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Modification and Characterization of Lactoferrin-Iron Free with Methylimidazolium N-ethylamine Ionic Liquid as Potential Drugs Anti SARS-CoV-2 †

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Abstract: Methylimidazolium N-ethylamine amine (MIE-NH_2) is synthesized successfully with excellent yield in the high performances and green chemical process, using N-methylimidazole and tert-butyl N-(2-bromoethyl) carbamate as starting materials. Following the mechanism of reductive amination, using this ionic liquid as a suitable ligand for modification, N-glycans contain the carbonyl group of the oligosaccharides, and the activity of an ionic liquid is disclosed by mass spectrometric techniques. This work illustrates that methylimidazolium N-ethylamine as an ionic liquid linked to carbohydrates, including N-glycans in lactoferrin and its derivatives, for example, lactoferrin (BL iron free), have been selected as examples of glycoproteins. The detection of profiling linked to oligosaccharides and glycoproteins is performed using UPLC/ESI-QTOF and MALDI-TOF mass spectrometry. Moreover, the ionic synthesis with active amino-group and employed as a multifunctional modification of the oligosaccharide, and using the products as applicable small molecules therapeutics linked to GlcNAc and its derivatives. Modifying glycoproteins by adding IL-MIE-NH_2 has improved ESI ionization efficiency and provided labeling results of N-glycans, even better than 2-AB derivatives. Relevantly, this ionic liquid is applicable as advancement and development in catalytic methods, N-glycosylation, and modification of small molecules as potential drugs against viral and microbial infections.

Keywords: ionic liquid; infections; lactoferrin; N-glycans; mass spectrophotometry

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1. Introduction

Lactoferrin (Lf-iron free) is a transferrin protein and the most abundant glycoprotein in human and ruminant milk resources. Lactoferrin contains 1–4 glycans with single-chain polypeptides of about 80,000 Da. lactoferrin is a multifunctional glycoprotein involving N-glycans have active with functional groups and, depending on the species, makes an important contribution to the host that defines the system [1–3]. In addition, lactoferrin carries many important biological functions, including N-glycans bonding to iron or other metals, being bioactive in cell proliferation and differentiation, as an anti-parasitic protein, an anti-bacterial, and anti-viral. These functions differ from lactoferrin's considerable attention as the primary nutritional contribution to iron-binding by the role of glycosylation [4,5]. Ionic liquids contain an N-active group that makes the critical role of carbonyl groups of glycan attractive and binding in bioprocesses very easily. The chain of saccharides-glycan moieties

in lactoferrin is likely to contribute significantly to the N-ionic liquids' roles by carbonyl of saccharides. Despite the high amino group of ionic liquid sequence homology in different with excellent results, which exhibits a unique N-glycosylation for heterogeneity of the biological properties and lactoferrin is chosen as a good example source of N-glycans [6,7].

Exploring and identifying the new characterization of novel ionic liquid provides reacting with oligosaccharides of glycoproteins. Several interesting studies are encouraging for discovering the new application of ionic liquids as potential antimicrobial drugs, including anti-viral. Studying the activity of glycoproteins is the assessment of the contributions of individual glycans to the observed bioactivities. This work examines how the study of N-link glycosylation in lactoferrin, which reacted with ionic liquid MIE-NH_2 increases the understanding of ionic liquid functionality [2,8,9].

