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Chemical polysialylation of recombinant human proteins

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Abstract

© Springer Science+Business Media New York 2015. All right reserved. Design of drug with prolonged therapeutic action is one of the rapid developing fields of modern medical science and required implementation of different methods of protein chemistry and molecular biology. There are several therapeutic proteins needing increasing of their stability, pharmacokinetic, and pharmacodynamics parameters. To make long-live DNA-encoded drug PEGylation was proposed. Alternatively polysialic (colominic) acid, extracted from the cell wall of E. coli, fractionated to the desired size by anionexchange chromatography and chemically activated to the amine-reactive aldehyde form, may be chemically attached to the polypeptide chain. Conjugates of proteins and polysialic acid generally resemble properties of protein- PEG conjugates, but possess significant negative net charge and are thought to be fully degradable after endocytosis due to the presence of intracellular enzymes, hydrolyzing the polysialic acid. Complete biodegradation of the polysialic acid moiety makes this kind of conjugates preferable for creation of drugs, intended for chronic use. Here, we describe two different protocols of chemical polysialylation. First protocol was employed for the CHO-derived human butyrylcholinesterase with optimized for recovery of specific enzyme activity. Polysialic acid moieties are attached at various lysine residues. Another protocol was developed for high-yield conjugation of human insulin; major conjugation point is the N-terminal residue of the insulin's light chain. These methods may allow to produce polysialylated conjugates of various proteins or polypeptides with reasonable yield and without significant loss of functional activity.

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Keywords

Bioscavenger, Butyrylcholinesterase, Chemical conjugation, Chemical polysialylation, Insulin, Long-acting therapeutic proteins, Polysialic acid