Immobilization of Papain on Crosslinked Polymer Supports: Role of the Macromolecular Matrix on Enzymic Activity

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SYNOPSIS

Papain was immobilized on polymer supports with spacer arms of varying nature and length. As the length of the spacer arm increased, there was a marked increase in the extent of enzyme immobilization and activity of immobilized enzymes. When a long, flexible and hydrophilic polyethylene glycol spacer was introduced between the polystyrene backbone and the functional group used for immobilization, the extent of coupling and enzyme activity increased. Dependence of enzyme activity on the nature and extent of crosslinking and on the nature of the polymeric backbone was investigated. Hydrophilic polyacrylamide-based supports were found to be more efficient supports for immobilization compared to hydrophobic polystyrene-based supports.

INTRODUCTION

Immobilized enzymes offer considerable advantages as easily removable and reusable biocalalysts that possess increased shelf life, operational simplicity, and thermal stability.[1-3] Enzymes covalently bound to water-insoluble organic polymer supports are used in several industrial processes and as components for various analytical and monitoring devices.[4,5] The physico-chemical properties of the polymeric support affect the properties of the immobilized enzymes and the selection of a suitable coupling procedure for immobilization. The chemical composition, morphology, hydrophilic / hydrophobic properties, distribution of the reactive groups, pore size, and the microenvironment of the attached functional group in the polymer sample affect the overall properties of immobilized enzymes.[6,7] The important factors in considering the use of immobilized enzymes are their retention of enzymic activity over long storage periods and under different conditions of reactions like temperature and pH. With proper methods of immobilization it is possible to obtain an enzyme derivative whose folded active structure is much more stable.[8,9]

Among the various methods available for immobilization of enzymes, the covalent binding method is particularly important as this leads to the preparation of stable enzyme derivatives. This method has been reported as a convenient method for immobilization of enzymes.[10-13] The irreversible attachment in this case is desirable as it avoids contamination of the product with detached enzymes. Immobilization involves coupling between functional groups present in the polymer support and the functional groups in the enzymes that are not essential for the biological activity.

In this work a number of polymeric supports varying in their nature and crosslinking were prepared, and the proteolytic enzyme, papain, was immobilized on these supports by the DCC coupling method. The method of immobilization, the extent of retention of enzymic activity on binding, conditions of immobilization and dependence of extent of coupling, and activity on the support structure are described here.

EXPERIMENTAL

The enzyme papain used was the commercially available sample (Sigma). Acrylamide, *N,N'*-methylene-bis-acrylamide (NNMBA), divinylbenzene (DVB), tetraethylene glycol dicrylate (TEGDA),

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polyethylene glycol (PEG) and dicyclohexylcarbodiimide (DCC) were purchased from Fluka. Copolystyrene–divinylbenzene beads (1, 2, 4, and 20% crosslinked, microporous, 200–400 mesh) and Merrifield's chloromethylated polystyrene (2% DVB crosslinked) were also purchased from Fluka, Switzerland.

Polystyrene carboxylic acid resin (**1a**) was prepared from chloromethyl polystyrene by oxidation with dimethyl sulfoxide and further oxidation with dichromate.[14] Carboxymethyl polystyrene resin (**2a**) was also prepared from chloromethyl polystyrene by treatment with potassium cyanide followed by acid hydrolysis.[15,16] Polystyrene–tetraethyleneglycol diacrylate (PS–TEGDA), polyacrylamide–divinylbenzene (PA–DVB) and polyacrylamide–*N,N'*-methylene-bis-acrylamide (PA–NNMBA) crosslinked supports were prepared by literature procedures.[17,18] The polystyrene-based resins were functionalized with amino groups by Friedel-Crafts reaction with *N*-chloromethylphthalimide followed by hydrazinolysis.[19] Polyacrylamide-based resins were converted to the amino resins by transamidation reaction with ethylenediamine.[20]

Preparation of Hexamethylenediaminomethyl Polystyrene (1)

Chloromethyl polystyrene (10 g) was suspended in chloroform (50 mL). A fivefold molar excess of hexamethylenediamine and pyridine (5 mL) was added. The reaction mixture was stirred at 70°C for 15 h. The resin was collected by filtration, washed with chloroform, dioxane, dioxane–water, ethanol, and methanol (50 mL, 3 times); drained and dried under vacuum. Amino group capacity, 0.5 mmol/g; yield 10.5 g.

