OBB-4

DEVELOPMENT AND APPLICATION OF HEPARIN-CONTAINING SUPPORTS

C.Gomez (1); C.Alvarez (1); D.Arrúa (1); J.Zarzur (2); M.Strumia (1)*
(1) Dpto. de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Edif. de Ciencias II, Ciudad Universitaria (5000) Córdoba, Argentina. Fax: 54-0351-4333030, e-mail: mcs@dqo.fcq.unc.edu.ar
(2) Laboratorio de Hemoderivados, Universidad Nacional de Córdoba, Av. Valparaíso s/n, Ciudad Universitaria (5000) Córdoba, Argentina.

Heparin is a sulfated glycosaminoglycan composed of 1,4-linked D-glucuronic acid and D-glucosamine residues. Different heparin samples have varied levels of N-sulfation in hexosamine residues and distinct extends of O-sulfation within hexuronic and hexosamine units[1]. Its polyanionic nature and the carbohydrate sequences supply the possibility of bind different substances by ionic interaction or specific binding sites, respectively. So, various proteins such as blood coagulation factors, heparin binding proteins, enzymes, etc., have been purified using immobilized heparin[1-2].

In this work, different matrices: poly (ethylene glycol dimethacrylate-2-hydroxyethyl methacrylate) [poly(EGDMA-co-HEMA)] and commercial agarose (Aga), were modified to couple heparin.

Poly(EGDMA-co-HEMA) matrix obtained in our labs by suspension copolymerization[3-4] constitutes a highly porous crosslinked network. Its total porosity value was 83%, defined as[5] $\%P = V_{p\ total}\ d_0 \times 100$. The total specific pore volume was 2.75 mL/g; the total specific surface area was 135.5 m^2/g and the sample apparent density (d_0) was 0.3 g/mL. These data were obtained from mercury intrusion porosimetry method (pore diameter between 4 - 400000 nm). Different samples of the matrix were activated with 1,4 butanediol diglycidyl ether (BDGE) or epichlorohydrin (ECH) in basic aqueous medium at room temperature, yielding poly(EGDMA-co-HEMA)-BDGE and poly(EGDMA-co-HEMA)-ECH products, that contain 98.6 and 107 μmol epoxy groups/g of dry sample, respectively. The epoxy-activated matrices were reacted with concentrated ammonia solution at 40 °C to generate terminal primary amine groups [1, 6-7]. Heparin (157 UI/mg) was then coupled on amino-matrices by reductive amination of its terminal formyl groups by incubation in phosphate buffer (pH 7) at room temperature for two days using sodium cyanoborohydride[6-8]. The amount of coupled heparin was determined by difference between the amount initially added to react and that remained in the reaction final supernatant. It was analyzed by UV-Vis spectrophotometry through the phenol-based method[8]. The remaining amino groups were acetylated with 0.2 M sodium acetate and acetic anhidride. The amount of coupled heparin on poly(EGDMA-co-HEMA) derivative in which BDGE was used, was 5.22 mg/g of dry support while when ECH was used as activating agent, no heparin was coupled. This was attributed at the difference in the length between both spacers used on the rigid poly(EGDMA-co-HEMA) matrix and the consequence in the steric restriction for heparin. So, the long-spacer BDGE was choice to attain the optimization in the coupling reactions to reach the maximum amount of bound heparin, by varying its concentration and/or sodium cyanoborohydride amount in the

reaction medium. This step is still under development.

Commercial Aga was reacted with ECH which acted as activating and crosslinking agent. The derivatization reactions were carried out following the steps previously described for activated poly(EGDMA-co-HEMA) matrices. Aga-ECH product contained 1154.9 μmol epoxy groups/ g of dry sample. In this case, the amount of coupled heparin on soft Aga derivative was 27.32 mg/g of dry support.

The products obtained and those in the optimization steps, will be compared in the retention and binding capacity of Antithrombin III. Besides it will be taking in account the chemical stability of supports for posterior sanitization procedure to remove bacterial endotoxins and re-use, for which they will be submitted at treatment with 0.1 N sodium hydroxide solutions assaying again the retention.

Acknowledgements

The authors thank Agencia Córdoba Ciencia, SECYT, and CONICET for their financial assistance. D.Arrúa also acknowledges receipt of a fellowship from Secretaría de Extensión Universitaria from Universidad Nacional de Córdoba.

References

1. Hermanson G, Mallia K, Smith P (1992) Immobilized Affinity Ligand Techniques. Acad Press, Inc. London
2. Farooqui A (1980) J Chromatogr 184:335
3. Gomez C, Alvarez C, Strumia M, Rivas R, Reyes P (2001) J Appl Polym Sci 79 (5):920
4. Gomez C, Alvarez C, Strumia M (2001) J Biochem Biophys Methods 49 (No 1-3): 141
5. Okay O, Soner E, Gungor A, Balkas T (1985) J Appl Polym Sci 30:2065
6. Funahashi M, Matsumoto I, Seno N (1982) Anal Biochem 126:414
7. Sasaki H, Hayashi A, Kitagaki-Ogawa H, Matsumoto I, Seno, N (1987) J Chromatogr 400:123
8. Lutkemeyer D, Bretshneider M, Buntemeyer H, Lehman J (1993) J Chromatogr 639:57