900 (95% ubahsuai) memberikan aktiviti tersekatgerak paling tinggi. Amberlite XAD7, XAD8 dan polikarboksilik asid (RCOOH) adalah polimer yang baik untuk menyekatgerakan enzim dalam kajian ini.

Suhu optimum lipase disekatgerak seperti ini didapati tidak bertukar. Walau bagaimanapun, pilihan terhadap asid lemak sebagai penderma kumpulan asil didapati bertukar. Asid lemak rantai sederhana lebih disukai berbanding dengan asid lemak rantai panjang apabila enzim disekatgerak. Aktivitinya juga tinggi di dalam pelarut organik yang lebih polar. Lipase tersekatgerak menunjukkan kestabilan terma, kestabilan dalam pelarut organik dan kestabilan penyimpanan yang lebih tinggi lagi daripada lipase natif dan lipase terubahsuai.