Preparation of Carboxyl Functional Resins 3a–6a, 8a, and 9a from Aminofunctional Polystyrene and Polyacrylamide-based Resins: Introduction of the Spacer Groups

A mixture of the aminomethyl resin (15 mmol) and a fourfold molar excess of glutaric anhydride or succinic anhydride (60 mmol) was suspended in dry DMF (600 mL). The suspension was stirred at 80°C for 10 h on a water bath. The resin was filtered hot, washed successively with dimethylformamide (DMF), CH_2Cl_2 and MeOH (50 mL, 4 times), and dried to constant weight to provide the carboxyl functional resins. IR (KBr): 3500 (NH, OH), 1720 (C=O, acid), 1640, and 1600 (C=O, amide) cm^{-1}.

Conversion of Aminofunctional Acrylamide–NNMBA Resin to Carboxyl Functional Resin (9a)

Poly(*N*-2-aminoethylacrylamide) (2 g, 5 mmol) was suspended in 0.1 *M* NaCl (50 mL). The suspension was stirred, while small portions of glutaric anhydride (20 mmol, fourfold excess) were added. The pH of the solution was kept near 4 throughout the addition of the anhydride by adding a few drops of 2 *N* NaOH. The mixture was stirred for 10 h. After the reaction, the resin was collected by filtration and washed with water till the filtrate was free from any glutaric acid. Finally the resin was washed with methanol, drained, and dried under vacuum. Yield was 2.7 g. IR (KBr): 3400–3500 (NH, OH) 1690 (C=O, amide), 1725 (C=O, acid) cm^{-1}.

Preparation of Carboxyl-Functionalized Polystyrene–Polyethylene Glycol Graft Copolymer (7a)

Carboxylated PEG_{600} (1.6 g, 4 mmol), DCC (0.42 g, 2 mmol), and CH_2Cl_2 (20 mL) were stirred in an ice bath at 0°C for 10 min. Hydroxymethyl polystyrene[21] (1 g, 2 mmol) was swelled in CH_2Cl_2 (20 mL) and added to the above solution. This was stirred for one more hour in an ice bath and then at room temperature for 10 h, filtered, washed with CH_2Cl_2 (20 mL, 3 times), $CHCl_3$ (20 mL, 2 times), and methanol (20 mL, 3 times). The polymer was drained and dried. Yield was 2.5 g; capacity of COOH group 0.78 mmol/g; IR (KBr): 1110 (PEG, C–O–C) and 1740 cm^{-1} (–C=O, ester).

Coupling of Carboxyl Functional Resins (1a–9a) with Papain: Preparation of Immobilized Papain Derivatives (1b–9b)

The carboxyl functional polystyrene resin (3.0 mmol) and DCC (0.62 g, 3 mmol) were taken in dioxane (15 mL) and stirred in an ice bath at 0°C for 30 min. Papain (200 mg) was separately dissolved in phosphate buffer pH (7.8, 10 mL) and the clear solution was added to the preactivated carboxyl resin in THF. The reaction mixture was stirred at 0°C for 2 h and at room temperature for a further 24 h. The resin was filtered and washed with buffer till the filtrate was free from any unbound enzyme as tested by the ninhydrin color reaction.[22] The resin was washed further with tetrahydrofuran (THF), dioxane, CH_3OH, CH_3OH–CH_2Cl_2 mixture (1 : 1), and finally with CH_3OH (20 mL, 2 times) until the resin was free from any dicyclohexylurea (DCU)

Table I Effect of Spacer Grouping on Enzyme Content, Activity, and Immobilization Yield of Immobilized Papain Derivatives

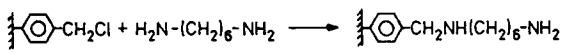
Immobilized Papain[a]	Enzyme (mg/g)[b]	Activity (units)[c]	Immobilization Yield (%)[d]
1b	5	0.1160	5.0
2b	9	0.2204	9.5
3b	18	0.6960	30.0
4b	20	0.7192	31.0
6b	11	0.3480	15.0
7b	32	1.253	54.0

[a] 2% Crosslinked polystyrene–DVB support was used for immobilization of papain.
[b] Duration of coupling: 24 h.
[c] Unit is defined as μmol of *p*-nitrophenol liberated/mg of enzyme/min.
[d] Expressed as the ratio of activity of immobilized enzyme to that of free enzyme, activity of free enzyme, 2.32 units.

precipitated during the reaction. The resulting immobilized enzyme was vacuum-dried.