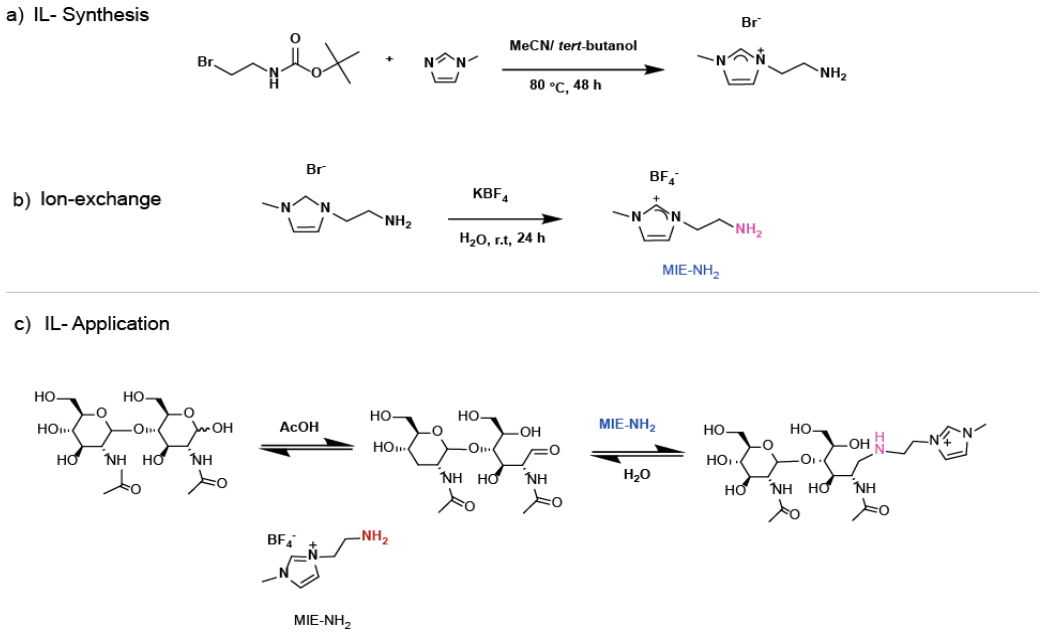
2. Experimental Section

2.1. Materials

Tert-butyl N-(2-bromoethyl) carbamate and N-methylimidazole were purchased from J&K (Shanghai, China). Acetonitrile and solvent used for HPLC were purchased from Merck (Ankara, Turkey). Lactoferrin-free iron and all other chemicals used in this study were bought at the highest grade from commercial suppliers without further purification or modification.

2.2. Synthesis of Ionic Liquid-[1-(2-Aminoethyl)-3-Methyl-1H-Imidazole-3-Ium] MIE-NH_2][BF_4^-])

Tert-butyl N-(2-bromoethyl) carbamate (246.5 mg, 1.1 mmol) was reacted with N-methylimidazole (82 mg, 1 mmol) in an anhydrous mixture solvent of CH_3CN and t-BuOH [5 mL (3/2, *v*/*v*)], reaction mixture refluxing at 80 °C for 2 days. Removing unreacted materials by washing with ethyl acetate three times and the product was dried under reduced pressure and obtained the light yellow viscous liquid (Scheme 1a) [10–13].



Scheme 1. Synthetic route of methylimidazolium N-ethylamine and derivation of aminoglycosides with GlcNAc [10]; (**a**) *IL-synthesis* (**b**) *Ion exchange* and (**c**) *ILs-application*.

The viscous liquid (1) IL-Br-1 (289 mg g, 85.7 mmol) was generated by stirring with KBF_4 (1.1 equiv.) in a water solution for 24 h at room temperature. Then, the reaction mixture was filtered and vacuum distilled, and washed the product by dichloromethane and ethyl acetate. The product was vacuum dried by a rotary evaporator at 55 °C to remove

the traces of dichloromethane and ethyl acetate. After drying for 6 h under vacuum at 80 °C, the expected ionic liquid [MIE-NH_2][BF_4^-] was obtained (Scheme 1b) [10,14,15].

2.3. Derivatization N-Glycans of Lactoferrin with ILs-NH_2

Derivatization solution contains 70 mM MIE-NH_2 and 0.1 M sodium cyanoborohydride in dimethyl sulfoxide/acetic acid solution (7:3, *v*/*v*)) was added to a sample of lactoferrin until completely dissolved. The derivatization mixture was mixed by ultrasonic for about 30 min and incubated at 70–90 °C for 4 h.

