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Hydrolysis and Esterification Reactions in Aqueous and Organic Medium: Study of the Selectivity Activity of an Acetonic Powder from the Dromedary Liver

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Abstract: The aim of this reaearch is the extraction of an enzyme from dromedary liver and the study of its activity opposite to hydrolyse, esterification reactions and also the study of reactionnal medium effect. In first time, to determine the susceptible ester which will be hydrolysed by our enzymatic preparation, a number of substrates have been tried. We have also studied the influence of the acyl and alkoxyl parts of the ester on the hydrolysis reactions. Hence, we have noticed that the butyrates of butyl and ethyl are the most reactives among the group of esters used in our work. The obtained result is the same to that reached with the acetonic powder of the horse liver. In second time, we have studied the esterifications reactions on different substrates: carboxylic acids and alcohols and this, in the aim to surround the extract specifity. Finally, we note that the presence of proteinic impurities in the case of acetonic powders allows a better organic solvent stability, which in turn, allows a conservation of the enzyme in a given conformation and a suitable rate of hydration by preventing it from having a direct contact with the solvent. It is the reason why we have decided to combine the Acetonic Powder from the Liver of Dromedary (APLD) in different used solvents with each of the solvents that we have used.

Key words: Biotransformations, hydrolases, enzymatic hydrolysis, enzymatic esterifications, organic solvent

INTRODUCTION

Molecular chilarity is an integral part of research on drugs as well as on the regulating processes of enzyme development. Its usefulness is embodied by a wide range of applications such as drugs, flavours, vitamins, pesticides, cosmetics, etc. The synthesis of chilarity-related enantiomerically pure molecules has become a major field of fundamental and applied research. Nevertheless, the process of obtaining these molecules happens to be often long and delicate, particularly using the ways of the traditional organic chemistry. On the other hand, there exist, in living creatures, catalysts that assist these syntheses with a very great effectiveness: The enzymes. They are produced by animals, plants and microorganisms (Yaha *et al*., 1996; Aires *et al*., 1994). These have been in use in laboratory as well as in industry for at least a decade.

The origin of the enzymes catalytic ability has always been of great interest to chemists and biochemists. These, being biocatalysts, possess therefore a great efficiency and, especially, a great selectivity. On one hand, the two regioselective and enantioselective properties of an enzyme, on the other hand, associated with the fact that it is a chiral molecule allow getting optically active substances. Moreover, (1) one enzyme can, in general catalyses no more than one type of reaction and (2) enzymes activate different reaction types and are classified accordingly in six groups by the International Union of Biochemists. These valuable advantages together with the existence in nature of biological enzymes make it that the research axis which is relative to the finding and the extraction of new enzymes of animal or microbial origin is a promising one.

In the present study, an enzymatic preparation has been extracted from a dromedary liver and tested, in various substrates, to get the right or appropriate esters. The solvents effects on the hydrolysis and esterifications reactions have also been studied.

EXPERIMENTAL TECHNIQUES

Hydrolysis reaction: Stotz's (1955) method was used here to extract the crude substance, that from which the enzyme is obtained. The esters hydrolysis reactions were

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carried out under agitation, at a stabilized temperature of 35°C for 30 min and in a phosphate buffer medium (0.2 M) with pH = 8 (Belair, 1990).

In each of these reactions (a) a quantity of 5 mmoles of substrate was used together with 20 mL of phosphate buffer and 2 g of crude extract and (b) the concomitant acid was neutralized by means of a solution of soda (0.1 M).

Finally, a graphical representation (this is not provided in this study) of V_{NaOH} as a function of time (t) has given a linear portion from which an initial reaction velocity was deduced with the help of:

$$V_0 = \frac{V_2 - V_1}{t_2 - t_1}. \quad (1)$$

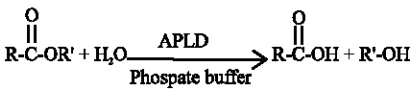
Other cases of hydrolysis reactions were also considered. In the particular cases of hydrolysis reactions employing a co-solvent (these are the ones that are relevant to the work presented here), we used the same medium (to which 2 mL of organic solvent were added) and the same experimental procedure.