Determination of the Amount of Enzyme Bound on the Supports: Lowry Protein Measurements[11,23]

Sodium carbonate (2% in 50 mL 0.1 *N* NaOH) and copper sulfate solution (0.5% in 1 mL of 1% sodium tartarate) were mixed well. To 2 mL of this reagent were added various amounts of papain (0.01–1 mg) dissolved in 0.1 *M* phosphate buffer (1 mL, pH 7.5). After 10 min 0.5 mL of 1 *N* Folin-Ciocalteau reagent was added, and the system was allowed to stand for 30 min. The absorbance at 750 nm was measured spectrophotometrically in each case. From the standard plot, the amount of enzyme present in the enzyme solution used for immobilization reaction and that remaining unbound in the filtrate was estimated. Finally, the amount of enzyme bound on the support was found by difference. Results are given in Tables I, II, and III.



Scheme I Preparation of hexamethylenediaminomethyl polystyrene (I).

Determination of Enzymic Activity of Free and Immobilized Papain Derivatives

p-Nitrophenyl acetate (50 mg) was dissolved in dioxane (5 mL) and water (60 mL) was added. Papain, free or immobilized (10 mg free papain or polymer containing 10 mg bound papain), EDTA solution (0.01 *M*, 10 mL), and phosphate buffer (0.1 *M*, 25 mL, pH 6.8) were added to the above solution. The *p*-nitrophenol liberated at different time intervals was measured spectrophotometrically at 405 nm. The activity was expressed as micromoles of *p*-nitrophenol liberated per milligram of enzyme (free or immobilized/minute).

RESULTS AND DISCUSSION

Preparation and Characterization of Immobilized Enzyme Derivatives

The polystyrene support with a $-CH_2NH(CH_2)_2$-$NHCO(CH_2)_3-$ spacer between the polymer matrix and enzyme functional amino group was prepared by the reaction of chloromethyl polystyrene with hexamethylenediamine (Scheme I).

However, the capacity of the amino resin (I) was low due to the double linkage between the two amino groups of hexamethylenediamine and chloromethyl polystyrene. The amino group capacity was found to be 0.5 mmol/g. But the resin was found to have a residual halogen content of 0.64 mmol Cl/g that accounts for 85% conversion.

Aminofunctional polystyrene and polyacrylamide

Table II Effect of Crosslink Density on Enzyme Content, Activity, and Immobilization Yields of Papain Immobilized on Polystyrene–DVB Resins Having Different Crosslink Densities

Immobilized Papain	Crosslink Density (%)	mg of Enzyme Bound/g Polymer	Activity (units)	Immobilization Yield (%)
$4b_1$	1	25	0.925	40.00
$4b_2$	2	22	0.784	33.79
$4b_3$	4	20	0.540	23.27
$4b_4$	20	0.1	—	—

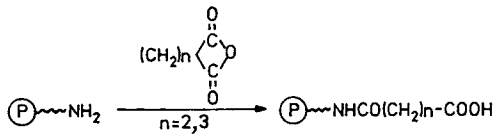
Table III Effect of Crosslink Density on Enzyme Content, Activity, and Immobilization Yields of Papain Immobilized on Polyacrylamide–DVB Resins with Different Crosslink Densities

Immobilized Papain	Crosslink Density (%)	mg of Enzyme/g of Polymer	Activity (units)	Immobilization Yield (%)
8b$_1$	5	41	1.68	72.4
8b$_2$	10	36	1.484	64.0
8b$_3$	15	20	0.7540	32.5
8b$_4$	20	15	0.5985	25.8

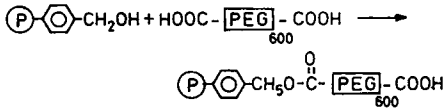
resins were treated with glutaric or succinic anhydride in DMF at 80°C to give carboxyl functional resins with a $-CH_2NHCO(CH_2)_3-$ spacer group between the functional carboxyl group and the polymer backbone. For the preparation of carboxyl functional acrylamide–NNMBA resin (**9a**) the above method was not satisfactory. In an alternative method, the resin was stirred with a fourfold molar excess of glutaric anhydride in 0.1 *M* sodium chloride at pH 4. The carboxyl functionalization of the amino polymers is represented generally in Scheme II.

