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Characterization of Galactosyl-neoglycoalbumin[1] by MALDI-TOF-MS

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Abstract: Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS, model AXIMA CFR+) was used to analyze galactosyl-HSA (human serum albumin) synthesized in our laboratory. It clearly showed that HSA covalently combined with galactoses. Galactosyl-HSA is pure and the ratio of galactosyl residues to protein is 48:1.

Keywords: Galactose, HSA, MALDI-TOF-MS, protein.

It was reported there was a membrane receptor, hepatic binding protein (HBP), which resides only at the cell surface of mammalian hepatocytes, selectively bind galactose-terminated glycoprotein for transporting to hepatic lysosomes[1]. According to this discovery, a special analog ligand, galactosyl-neoglycoalbumin(NGA), was prepared as hepatic targeting drug carrier and hepatic screening agents. The ligand was synthesized by covalent coupling of carbohydate bifunctional reagent, 2-imino-2-ethyloxymethyl-1-thiogalactose, to human serum albumin. Since HSA has a large molecular weight, it is difficult to assay the glycosyl-density of NGA and judge the combining state of galactose with protein. Some reports suggested[2,3] that the problem was resolved by means of SDS-PAGE and DTA (differential thermal analysis). It is obvious that this is inadequate to the purpose intended.

Modern bio-mass spectrometry such as MALDI-TOF-MS is a powerful mass technology for study structure of protein[4]. With the use of bio-mass spectrometry, the protein molecular weight, combining state of protein with galactose and the protein site of modification with glycose can be studied. In this paper, we synthesized the different glycosyl-density NGA and studied its coupling state and glycosyl density by MALDI-TOF-MS.

Materials and Methods

Galactosyl-neoglycoalbumin was prepared in our laboratory. Human serum albumin (HSA) was bought from Pharmaceutical Corporation of Shuanling, Zhanjiang

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Guangdong. A portion of the protein sample (0.7 mg) was re-suspended in 70 μL water, a further 50-fold dilution was performed.

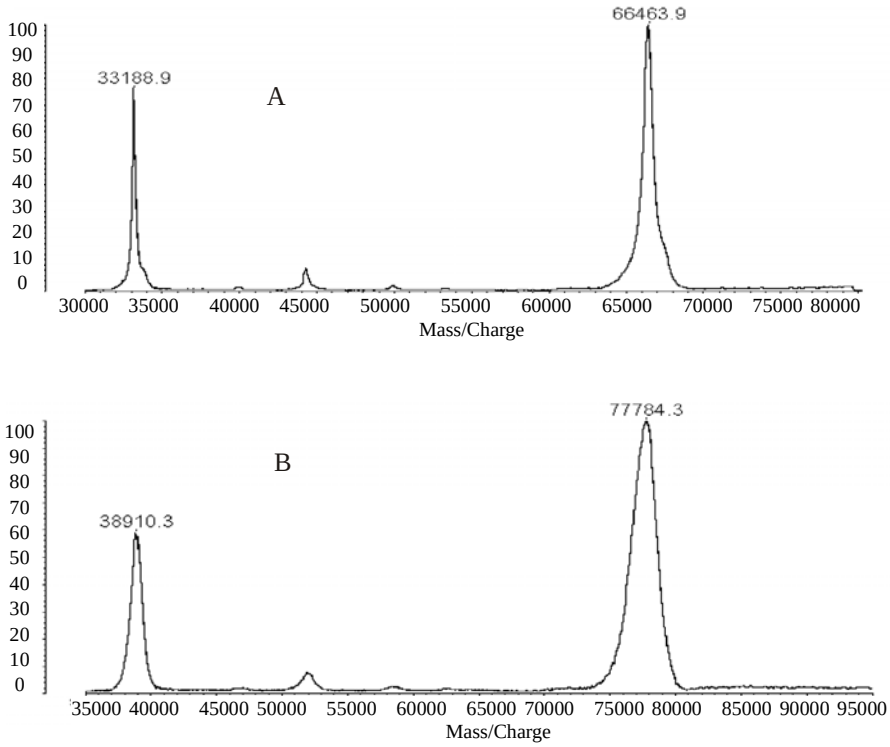
0.5 μL of the final dilution (about 0.2 mg/mL) was deposited on an stainless steel MALDI target, 0.5 μL of CHCA (10 mg/mL in 50% acetonitril : 0.1% TFA (v/v) solution) was added to the deposited sample and the solution was allowed to air-dry.

Samples were analyzed using AXIMA-CFR plus (Shimadzu Biotech) in positive-ion mode. The protein samples were analyzed in linear mode. Samples were calibrated using a 2-point close external calibration procedure using bovine serum albumin. A pulsed extraction value of 66000 was used.

Results and Discussion

The mass spectra of the human serum albumin were presented in **Figure 1A**, and the neoglycoalbumin in **Figure 1B**. It showed that the HSA molecular weight was 66463 Da, while the molecular weight of NGA was 77784Da. The mass shift was about 11300 Da.

Figure 1 The HSA and NGA spectra of MALDI-TOF-MS



A). The spectrum of HSA B). The spectrum of NGA

In the **Figure 1**, it showed that galactose coupled the HSA by covalent bonds, so the NGA molecular weight was higher than HSA. If the galactose did not combine the HSA by covalent bonds but by the others, the composition would not increase in molecular weight and possessed any function of hepatic targeting.

The glycosyl density of NGA could be calculated by the data of **Figure 1**. The molecular weight difference of NGA and HSA was about 11300Da, and the molecular weight of galactosyl-coupler was 236Da, indicating the glycosyl density were 48:1. It was much higher than the reported[5,6]. The study showed, the higher glycosyl density of NGA, the better hepatic targeting action.

It was a beneficial condition to synthesize glycoprotein of high glycosyl density that the ratio of protein to IME(2-imino-2-methoxyethyl 1-thioglycosides) was 1 : 150 at 20℃ and pH=8.0-8.6 for 24 h.

There is no peak in the 66463Da in the **Figure 1B**, which means that no HSA remainder was in NGA. If the NGA mixed with HSA, the function of NGA as the hepatic targeting drug carrier would be decreased as the HSA could couple with the drug.

In conclusion, MALDI-TOF-MS is a powerful tool for studying glycoprotein on its coupling state, purification and glycosyl density. It is suitable for the quality control of neoglycoalbumin of hepatic targeting drug carrier.

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