3. Results and Discussion

The glycoproteins in bovine lactoferrin were chosen as substrates to prepare N-glycans with a high structure, including saccharides. The high proportion of glycosylation verifies the methodology of MIE-NH_2, which was used for labeling N-glycans [10]. According to our previous studies, lactoferrin-iron free (LF-iron free) is well-known as a good resource of glycoprotein and found proximity 42 types of N-glycans with five potential sits N-glycosylation, the different N-glycans with all structures sites [16]. The lactoferrin was modified by using an ionic liquid; there are 14 different MIE-NH_2 derivative lactoferrin-N-glycans were deduced according to the UPLC profile and MS spectrum (see Figure 1). The corresponding structures of lactoferrin-MIE-NH_2 were assigned, as shown in Figures 1 and 2. The results of the detection in Figure 1 suggested the possible structures of compounds were modified by IL-MIE-NH_2, and this result was confirmed by MALDI ToF analysis (Figure 2). The m/z values of structures either with mono-charge or di-charge were calculated related to the signals of MIE-NH_2 linked to N-glycans was observed. In extracted ion chromatogram of the products of N-glycans linked to MIE-NH_2 from lactoferrin by HPLC and two peaks exhibited the same m/z value of new products 1716.50, which assigned and identified with theoretical m/z = 1716.70 $[m]^+$). In this case, we suggested the new product is MIE-NH_2 linked monofucosylated monogalactosylated bi-antennary complex N-glycan isoforms [10,17]. For example, from LC-MS analysis, it was founded the peak of 13.7 min was assigned as MIE-NH_2-FA2G1, and the peak at 14.6 min was derivate as MIE-NH_2-A2G1F. This work demonstrated the catalytic mechanism of the derivatization of lactoferrin-N-glycans with ionic liquid MIE-NH_2 following the reductive amination [10,16]. The free aldehyde realized in the acidic medium and by reducing ligand as sodium cyanoborohydride, which possesses significant converted the carbonyl to an imine by the NH_2 group of MIE-NH_2 (Scheme 1c) [10].

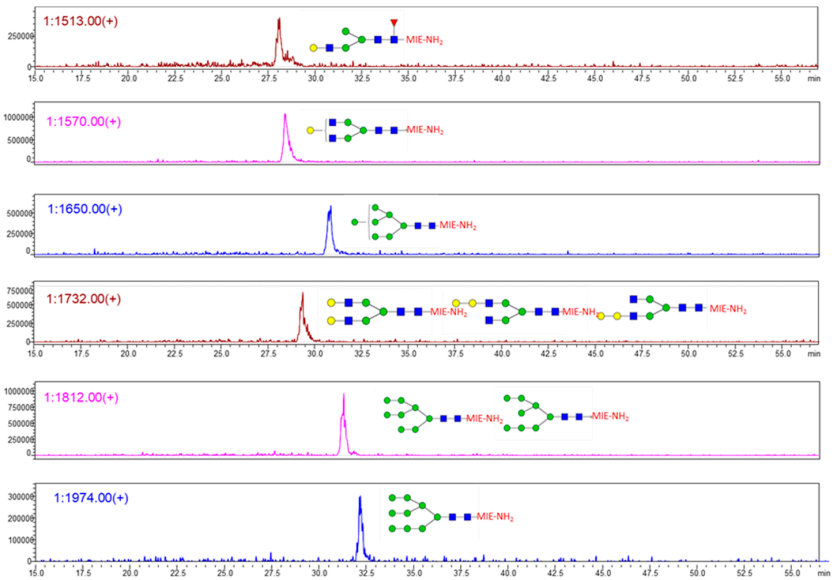


Figure 1. LCMS result of derivatization of different N-Glycans from lactoferrin and linked with MIE-NH_2.