Carboxyl functionalized polystyrene–PEG graft copolymer was prepared by the reaction between hydroxymethyl polystyrene and carboxyl-terminated PEG_{600} (Scheme III).

The structures of the different carboxyl functional supports prepared are given in Figure 1. They differ in the nature and length of the spacer arm, nature of the polymer backbone, and nature of the crosslinking agent. Structures **1a–7a** show a difference in the length and nature of the spacer arm; **7a** has a long, flexible, and hydrophilic polyethylene glycol spacer between the carboxyl function and the crosslinked polystyrene matrix. Structures **1a–4a, 6a,** and **7a** have divinylbenzene crosslinks whereas **5a** has tetraethyleneglycol diacrylate crosslinks that are hydrophilic and flexible in nature compared to the rigid hydrophobic divinylbenzene. The support **8a** has hydrophobic divinylbenzene and **9a** has hydrophilic *N,N'*-methylene-bis-acrylamide as crosslinking agents.



Scheme II Preparation of carboxyl functional resins **3a–6a, 8a,** and **9a** from aminofunctional polystyrene and polyacrylamide-based resins.



Scheme III Preparation of carboxyl functionalized polystyrene–PEG graft copolymer.

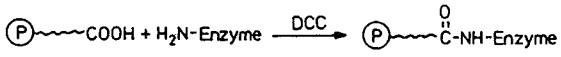
These carboxyl functional polystyrene and polyacrylamide resins were reacted with papain in phosphate buffer at pH 7.8 using DCC as the coupling reagent (Scheme IV) to get immobilized papain derivatives **1b–9b.**

After the completion of the immobilization process, the resins were washed several times with phosphate buffer to remove the unbound enzyme. The filtrate, together with the washings, was collected and the enzyme concentration in it was measured using Folin's-Ciocalteau reagent at 750 nm (Lowry protein measurements). The amounts of papain bound on different supports were found to be in the range of 5–50 mg of papain/g of polymer. This was further confirmed by Kjeldahl's nitrogen estimation procedure.[24]

The activities of these immobilized enzyme preparations were determined by following the hydrolysis of the chromogenic substrate *p*-nitrophenylacetate spectrophotometrically at 405 nm.[25] The activity was expressed as micromoles of *p*-nitrophenol liberated per milligram of enzyme per minute.

Role of Structural Parameters on the Activity of Immobilized Enzymes

Support features influence the activity of immobilized enzymes. Different parameters like the effect



Scheme IV Immobilization of papain on carboxyl functional polymer supports.

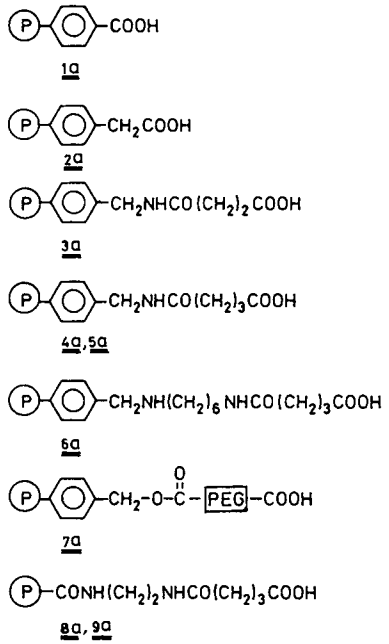


Figure 1 Structures of the carboxyl functional resins used for immobilization of papain **1a–4a, 6a, 7a:** Polystyrene–DVB support, **5a:** Polystyrene–TEGDA support, **8a:** acrylamide–DVB support, **9a:** Acrylamide–NNMBA support.

of spacer grouping, crosslink density, microenvironment of the attached species, hydrophilic–hydrophobic balance of the system, and pore size have significant influence in polymer-supported reactions. The effect of these factors on the overall reactivity of attached enzyme was investigated.

