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一种温敏性水凝胶复性体系：聚N-异丙基丙烯酰胺的制备及其在溶菌酶复性中的应用

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摘要 Temperature-sensitive hydrogel—poly(N-isopropyl acrylamide) (PNIPA) was prepared and applied to protein refolding. PNIPA gel disks and gel particles were synthesized by the solution polymerization and inverse suspension polymerization respectively. The swelling kinetics of the gels was also studied. With these prepared PNIPA gels, the model protein lysozyme was renatured. Within 24h, PNIPA gel disks improved the yield of lysozyme activity by 49.3% from 3375.2U·mg^-1 to 5038.8U·mg^-1. With the addition of faster response PNIPA gel beads, the total lysozyme activity recovery was about 68.98% in 3h, as compared with 42.03% by simple batch dilution. The novel refolding system with PNIPA enables efficient refolding especially at high protein concentrations. Discussion about the mechanism revealed that when PNIPA gels were added into the refolding buffer, the hydrophobic interactions between denaturedproteins and polymer gels could prevent the aggregation of refolding intermediates, thus enhanced the protein renaturation.

关键词 酶复性； 温度性； 水凝胶； 聚合酶； DNA； 蛋白质； 溶菌酶复性体系

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A Temperature-sensitive Hydrogel Refolding System: Preparation of Poly (N-isopropyl acrylamide) and Its Application in Lysozyme Refolding

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Abstract Temperature-sensitive hydrogel—poly(N-isopropyl acrylamide) (PNIPA) was prepared and applied to protein refolding. PNIPA gel disks and gel particles were synthesized by the solution polymerization and inverse suspension polymerization respectively. The swelling kinetics of the gels was also studied. With these prepared PNIPA gels, the model protein lysozyme was renatured. Within 24h, PNIPA gel disks improved the yield of lysozyme activity by 49.3% from 3375.2U·mg^-1 to 5038.8U·mg^-1. With the addition of faster response PNIPA gel beads, the total lysozyme activity recovery was about 68.98% in 3h, as compared with 42.03% by simple batch dilution. The novel refolding system with PNIPA enables efficient refolding especially at high protein concentrations. Discussion about the mechanism revealed that when PNIPA gels were added into the refolding buffer, the hydrophobic interactions between denaturedproteins and polymer gels could prevent the aggregation of refolding intermediates, thus enhanced the protein renaturation.

Key words poly (N-isopropyl acrylamide) hydrogel; free radical polymerization; protein refolding; lysozyme.

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