Esterification reaction: We have mixed in closed hermetic pot: 10 mL of solvent, 2.5 m moles of acids and 4.0 m moles of alcohol with the presence of 0.5 g of molecular sieve (in order to fix the water since its formation) and 1 g of crude extract of APLD. The reaction mixture was carried out at 35°C with vigorous magnetic stirring. After 24 h of agitation, the acid which has not reacted is dosed with the help of soude solution (0.1N) in the presence of phenolphetaleine.

RESULTS AND DISCUSSION

We carried out various tests in order to determine the optimum activity conditions for this enzyme. These, found to be (see Fig. 1-3): pH = 8, T = 35°C, quantity of enzyme = 2g, were adopted for all hydrolysis reactions performed in the process of determination of the right or correct esters.

Influence of substrate structure on a hydrolysis reaction:



Influence of the ester's acyl part structure on the hydrolysis reactions: An analysis of the data provided in Table 1 (see below) allows saying (I) that the enzyme

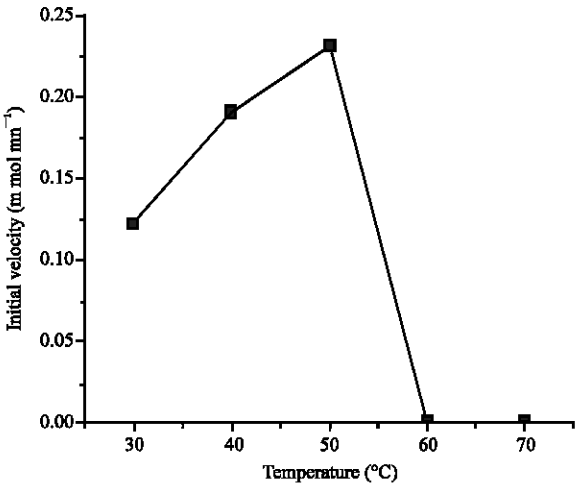


Fig. 1: Example of influence of temperature on hydrolysis reactions

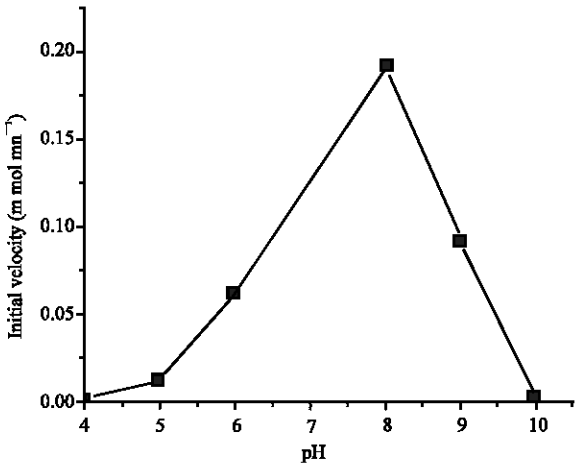


Fig. 2: Influence of pH on a hydrolysis reaction

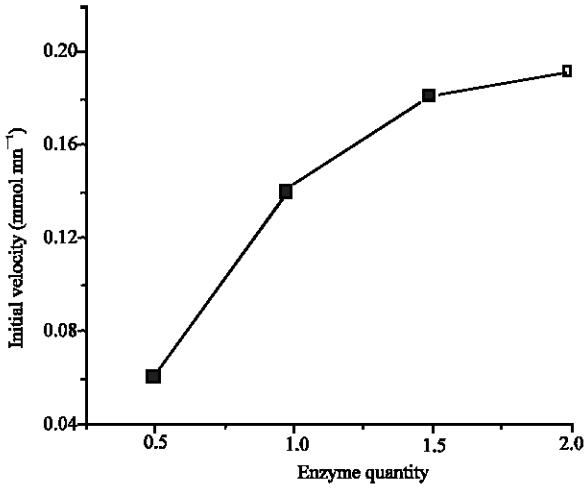


Fig. 3: Influence of enzyme quantity on a hydrolysis reaction

activity decreases with decreasing number of carbon atoms in the substrate chain and (ii) that, as suggested by the results for the two tests one and two, ramification also results in a decrease in the enzyme's activity. It seems therefore that the enzyme is sensitive to lypophilie as well as to the obstruction of the ester's acyl part.