Effect of Spacer Grouping

The amount of the bound papain, activity, and the corresponding immobilization yields of papain immobilized on polystyrene supports (2% crosslinked with divinylbenzene) through spacer arms of varying length and nature are given Table I. For resin **4b** in which the enzyme is attached through a $-CH_2NHCO(CH_2)_3-$ spacer arm, the enzyme content was found to be 20 mg papain/g of polymer with an immobilization yield of 31% whereas for resin **1b** without any spacer, the enzyme content was only 5 mg of papain/g of polymer. For resin **6b** the enzyme content was only 11 mg papain/g of polymer. The activities and the immobilization yields also were low for this resin, 0.3480 and 15%, respectively. This is quite a low value even though the polymer has a long $-CH_2NH(CH_2)_6-NHCO-(CH_2)_3-$ spacer group. The plausible explanation for this low value is that during the preparation of amino resin there are chances of double linkage, and so steric hindrance to the approaching enzyme is greater for this resin. Hence the enzyme content and activity are not as high as expected. For the resin **7b,** where there is a long, flexible hydrophilic spacer group between the crosslinked polymer matrix and the attached enzyme, the enzyme content was quite high (32 mg per g of polymer). The corresponding activity was 1.253 units and 54% immobilization yield was obtained. Thus the hydrophilic spacer arm reduced the steric effects in the medium and the spacer arm renders the support more compatible with the enzyme.

Effect of Crosslinking

The extent of crosslinking exerts a striking influence on the reactivity. Papain immobilized by DCC-mediated coupling on polystyrene–DVB resins of varying crosslink densities (1, 2, 4, and 20%, $\mathbf{4b_1}$–$\mathbf{4b_4}$), and acrylamide–DVB resins of 5, 10, 15, and 20% ($\mathbf{8b_1}$–$\mathbf{8b_4}$) crosslink densities were used for the studies. The activities, immobilization yields, and enzyme contents are given in Tables II and III.

For polystyrene–DVB resins, 1% crosslinked resin ($\mathbf{4b_1}$) gave an enzyme content of 25 mg/g of resin, and the enzyme content gradually decreased with increasing crosslink density (Table II). For 20% crosslinked resin ($\mathbf{4b_4}$) the enzyme content was only 0.1 mg/g of resin. This is due to the rigid nature of the resin at higher crosslink densities. For studying the effect of crosslink density on activity and immobilization yields, different weights of crosslinked resins containing the same amount of bound enzyme (10 mg) was taken. Studies indicate that activity is dependent on the rigidity of the matrix, since, for 20% crosslinked resin no appreciable activity was observed, whereas for 1% crosslinked resin an activity of 0.925 units and 40% retention of original enzymic activity was observed.

Divinylbenzene-crosslinked acrylamide resins ($\mathbf{8b_1}$–$\mathbf{8b_4}$) also showed similar behavior (Table III). But here, even the 20% crosslinked resin ($\mathbf{8b_4}$) contained 15 mg enzyme/g of polymer. The activities decreased with increasing crosslink densities. For 5% crosslinked resin ($\mathbf{8b_1}$) an activity of 1.68 units and immobilization yield 72.4% was observed, whereas, for 20% crosslinked resin ($\mathbf{8b_4}$) activity was 0.5985 units and immobilization yield was 25.8%. The increased activities and enzyme contents for these resins can be attributed to the increased hydrophilic nature of the support. Figure 2 gives a comparison between immobilization yield obtained for polystyrene–DVB resins and polyacrylamide–DVB resins with different crosslink densities.

