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THE PREPARATION OF BEAD-SHAPED TRISACRYL, AND ITS USE AS A CARRIER FOR IMMOBILIZING ARGINASE AND INVERTASE

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Enzymes are frequently immobilized on agarose. Unfortunately, the latter is very expensive and is liable to microbial degradation, which precludes its use on a large scale. For this reason, we sought to develop a new type of hydroxylated carrier that would be reasonably cheap, being thermally, chemically and biologically stable, and that would have also the well-known qualities of agarose. In a previous paper (1), we considered using gels corresponding to the idealized structure 1. These gels, called TRISACRYL, were obtained by aminolysis of poly (methyl acrylate) with the amine 2. But, they were very soft and consequently unfit for use in column. Therefore, we next tried to develop the preparation of TRISACRYL under the form of spherical beads having satisfactory mechanical properties. We describe here our results.

1 $-(CH_2-CH)_n-$ with $CO-NHC(CH_2OH)_3$ 2 $H_2N-C(CH_2OH)_3$ 3 $CH_2=CH-CONHC(CH_2OH)_3$

Treatment of acryloyl chloride with 2 equ. of amine 2 in iso-propanol afforded the known amide 3, m.p. 132° (acetonitrile)(2). By inverted emulsion copolymerization at room temperature, in a water/oil of paraffin mixture, of the monomer 3 with N,N'-methylene-bis-acrylamide (BIS), using ammonium (or potassium) persulfate/N,N,N',N'-tetramethylethylenediamine as a catalyst, the copolymer 1 was obtained (conversion rates ~ 75 %), under the form of very hydrophilic spherical beads which can be filtered easily, and can withstand heating at 120° for 10 mn in a sealed tube, and are not modified by slow stirring in acidic (pH 2) or alkaline (pH 13) solutions for 4 hrs at room temperature. The aspect of the beads is not modified by treatment with dioxan, DMF, acetone, EtOH or MeOH. The activation of the beads was performed using BrCN or p-benzoquinone. 10 mg samples of arginase or invertase were immobilized on aliquots of TRISACRYL corresponding to 100 mg of dried polymer. We use the following parameters : m_1 (mg), mass of

enzyme actually immobilized ; m_2(mg), mass of native enzyme having the same activity as the insoluble derivative obtained ; τ (%), yield of the coupling reaction ; REA (%) and TA (%), percentages of residual specific activity and total residual activity of the insoluble derivative ; clearly, τ (%) = 10 m_1 ; REA (%) = 100 m_2/m_1 ; TA (%) = REA x τ/100.

The immobilization of arginase (from KOCH-LIGHT, activity : 15 U/mg) on the beads of TRISACRYL was carried out in a maleate/$MnCl_2$ 0.05 M buffer, pH 7.2 (4 cm^3) at 4°C for 20 hours. The experiments were performed with samples of TRISACRYL containing various amounts of cross-linking co-monomer (BIS), and activated with BrCN (a) or p-benzoquinone (b). Our results are summarized in the following table. The insoluble arginase/trisacryl derivatives retained 81 % of their initial activity after 3 months storage in the maleate/$MnCl_2$ buffer pH 7.2 at 4°C.

ratio of BIS in the co-monomers	τ (%)	REA (%)	TA (%)
5 % (a)	60	50.5	30.3
10 % (a)	54	57.2	30.9
20 % (a)	47	62.5	29.4
10 % (b)	46.8	48.5	22.7

The immobilization of baker's yeast invertase (100 U/mg) on TRISACRYL beads was carried out in AcONa/AcOH 0.05 M buffer, pH 5, at 4°C for 20 hrs. The following results were obtained using a TRISACRYL prepared with 10 % BIS in the co-monomers mixture, and activated with BrCN : τ = 80 and 80 % ; REA = 95 and 104 % ; TA = 76 and 83 %. The insoluble invertase/TRISACRYL derivatives retained 64 % of their initial activity after 2 months'storage at 4°C in the acetate buffer, pH 5, the activity decreasing regularly as a function of time.

Conclusion. - Immobilization of enzymes, such as arginase and invertase, on beads of TRISACRYL affords water-insoluble derivatives having high residual enzymatic activities, as well as having a good stability in time. We found that the above synthetic copolymer seems to be a choice material, liable to replace favourably the traditional carriers of natural origin (such as cellulose, Sephadex or agarose), as far as enzyme immobilization is concerned (3).

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