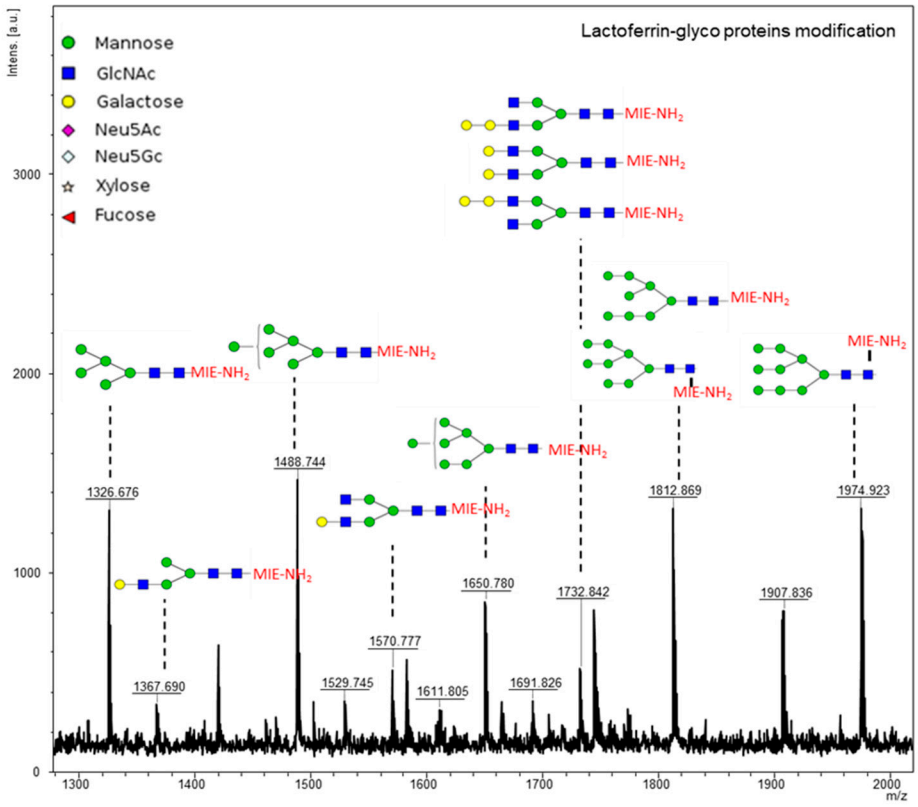


Figure 2. MALDI-TOF-MS results confirm the modulation of N-glycans from lactoferrin linked with MIE-NH_2.

Recent scientific research focused on the progress in protein-based nanomedicine, albumin-paclitaxel as nanoparticles have been introduced in novel therapeutics and used for the treatment of cancer and viral infections. However, specific drug targeting of SARS-CoV-2 is almost challenging and absent until now, premature drug release and supports the poor pharmaceuticals stores for resistance to COVID-19 and its mutations. Therefore, some studies with alternative protein-based nanomedicines have opened the eyes to the use of ionic liquids for extending and developing a novel of small molecules form glycoproteins. Regarding this challenge, lactoferrin (Lf-iron free) offers a promising bioactive well as potentially therapeutic and drug nanocarrier. In this work, we focused on the major pharmacological actions of modified glycoproteins form lactoferrin with ionic liquids to produce new molecules, including anti-viral, anti-cancer, and/ or improved immunology.

For enhancing the efficacy of glycoproteins as potential anti-COVID-19 drugs, it was functionalization of N-glycans with an emphasis on lactoferrin. Besides this technique in the wide application of small molecules therapeutics, we depended on the recent advances of ionic liquids-Lf-based small molecules as efficient platforms for delivering novel drugs and anti-viral drugs, particularly for treating the SARS-CoV-2 infections.

4. Conclusions

This conclusion confirmed the modification and application of ionic liquid methyl imidazole ethyl amine ionic liquid MIE-NH_2 derivatization of lactoferrin (Lf-iron free). The detection of N-glycans from lactoferrin-glycoprotein promoted to use of the ionic liquid methyl imidazole ethyl amine IL-MIE-NH_2 (IL-MIE-NH_2) for producing small molecules ionic liquids, which could provide novel drugs anti-viral, this study explored the available strategy for modifying glycoproteins by ionic liquids follows the reductive amination mechanism, this study suggested new drugs by modifying carbohydrates ionic liquid with potential bioactive, the separation of MIE-NH_2 shows the high selectivity of the carbonyl group of sugars which could be accomplished by the hydrophilic interaction

chromatography. This study suggested that new small molecules of lactoferrin containing methyl imidazole ethylamine contain antimicrobial and anti-viral potential, including for SARS-CoV-2 treatments.

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Conflicts of Interest: The authors declare no conflict of interest.

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