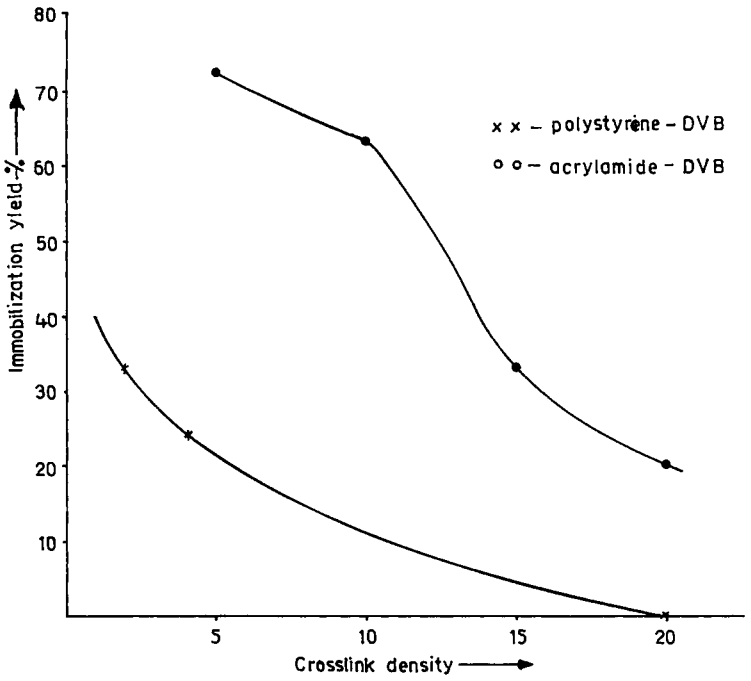


Figure 2 Comparison of immobilization yields of papain immobilized on different crosslinked polystyrene–DVB and polyacrylamide–DVB supports.

Influence of Nature of the Support

Support characteristics significantly influence the immobilization reactions. Nature of the support was varied by changing the hydrophilic–hydrophobic balance in the system. Papain attached by DCC-mediated coupling on polystyrene crosslinked with divinylbenzene or tetraethyleneglycol diacrylate and polyacrylamide crosslinked with divinylbenzene or *N,N'*-methylene-bis-acrylamide type supports were used for the present studies.

In polystyrene-based supports, as the crosslinking agent was changed from divinylbenzene to tetraethyleneglycol diacrylate, a marked difference in enzyme content and activity was observed. The enzyme content in acrylamide–DVB resin in which the enzyme is attached through a $-CH_2NHCO-(CH_2)_3-$ spacer arm (**4b**, 5% crosslinked) was 20 mg/g of polymer, whereas, for 5% crosslinked polystyrene–tetraethyleneglycol diacrylate resin (**5b**) with the same spacer arm, the enzyme content was 28 mg/g of polymer. For comparing the effects of these polymer supports on activities, different weights of resins containing the same amount of bound enzymes (10 mg each) were taken, and their activities were studied following the hydrolysis of *p*-nitrophenyl acetate spectrophotometrically as described earlier. For polystyrene–DVB resin, an

Table IV Effect of the Nature of Supports of Enzyme Content, Activity, and Yield in Various Polystyrene and Polyacrylamide-Based Resins

Immobilized Papain[a]	mg of Enzyme/g of Polymer	Activity (units)	Immobilization Yield (%)
4b	20	0.54	23.27
5b	28	1.044	45.0
8b	41	1.680	72.4
9b	50	1.793	78.0

[a] **4b** Polystyrene–DVB resin, **5b** polystyrene-TEGDA resin, **8b** acrylamide-DVB resin, **9b** acrylamide-NNMBA resin.

activity of 0.54 units and immobilization yield 23.2% were observed whereas for polystyrene–TEGDA resin the observed activity was 1.044 units and 45% immobilization yield. The results clearly indicate the effect of a hydrophilic flexible crosslinking agent on the extent of immobilization and activity. The long tetraethyleneglycol chains act as spacer grouping between the polymer matrix, thus reducing the overall steric effects, and at the same time due to the hydrophilic property of the tetraethyleneglycol diacrylate, resins are more compatible with the enzyme.

