EVALUATION OF KAPs IN THE RESOLUTION OF DL-N-ACETYLPHENYLALANINE

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Introduction. It is clear the great importance of the amino acids, natural and synthetic, in organic synthesis, so any contribution to the methodology for its preparation will be welcomed (1).
The use of crude biocatalysts have the advantage of saving in the cost and time associated to the isolation and purification of the enzyme involved in the biotransformation; besides it is well known that the presence of other enzymes do not interfere with the desired process or biotransfomation.
Regardless the ubiquity of acylases, which are present in most of animal tissues, have been less studied than lipases. Actually microorganisms are the main source for these kind of enzymes used in organic chemistry, but it is recognized its abundance in liver and specially kidney (2).
The objective of this work is to determine the effect of the amino acid structure on the acylase activity of the kidney acetone powders (KAPs) from cow, pig, sheep and guinea pig using (D,L)-*N*-acetylphenylalanine as substrate, compared with that previously determined for (D,L)-*N*-acetylmethionine (3).

Methods. The (D,L)-*N*-acetylphenylalanine was prepared following a reported procedure (4). The crude kidney acetone powders were prepared by blending the organs with acetone, to obtain a fine dry powder.
The enzymatic hydrolysis reactions were performed by stirring the (D,L)-*N*-acetylphenylalanine in buffer containing $CoCl_2$ at 37° C, with the corresponding KAP (Fig. 1). The reactions were monitored by chiral HPLC.

Results. The reaction of the crude biocatalysts (KAPs) was assayed with N-acetylmethionine as reference substrate for the determination of acylase activity, and used it for this comparative study of the influence of the amino acid structure in the enzymatic activity of the KAPs. The results are shown in the Graphs 1 and 2.

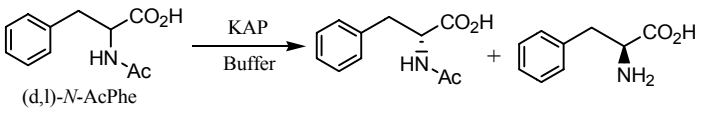
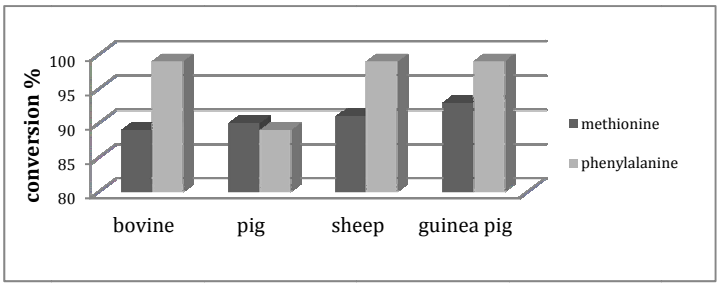
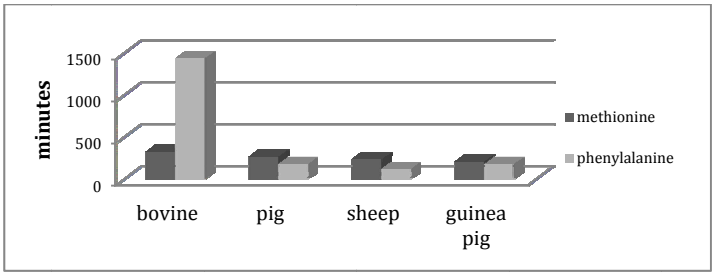


Fig.1 Phenylalanine hydrolysis reaction.

In the Graph 1 are only show the conversion of the L-enantiomer of each amino acid because the D-enantiomer did not suffer any hydrolysis during the reaction. This fact demonstrated the enantiospecificity of the reaction.



Graph 1. Comparison of the conversion percentage of the hydrolysis reaction of (L)-*N*-acetylmethionine and (L)-*N*-acetylphenylalanine with the different KAPs.



Graph 2. Comparison of the reaction time needed to reach the equilibrium in the hydrolysis of L-*N*-acetylmethionine and L-*N*-acetylphenylalanine with the different KAPs.

Respect to the time to reach the equilibrium, the reactions were slightly faster for *N*-AcPhe than *N*-AcMeth (Graph 2), and greater the conversion.

Conclusions. All the reactions with *N*-acetylphenylalanine were slightly faster than with *N*-acetylmethionine, except for the bovine KAP where, although the reaction was initially fast, it took 24 h to reach the equilibrium. It is worth to mention that the reactions conditions were not optimized.

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