Influence of the ester's alkoxyl part structure on the hydrolysis reaction: Generally, the influence of the ester alkoxyl part on the enzyme's activity is less significant compared with that of the acyl part. Effectively, as it may be noticed from Table 2, the difference in activity for the various couples (1, 2), (3, 4) and (5, 6) is relatively weak.

Influence of a co-solvent on the hydrolysis reaction: In order to study the effect of various solvents on the hydrolysis reactions with our enzyme, several solvents ranging from polar to nonpolar were screened. The log P value of the solvents is the widely used parameter to describe solvent polarity and their possible effects on enzyme activity, where P is the partition coefficient of a given solvent between water and octanol in a two phase system (Laane *et al*., 1987). The choice of an organic co-solvent is often made on the basis of its capacity to solve the substrate and to increase the reaction speed.

Finally, the APLD has been combined with each of the organic solvents that we have used in order to improve its stability and thus to preserve the enzyme in a particular (or given) conformation and a suitable rate of hydration by preventing the occurrence of a direct enzyme-solvent contact; this is because of the presence of proteinic impurities, in the case of acetone powders, yields a better stability of the organic solvent (Belair, 1990).

An examination of Table 3 shows that an addition in the buffer solution of an organic solvent having a log P higher than 4 slightly improves the reaction speed; so hydrophobic solvent such as heptane and octane preserve the catalytic activity without disturbing the micro aqueous layer of the enzyme. On the contrary, solvents with a log P smaller than 3 cause a significant speed reduction. This may be explained by the fact that the solvents which are less hydrophobic tend to strip the essential water from the enzyme and thus distort the catalytic conformation leading to enzyme inactivation. Our observations are agreement with the literature reports (Hari *et al*., 2001).

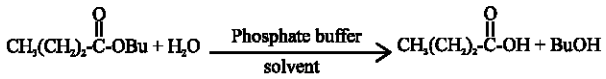


Table 1: Influence of the ester's acyl part structure on the hydrolysis reactions

Test	Substrate	V_0 relative
1	$CH_3CH_2CH_2$-CO_2Et	0.88
2	$(CH_3)_2$-CH-CO_2Et	0.77
3	CH_3-CH_2-CO_2Et	0.66
4	CH_3-CO_2Et	0.22

Table 2: Influence of the ester's alkoxyl part structure on the hydrolysis reaction

Test	Substrate	Relative speed
1	$CH_3CH_2CH_2$-CO_2Et	0.88
2	$CH_3CH_2CH_2$-CO_2-$(CH_2)_3CH_3$	1.00
3	$(CH_3)_2$-CH-CO_2Et	0.77
4	$(CH_3)_2$-CH-$CO_2(CH_2)_3CH_3$	0.72
5	CH_3CO_2Et	0.22
6	CH_3-$CO_2CH(CH_3)_2$	0.27

Table 3: Influence of the co-solvent on the hydrolysis reaction

Tests	Co-solvent	Log P	Relativespeed
1	CH_3CN	-0.34	0.27
2	t-BuOH	0.89	0.38
3	t-BuOMe	0.94	0.28
4	Ccl_4	2.83	0.55
5	Bu_2O	3.21	0.53
6	$CycloC_6H_{12}$	3.44	0.60
7	C_7H_{16}	4.57	0.65
8	C_8H_{18}	5.18	1.00

Influence of acid nature on esterification reaction: Natural flavor esters extracted from plant materials are often in short supply and those produced by fermentation are at present too expensive for commercial exploitation (Langrand *et al*., 1988). Enzymatic synthesis can be attractive as they are typically very selective and are performed at moderate temperatures and pressures compared with chemical synthesis. Esters produced through biocatalysis can be considered close to natural and can potentially satisfy the recent consumer demand (Prapulla *et al*., 1992).

The present study is aimed at realizing esterification reaction from different acids mixed with butanol. The chemical yield is determined by neutralizing of the acids remaining after 24 h of incubation. The obtained results are shown in Table 4.

We notice that the acetic and oleic acids are not esterified by this enzyme while we obtain a good yield with the hexanoic acid; thus we have adopted this acid for the following reactions in order to study the influence of the alcohol nature on the enzyme yield.