In polyacrylamide-based supports the crosslinking agents used for the investigation were divinylbenzene and *N,N'*-methylene-bis-acrylamide. Divinylbenzene crosslinks reduce the extreme hydrophilic nature of the polyacrylamide compared to *N,N'*-methylene-bis-acrylamide, which is hydrophilic in nature. Five percent crosslinked resins were used for investigations. For acrylamide–DVB resin (**8b**) the enzyme content was 41 mg/g of polymer, whereas, acrylamide–NNMBA resin (**9b**) contained 50 mg of enzyme/g of polymer after immobilization. The activity and immobilization yield in the former were 1.68 units and 72.4% whereas the latter had 1.793 units of activity and 78% immobilization yield, respectively. The enzyme contents, activities, and immobilization yields of various polystyrene-based

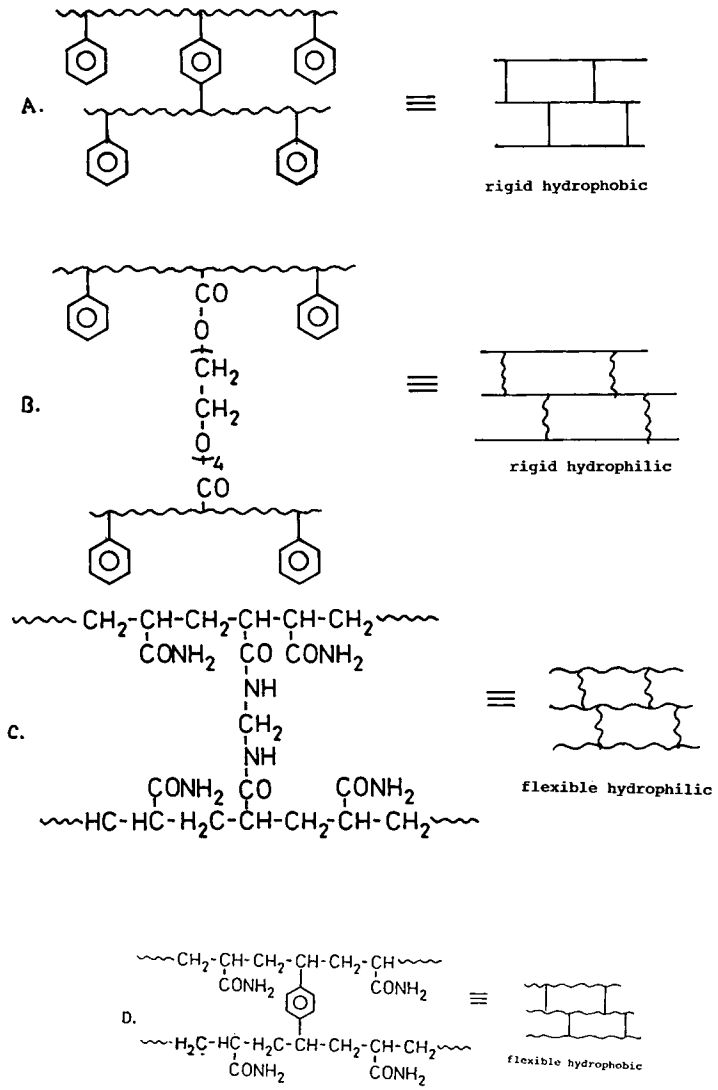


Figure 3 Relative rigidity and flexibility of polystyrene-based and polyacrylamide-based supports having different crosslinking agents. (A) Polystyrene–DVB, (B) polystyrene–TEGDA, (C) polyacrylamide–NNMBA, (D) polyacrylamide–DVB.

supports and polyacrylamide-based supports are given in Table IV. The results show that hydrophilic supports are more efficient in bringing about immobilization reactions, and they have more affinity toward substrates as indicated by their activity values.

The relative rigidity and flexibility of polystyrene-based and polyacrylamide-based supports with the different crosslinking agents are schematically represented in Figure 3.

