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论文

丝素纳米颗粒的制备及应用于*L*-天冬酰胺酶的固定化

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摘要:

丝素蛋白纤维溶于高浓度中性盐溴化锂溶液或氯化钙-乙醇-水三元溶剂中, 经过透析和纯化可以制成3种液态丝素. SDS-PAGE分析结果表明, 其分子量分布范围明显不同. 应用能与水混溶的有机溶剂如丙酮等可将这种丝素制成丝素纳米颗粒, 用SEM观察到丝素纳米颗粒粒径分布范围为50~120 nm. 以戊二醛为交联剂, 将治疗急性淋巴性白血病常用酶制剂*L*-天冬酰胺酶共价结合在丝素纳米颗粒上. 酶活性分析结果表明, 由肽链断裂较少的丝素制备的纳米颗粒更适合于酶的生物结合. 酶动力学研究结果表明, 这种固定化酶活性回收率为44%, 热稳定性较游离酶有明显提高, 最适pH值范围加宽为6.0~8.0, 最适反应温度提高10 ℃; 抗胰蛋白酶水解能力明显增强. 结果表明, 丝素纳米颗粒与丝素蛋白膜一样, 是一种酶固定化的良好载体, 在药物缓释系统方面具有潜在的研究和开发价值.

关键词: 丝素纳米颗粒 *L*-天冬酰胺酶 固定化酶 交联

Preparation of Silk Fibroin Nanoparticles and Their Application to Immobilization of *L*-Asparaginase

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Abstract:

After the degummed fiber of silk fibroin derived from *Bombyx mori* cocoon was dissolved in $CaCl_2$ ternary solvent system or highly concentrated LiBr solution, three kinds of silk fibroin in liquid could be obtained by means of dialysis. SDS-PAGE analysis results show that three kinds of silk fibroin are of different molecular ranges. The silk fibroin nanoparticles were prepared rapidly from the liquid silk by using water-miscible organic solvents such as acetone. These nanoparticles are insoluble in water but well dispersed and stable in aqueous solution and are globular particles with a size range of 50—120 nm in diameter by means of SEM. *L*-Asparaginase as a model enzyme was bioconjugated with these nanoparticles by cross-linking agent glutaraldehyde. Activity analysis results indicate that silk fibroin nanoparticles derived from the fibroin by less breakage of peptide chain are more suitable for the bioconjugation of enzymes. The results show that the recovery of the immobilized *L*-asparaginase was about 44%. Its theromal stability increased evidently and the optimal scale of pH was much wider(pH 6.0—8.0) than that of native L-asparaginase. And the optimal reaction temperature of the modified enzyme was increased about 10 ℃. These preliminary results above indicat that the silk protein nanoparticles are also a good support as silk fibroin membrane. Therefore, the silk fibroin nanoparticles as a new drug release system are of potential values for study and development.

Keywords: Silk fibroin nanoparticle *L*-Asparaginase Immobilization enzyme Cross-linking

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