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COMPARISON OF CATALYTIC ACTIVITIES BETWEEN ESTERASE AND LIPASE IN THE SYNTHESIS OF DRUG, FLAVOR AND AMIDE COMPOUNDS

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ABSTRACT

Synthesis of drugs, flavoring and amide compounds by PLE (Pig Liver Esterase) and PPL (Pig Pancreatic Lipase) prepared earlier in this laboratory have been reviewed and catalytic activities between the enzymes in the synthesis of compounds have been compared. Paracetamol is a well known analgesic drug. The complicated chemical steps for synthesis of paracetamol starting from unstable p-aminophenol has been overcome by using PLE and PPL. Geraniol is a flavoring agent used in food and perfume industries. The stability of geraniol has been increased to convert geraniol to geranyl acetate. New route for enzymatic transesterification of geraniol has been efficiently carried out by PLE and the result was better than PPL.

Key words: Analgesic, Anticancer, Flavor, PLE, PPL.

INTRODUCTION

Within the wide class of enzymes catalyzing the hydrolysis of various esters, one differentiates between lipase and esterase on the basis of their relative preferential substrate specificity, though all lipases as well as the majority of esterases share α/β hydrolase fold[1].

Microbial esterase or lipase can be used in the form of cells from both bacteria and fungi to catalyze the reactions. But the problem is faced to produce the microbial cells in a suitable medium and sometimes, it becomes more complicated to control the reaction.

The enzyme-catalyzed hydrolysis of the corresponding racemic esters of (±) ibuprofen and (±) naproxen using PPL has been reported[2]. It was found that hydrolysis of corresponding acetate to the corresponding S (-) alcohol was 70 % by PPL whereas with PLE, it was unfavorable and yield was only 20 %. Then the hydrolysis reaction was

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extended to the carbinol acetate derived from (±) flurbiprofen; and in all cases, the enantiomeric excess was very high (as judged from the specific rotation). The oxidation of the (+) alcohols was carried out with PDC using DMF as solvent to make targeted chiral drug. This is a simple chemoenzymatic preparative route of anti-inflammatory drugs.. The method is operationally simple and produces pharmacologically active form in high enantiomeric purity.

During hydrolysis of β-lactam acetates, the PPL preceded with high degree of enantioselectivity, while PLE failed to show such desired selectivity as observed by Basak et al.[3,4]

The synthesis of DNA and cleavage of various enediyne[5] by PPL (failed by PLE) has also been reported. We first synthesized the various enediyne diols via Pd (0) –catalyst coupling with propergyl and/or homopropergyl alcohol. These alcohols were then acetylated and the diacetates were subjected to PPL–catalyzed hydrolysis. Interestingly, there was no formation of the diol upto 40-65 % conversion (based on the consumption of starting materials); only the monoacetates were formed at this stage. The monoacetates and diacetates were separated by column chromatograph. The monoacetates were then successfully converted to the N-containing 10-membered cyclic enediynes. These enediynes have been shown to be powerful DNA-cleaving agents.

Fatty acid amides have a broad spectrum of use as detergents, shampoos, cosmetics, lubricants, foam control agents, fungicides, corrosion inhibitors and water repellent[6]. The desired amides[7] can be prepared by different chemical processes that are tedious and require large amounts of energy. In view of these observations, an enzymatic process was developed by Nag et al.[8], which is likely to be an alternate low-cost and low–energy consuming industrial process. They prepared amide from fatty acid and amine using PPL but PLE was ineffective in that reaction.

Among lipase and esterase, it was also demonstrated by Nag et al.[9] that PPL was the best to catalyze the resolution of dl-menthol in organic solvent. The enantiomeric excess in the case of lipase and esterase were 95 ± 5 and 45 ± 3.

In this paper, a comparison of enantioselectivity and yield of important analgesic drug and flavor compounds by PLE and PPL have been reported.

Paracetamol is a common analgesic drug and geraniol is a well known flavoring agent used in food and perfume industries. But geraniol deteriorates very fast on prolonged

keeping in presence of light or air. If the hydroxyl group is protected, the product gains stability. This can be achieved chemically as well as enzymatically, but the latter being faster and economic.

EXPERIMENTAL

Preparation of paracetamol

Pig Liver Esterase (PLE) and Pig Pancreatic Lipase (PPL) were isolated as acetone powder from fresh pig liver and pancreases[12, 13] as discussed earlier. Hydrolysis activity of the enzyme was measured by the hydrolysis of *p*-nitrophenyl butyrate as substrate. One unit of enzyme activity was defined as an amount of enzyme necessary, which produced 1 micromole of p-nitrophenol per minute at 37^0 C at pH 7.0. The hydrolysis activity of PLE was experimentally verified using p-nitrophenyl butyrate in which 200 units per gram of protein was present[5].