From the foregoing results, it can be seen that the efficiency of the methods to be employed for the immobilization of enzymes on synthetic polymeric supports, the yield of immobilization, and the retention of enzymic activity in the immobilization process depend on a number of factors characteristic of the macromolecular matrix. The factors to be considered in this respect are the hydrophilic–hydrophobic nature of the polymer matrix, the nature and extent of crosslinking, nature of the building blocks of the macromolecular backbone, and the distance of the functional group employed for coupling from the crosslinked polymeric network. The extent of immobilization was found to increase gradually as the length of the spacer arm increases in the case of polystyrene resins. The activities of the resins were also found to increase in this order. This appears to be due to relief of steric restrictions imposed by the crosslinked polymeric backbone and also due to increased flexibility and mobility of the attached macromolecular enzyme as it protrudes farther into the continuous phase. The extent of immobilization and activity of the attached enzyme was also found to be dependent on the hydrophilicity of the polymer matrix. Divinylbenzene-crosslinked polyacrylamide resins were found to be more efficient as supports as they could be functionalized readily compared to *N,N'*-methylene-bis-acrylamide crosslinked polyacrylamide supports. Polystyrene–polyethyleneglycol graft copolymers and polystyrene–tetraethyleneglycol diacrylate copolymers were found to be more suitable for the preparation of immobilized enzymes compared to polystyrene–divinylbenzene supports. In the case of divinylbenzene-crosslinked polystyrene and polyacrylamide resins, it was found that the activity and extent of immobilization decrease as the crosslink density increases.

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REFERENCES

1. L. Goldstein and E. Katchalski, in *Applied Biochemistry and Bioengineering,* L. B. Wingard, Jr., L. Goldstein, and E. Katchalski, Eds., Academic Press, New York, 1976, p. 1.
2. I. Chibata, Ed. *Immobilized enzymes—Research and Development,* Halsted, New York, 1978.
3. G. Manecke, *Naturwissenchaften,* **51,** 25 (1964).
4. H. U. Bergmeyer, Ed. *Methods of Enzymatic Analysis,* 3rd ed. Verlag-Chemie, Weienheim, 1986.
5. G. M. Whitesides and C. H. Wong, *Angew. Chem. Int. Ed. Engl.,* **25,** 617 (1985).
6. J. Tramper, in *Solid Phase Biochemistry* W. H. Scouten, Ed. Wiley, New York, 1983, p. 393.
7. R. Goldman, L. Goldstein, and E. Katchalski, in *Biochemical Aspects of Reactions on Solid Supports,* G. R. Stark, Ed., Academic Press, New York, 1971, p. 1.
8. A. Konnecke, V. Schellenberger, and H. O. Jakubke, *Z. Chem.,* **24,** 115 (1985).
9. A. G. Demers and S. S. Wong, *J. Appl. Biochem.,* **7,** 12 (1985).
10. L. Tarhan and B. Perkin, *Biotechnol. Bioeng.,* **25,** 2773 (1983).
11. H. R. Allcock and S. Kwon, *Macromolecules,* **19,** 1502 (1986).
12. M. Kotorman, L. M. Simon, B. Szajani, and L. Boross, *Biotechnol. Appl. Biochem.,* **8,** 53 (1986).
13. C. J. Findlay, K. L. Parkin, and R. Y. Yada, *Biotechnol. Lett.,* **8,** 649 (1986).
14. J. M. J. Frechet and K. E. Haque, *Macromolecules,* **8,** 130 (1975).
15. J. M. J. Frechet, M. D. de Smet, and M. J. Farral, *J. Org. Chem.,* **41,** 1974 (1979).
16. J. T. Ayres and C. K. Mann, *J. Polym. Sci. Polym. Lett.,* **3,** 505 (1965).
17. K. S. Devaky, Ph.D. Thesis, Calicut University, Kerala, 1989.
18. S. R. Sandler and W. Karo, Eds., *Polymer Synthesis,* Academic Press, New York, Vol. 1, p. 343.
19. R. B. Merrifield, *Science,* **232,** 343 (1986).
20. J. K. Inman and H. M. Dintzis, *Biochemistry,* **8,** 4074 (1969).
21. R. L. Letsinger and I. Kalus, *J. Am. Chem. Soc.,* **81,** 300 (1964).
22. D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.,* **30,** 1190.
23. O. H. Lowry, N. J. Rosebrough, A. I. Farr, and R. J. Randall, *J. Biol. Chem.,* **193,** 265 (1951).
24. S. Dumitriu and M. Popa, *J. Bioactive Compatible Polym.,* **1,** 285 (1986).
25. M. L. Bender and L. J. Brubacher, *J. Am. Chem. Soc.,* **88,** 5890 (1966).

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