Abstract No.7

The Stability Study of Recombinant Human Granulocyte-Colony-Stimulating Factor

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Growth and differentiation of various blood cell lines from progenitor stem cells are regulated by a group of proteins known as hematopoietins including granulocyte-colony-stimulating factor (G-CSF). G-CSF is a 19.6 kDa glycoprotein consisting of 174 amino acid residues. G-CSF, produced mainly by macrophages, induces proliferation of neutrophil colonies and differentiation of precursor cells to neutrophils, and stimulates the activity of mature neutrophils. Recombinant human granulocyte-colony stimulating factor (rh-G-CSF) is a therapeutic unglycolysated protein is produced in E.coli in our institute and used primarily to reduce incidence and duration of severe neutropenia and its associated. The stability of recombinant proteins has become an increasingly important consideration as more protein therapeutics are developed.In this study, the purified rh-G-CSF was characterized by following the changes on the structure, purity, dimerization and aggregations of protein in time of 0-3 months and two temperatures (4 and 25 ℃) by using biochemical techniques including reverse-phase (RPC), size-exclusion chromatography (SEC), electrophoresis and circular dichroism. The results were compared with Neupogen filgrastim as reference standard. The purity of samples and the stability of the proteins against aggregations were measured by SEC and SDS-PAGE electrophoresis. According to the inspection chromatogram, obtained peak conforms to molecular weight of rh-G-CSF without any aggregation forms in the protein structure (less than 1%) and disulfide bonds are in correct position. RPC results showed the similar hydrophobicity for rh-G-CSF and reference standard. CD results showed the same secondary and tertiary structure of G-CSF and reference standard in addition to the fact that the G-CSF secondary structure is predominantly helical. The obtained results approved that the rh-G-CSF was highly pure and comparable with the innovator products, Neupogen filgrastim and has a proper thermal stability in 4 and 25 ℃ in period of 3 months after production.

Keywords: Rh-G-CSF, Stability, SDS-PAGE Electrophoresis, Circular Dichroism, Chromatography.

Abstract No.8

Study on the Interaction of Lysozyme whit SDS and SOS by Fluorescence Spectroscopy

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Lysozyme, also called muramidase, a small monomeric globular protein first discovered by Alexander Fleming in 1922, is a basic protein belongs to the class of enzymes that lyse the cell walls of bacteria by hydrolyzing the bond between N-acetylmuramic acid and N-acetylglucosamine of the peptidoglycan. Lysozyme is antimicrobial protein widely distributed in various biological fluids and tissues including avian egg and animal secretions, human milk, tears, saliva, airway secretions, and secreted by polymorphonuclear leukocytes. In this paper, we designed to examine the effect of sodium dodecyl sulfate (SDS) and sodium octyl sulfate (SOS) on the solution structure of lysozyme using fluorescence and UV/Vis at different temperatures and pHs. All studies were carried out in quartz cells containing 0.1mM lysozyme and different Concentration SDS and SOS. The fluorescence spectrum of each suspension was measured, where the excitation wavelength was at 280 nm and the emission wavelength was between 290 and 450 nm (using 5 and 3 nm of slit width). The interaction of (SDS and SOS) with lysozyme was investigated by fluorescence spectroscopic techniques under physiological conditions. The results revealed that SDS and SOS caused the fluorescence quenching of lysozyme through a static quenching procedure. It was reported that 80% of lysozyme fluorescence was due to Trp62 and Trp108, and the oxidation of either Trp62 or Trp108 was accompanied by a drastic decrease in fluorescence intensity. In addition, Trp62 emission was in a higher proportion than that of Trp108 when lysozyme molecule was excited at 280 nm. Therefore it was reasonably proposed that SDS and SOS quenched the fluorescence of both Trp62 and Trp108 residues of lysozyme.

Keywords: Lysozyme, Sodium Dodecyl Sulfate, Sodium Octyl Sulfate (SOS), Protein.