N,O-Diacetyl p-aminophenol was prepared from *p*-nitrophenol as starting material by addition of equal volume of acetic anhydride and acetic acid mixture and followed by zinc dust, m. pt. = 124ºC (1.95 g ; yield = 72 %).

N, O-Diacetyl *p*-aminophenol (0.5 g, 2.59 mmol) and 3 mL acetone was taken in a 100 mL round bottom flask. Then 0.5g PLE was added followed by 25 mL phosphate buffer. The mixture was stirred for 6 hr and monitored for completion of reaction by TLC. The solution was filtered on celite under vacuum. The filtrate was acidified (pH = 2.4) with 1 N HCl and was extracted with ethyl acetate (2 x 40 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to leave the compound as light colored solid, crystallized from ethyl acetate solution m. pt. = 168 ^{0}C (lit 169^{0}C; Yield = 0.35 g, 70 %).

The identity of compound (paracetamol) was confirmed by: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.48 (s, 1H, -O<u>H</u> φ-OH), 7.45 (d, 2H, J = 8Hz), 6.8 (d, 2H, J = 8 Hz), 2.2 (s, 3H, -$NHCOCH_3$).

Geranyl acetate synthesis

It was carried out in screw-capped vials containing geranial, hexane, ester and the enzyme. The reaction mixture was incubated at 37^0 C at 1200 rpm in an orbital shaker. Samples were withdrawn to check the progress of the reaction by TLC .The enzyme was purified by filtration and the solvent hexane and ester was removed in vacuum. The

geraniol and geranyl acetate were removed by flash chromatography on silica to get the pure product and was confirmed by ^{1}H NMR (200MHz): δ_H 1.25 (6H,s), 1.601.60-1.68 (4H,m), 2.01(6H,s), 4.51 (3H, J= 7.1 Hz). 5.00 (7H,d J=6.9 Hz), 5.28(1H, t J=6.9Hz).

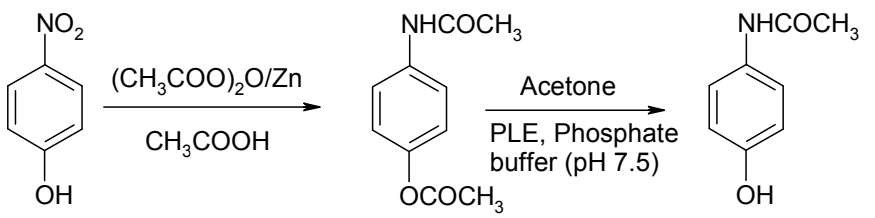
RESULTS AND DISCUSSION

(a) The commercial preparations of paracetamol are patented and some of the methods are tedious[10,11]. During preparation of paracetamol from *p*-aminophenol, the following difficulties are encountered:

(i) p-aminophenol is an unstable compound and undergoes oxidation[11] to *p*-quinone and related compounds and thus, the isolation and purification of the desired product may be tricky.

(ii) Selective acetylation of amino group in preference over phenolic hydroxyl group is a difficult proposition.

To overcome the above difficulties, p-nitrophenol rather p-aminophenol was selected to prepare N,O-diacetyl p-aminophenol, which was resistant to oxidation. Selective hydrolysis of O-acetyl in preference over N-acetyl group was achieved successfully by pig liver esterase (PLE) avoiding unstable compound p-aminophenol (Fig.1).



Scheme1

Fig. 1: Enzymatic synthesis of paracetamol

(b) The yield of geranyl ester synthesized by directed esterification by PLE was found to be very low. Hence, transesterification reaction was attempted with propyl acetate, ethyl acetate and vinyl acetate. The results showed (Table 1) that geranyl acetate transesterification with esters was highest with vinyl acetate followed by propyl acetate and ethyl acetate. The yield was also better than PPL in each reaction (Table 1).

Table 1. Transesterification yield of geraniol by PLE or PPL

Reaction condition	PLE	PPL
Geraniol + Propyl acetate	28	13
Geraniol +Vinyl acetate	70	38
Geraniol + Ethyl acetate	10	8

Although the role of water in enzymatic reaction in organic solvent is still not clear, some amount of water seems to be absolutely essential in enzymatic activity. The optimum amount of water 2.5 microliter gave the maximum conversion of geraniol acetate (Fig. 2). Experiment was carried out at various time and the maximum conversion was achieved after 72 hr.

