IMMOBILIZATION OF D-HYDANTOINASE ON EUPERGIT C

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D-hydantoinase from *Vigna angularis* was immobilized by covalent linkage to the oxirane polimer Eupergit C. This procedure obtain an enzyme preparation stable and with high activity for the preparation of non-proteinogenic amino acids. The target product were: D-*p*-hydroxyphenylglycine and D-*p*-fluorophenylglycine. These target products were obtained by enantioselective hydrolysis of the respective racemic hydantoins. Enzymatic activity was measured at pH 9.0 (buffer: 100mM H_3BO_3/KCl), 30^oC with 50mM of substrate and an enzyme concentration required to maintain the reaction under initial velocity conditions, for 5min at least. The product formed was detected with a colorimetric assay using *p*-dimethyl aminoabenzaldehyde as reagent. Immobilization in epoxy-activated support was performed with a procedure involving to steps. 600mg of support, suspended in 5mL of 1M H_3BO_3/KCL, pH 9.0 buffer, at 25^oC, under orbital shaking with 250mg of D-hydantoinase during 20h. The immobilized enzyme was then submitted to a multipoint attachment to the support by incubation at 20^oC in $KHCO_3/K_2CO_3$ buffer, pH 11.0. The yield of both step was 100% approximately. D-hydantoinase with multipoint covalent attachment displays an activity with *p*-fluorphenylhydantoin that was 30% greater than with the hidantoin, the best substrate of the soluble enzyme. The immobilized D-hydantoinase transformed 10mM *p*-fluorphenylhydantoin with 100% conversion after 7h reaction with very good stability. No appreciable activity loss was observed after five repeated batch reactions at 30ºC. The enantiomeric excess after each cycle was maintained close to 99% as revealed by chyral HPLC chromatography.