Influence of alcohol nature on esterification reaction: As it can be seen in Table 5, we notice that this enzymatic preparation catalyses with an efficient manner the reactions that throw in the aliphatics alcohols; thus the yield increases when we go from ethanol to propanol than to butanol, but decreases with pentanol and cancel each other out with heptanol and octanol. Also, it seems therfore that enzyme is inactive opposite to the

Table 4: Influence of the acids nature on the esterification reaction

Test	R	Yield (%)
1	CH_3-	--
2	CH_3-CH_2-	60
3	CH_3-CH_2-CH_2-	62
4	$(CH_3)_2$-CH-	80
5	CH_3-$(CH_2)_3$-CH_2-	86
6	CH_3-$(CH_2)_{15}$-CH_2-	--

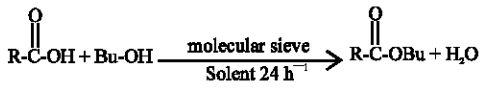
Table 5: Influence of the alcohols nature on the esterification reaction

Test	R'	Yield (%)
1	Cyclo C_6H_{11}-	--
2	$PhCH_2$-	--
3	CH_3-CH(CH_3)-CH_2-CH_2-	--
4	CH_3-CH_2-	56
5	CH_3-CH_2-CH_2-	80
6	CH_3-$(CH_2)_2$-CH_2-	86
7	CH_3-$(CH_2)_3$-CH_2-	56
8	CH_3-$(CH_2)_5$-CH_2-	--
9	CH_3-$(CH_2)_6$-CH_2-	--
10	$(CH_3)_2$-CH-	22

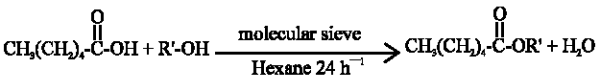
Table 6: Influence of the solvents on the esterification reaction

Test	R'	Yield (%)
1	CH_3CN	--
2	t-BuOH	--
3	t-BuOMe	--
4	Ccl_4	--
5	Bu_2O	50
6	CycloC_6H_{12}	67
7	C_7H_{14}	77
8	C_8H_{18}	80

obstruction of the alcohols such as the benzylic alcohol and the cyclohexanol and the ramification of the chain carries a decrease of the activity of this enzyme.



Influence of the solvent on esterification reaction: As all catalyst, an enzyme doest not modify the position of reactionnal equilibrium which is settled by intangible thermodynamics contraints. Hence, in the esterifications reactions in aqueous medium, the formation of water presents a problems as it favors the reverse (ester hydrolysis) reactions. Activated molecular sieves or salt hydrates can be added to the system to remove the water produced by the reaction (Fonteyn, 1994). The enzyme catalyzing this reaction will favorize the hydrolysis and will behave as hydrolase. In order to study the effect of the solvent nature on this reaction, we have realized different tests in different solvents. It is generally recommended that the use of solvent with a log p>4.0 (nonpolar) result in better esterification (Hari *et al.*, 2001). Table 6 shows the effect of various solvents on the synthesis of different e sters. It can be seen that the solvents with log P value above 3.0 supported esterification and gave excellent conversions. Other solvents with log p<3.0 tend to give poor esterification. These results confirm those obtained in the hydrolysis reaction.



CONCLUSION

A study of the selectivity of the Acetonic Powder from the Liver of Dromedary (APLD) carried out on two different hydrolysis mediums (one aqueous and the other organic) has made it possible to conclude that the APLD:

- may be considered as specific for the hydrolysis of aliphatic (i.e., with non ramified chains) esters with a fairly long chain, in particular a strong effectiveness is observed for the butyric chains;
- hydrolysis acetates but with a weak activity;
- is functional in presence of organic co-solvents such as the octane for which we have obtained the best yield in hydrolysis reactions.
- The none ramified alcohols and aliphatic acids with an average longer chain are the most reactives and the high-efficacity was observed with the caproïc-butanol acid couple; on the other hand, it is inactive opposite to acetic and oleic acid.
- The organic solvents influence the esterification reaction and it was better to work with the hydrophobics solvents.

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