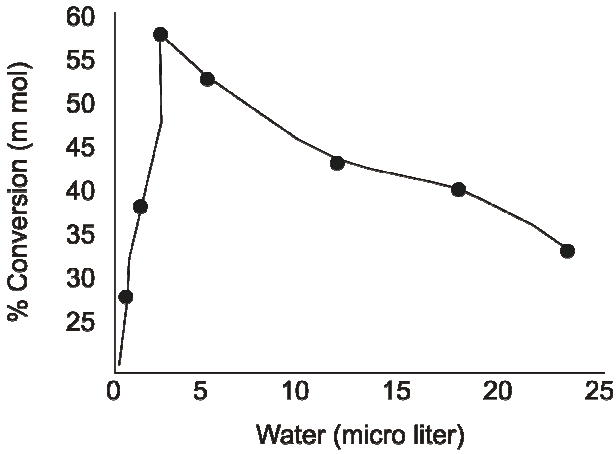


Fig. 2: Effect of water content in the geranyl acetate synthesis

CONCLUSION

Animal esterases play an important role in the synthesis of different drugs and flavoring compounds but they show some limitations on the enantioselectivity and/or yield of the product. Lipase showed advantages over esterase. For synthetic purpose, one may not restrict to the use of esterase and can take the help of both; esterase and lipase.

REFERENCES

1. P. Fozar, H. Johnson Per, T. N. M. Person and B. S. Petersen, What Distinguishes an

Esterase from Lipase; A Novel Structural Approach, Biocheme, **42**, 1033 (2000).

2. A. Nag, S. Nag, S. Mondal and A. Basak, Chemoenzymatic Synthesis of Anti-inflammatory Drugs in Enantiomerically Pure form Tetrahedron Asymmetry, **8**, 401 (2000).
3. A. Nag, T. Mahota, K. Ghosh and A Basak, Studies on PLE Catalyzed Hydrolysis: Dependence on Substituent Flexibility Biotech. Lett., **17(10)**, 1099 (1995).
4. A. Basak, K. Rudra, H. M. Bodur and J. Dasgupta, Active Site Geometry of Porcine Pancreatic Lipase, Bioorg. Med. Chem. Lett., **11**, 305-30 (2001).
5. A. Basak, H. M. Bdour, J. C. Shain, S. Mondal and S. Nag, DNA- Cleavage Studies on N-substituted Monocyclic Enediynes: Enhancement of Potency by Incorporation of Intercalating or Electron Poor Aromatic Ring, Bioorg. Med. Chem. Lett., **10**, 4954 (2000).
6. R. A. Rack, in Fatty Acids in Industries; R.W. Johnson, E. Fritz (Eds.) Decker; New York, (1989) pp. 177-199.
7. S. H. Shapiro, Commercial Nitrogen Derivatives of Fatty Acids in Fatty Acids and Their Industrial Applications; Witco Chemical Corp., E. Scott (Eds.); Decker, New York (1968) pp. 77-154.
8. A. Nag, G. Lee and Jei – Fue Shaw, Lipase – Catalyzed Synthesis of Fatty Acid Diethanolamides, J. Am. Agri. Food Chem., **49**, 12 57 (2001).
9. A. Nag, G. Lee, Jei–Fue Shaw, Factors Affecting the Resolutions of DL–Menthol by Immobilized Lipase-Catalyzed Esterfication in Organic Solvent. J. Am. Agri. Food Chem., **50**, 262 (2002).
10. Y. Fang, W. Liu and F. Zang, Acetylation of Amino Compounds Xaandia Huagong, **8**, 37 (2000).
11. Z. Dhanutirto, H. Suwamo and F. V. Setiwen, Effects of Analgesic Drugs Pharmaceutics. Indonesia, 179 (1984).
12. A. Nag, G. Bhattacharjee and A. Basak, Selectivity in Enzyme-Catalyzed Reaction: Preferential Hydrolysis of Saturated Methyl Ester Over the Corresponding Unsaturated Ester by Pig Liver Esterase, Biotech Lett., **15(1)**, 22 (1993).
13. A. Nag, G. Bhattacharjee and A. Basak, Selectivity in Enzyme Catalyzed Reactions Preferential Hydrolysis of a Phenolic Acetate in the Presence of an Aromatic Methyl Ester by Pig Liver Esterase, Biotech. Lett., **15(5)**, 469 (1995).

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