Research Article

Nucleophilic Displacement Reaction on Tosyl Cellulose by L-Methionine to the Synthesis of Novel Water-Soluble Cellulose Derivative and Its Antibacterial Activity

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A novel ampholytic cellulose derivative, cellulose-L-methionine, has been synthesized by means of an esterification reaction of microcrystalline cellulose with tosyl chloride (p-TsCl) in DMAc/LiCl (8%) at 8°C that was followed by nucleophilic displacement (SN) of the tosyl group by the L-methionine amino acid. The resulting structure of cellulose-L-methionine has been characterized by elemental analysis (CHNSO), Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (1H-NMR), and scanning electron microscopy (SEM). The antibacterial activity of the synthesized product was screened against Gram-positive and Gram-negative microbial strains such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, by the agar well diffusion method, and compared with commercial antibiotics such as ampicillin and chloramphenicol. It was found that antibacterial experiment revealed excellent antibacterial activity of the cellulose-methionine with respect to a minimal inhibitory concentration (MIC) reference.

1. Introduction

Cellulose and its derivatives are of huge importance for various applications areas, food medical products, pharmaceutical industries, packaging, textile, etc., because of their very interesting chemical and physical properties, such as biodegradability, biocompatibility, bioactivity, and nontoxicity property [1, 2]. Cellulose is the most abundant biopolymer in nature. Cellulose is insoluble in common organic solvents and in water [3]. This is due to the fact that the hydroxyl groups are responsible for the extensive hydrogen bonding network forming both intra- and intermolecular hydrogen bonding. However, special solvents were developed for the dissolved cellulose and for preparing a broad variety of cellulose derivatives by homogeneous chemical modification of the biopolymer [4–8]. By a chemical functionalization of cellulose, a wide variety of new macromolecules have been obtained [9–11]. For several years, great effort has been devoted to the synthesis of water-soluble cellulose derivative as promising biomaterials in high-end applications for drug delivery such as potential antibacterial activity [12, 13]. For example, the synthesis of tosyl cellulose derivative intermediates through the tosylation of the primary hydroxyl group at C-6 of cellulose has been reported since the tosyl group is a good electrophile and leaving group in nucleophilic displacement (SN) reactions or as protecting group in further reactions of the remaining free hydroxyl (-OH) groups [13–15]. In our previous work, we were able to synthesize tosyl cellulose with different degrees of substitution (DS) by reacting microcrystalline cellulose with p-tosyl chloride, and we have studied the effects of reaction parameters on DS by response surface methodology (RSM) [16]. Ampholytic biomaterials are semisynthesized biomacromolecular carrying both the ionic and cationic groups; they offer beneficial applications...
in drug delivery, protein separation, surfactants, and chelation with metal ions because of their structure specificity [17–20]. Several routes have been reported for the preparation of ampholytic cellulose such as the synthesis of cellulose sulfates functionalized with quaternary ammonium salt [21], synthesis of 6-deoxy N-sulfonated and N-carboxymethylated cellulose [22], and cellulose zwitterions [23].

Up to now, although researchers have synthesized some cellulose derivatives that are functionalized with the amino groups [12, 13, 24, 25], there are no published studies related to the synthesis of cellulose-L-methionine and its antibacterial activities to our knowledge. In our concept, the key reaction is the nucleophilic displacement ($S_N$) reaction of the tosyl cellulose by L-methionine to synthesize new water-soluble ampholytic cellulose derivative with interesting molecular structure and properties, cellulose-L-methionine, and evaluated its antibacterial activities against a Gram-positive and Gram-negative microbial strains such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus fasciens. The tosyl cellulose with $DS \approx 1$ was chosen as an intermediate to yield new water-soluble ampholytic cellulose by $S_N$. The reaction proceeds homogeneously in DMF at $80^\circ$C for 24 h. The chemical structure and properties of the synthesized product were characterized by elemental analyses, Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance ($^1$H-NMR), and scanning electron microscopy (SEM).

2. Experimental Part

2.1. Materials and Reagents. Microcrystalline cellulose commercial (Powder Aldrich Chemical) with a degree of polymerization $DP = 280$ was used as a starting polymer. $N,N$-Dimethylacetamide (DMAc), anhydrous lithium chloride (LiCl), tosyl chloride ($p$-TsCl), $N,N$-dimethylformamide (DMF), L-methionine, potassium hydroxide (KOH), ethanol (EtOH), and triethylamine (TEA) were purchased from Sigma-Aldrich and were used without further purification.

2.2. Microorganisms and Inoculum Preparation. The tested microorganisms included the following bacteria: Escherichia coli ATCC 4157, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and Streptococcus fasciens 29212.

All pathogenic microorganisms isolated from patients were stored at the culture collection of the Biology Department (Microtech Unity) at the Faculty of Science, Rabat, Morocco. They were maintained in brain heart infusion (BHI) at $-80^\circ$C. Prior to the experiment, cultures were prepared by subculturing 1 mL of each culture stock in 9 mL of BHI broth.

2.3. Methods of Material Analysis

2.3.1. Spectroscopic Measurements. The apparatus used for infrared spectroscopy characterization is a Bruker Tensor 70, which operates in transmittance mode. This apparatus is equipped with a Glabor source that emits radiation in the region of midinfrared and of a DLaTGS detector. The acquisition spectrum was recorded from a sample in solid form prepared as pellets to 1% by weight of product dispersed in KBr.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance 300 MHz spectrometer with 16 scans for $^1$H-NMR at $25^\circ$C in D$_2$O or DMSO and a sample concentration of 30 mg/mL. The chemical shifts (δ) were expressed as part per million (ppm) against tetramethylsilane (TMS) as internal reference.

2.3.2. Scanning Electron Microscopy (SEM) Analysis. The surface morphology of the microcrystalline cellulose and the tosyl cellulose was analyzed by scanning electron microscopy (SEM), using a FEI Quanta 200 microscope. The samples were air dried for 24 h before imaging and were coated with a carbon layer to increase their conductivity. The images of samples were obtained using an accelerating voltage of 30 kV.

2.3.3. Elemental Analysis. Elemental analysis was performed by Euro EA - CHNSO Elemental Analyzer.

2.4. Typical Cellulose Dissolution Process in DMAc/LiCl Solvent System. In brief, 0.5 g of oven-dried microcrystalline cellulose (3.08 mmol) was added to 20 mL of DMAc and the suspension was heated at $130^\circ$C for 2 h. The resulted slurry was cooled down to $100^\circ$C, and 1.6 g of anhydrous LiCl (8%) was added to the mixture with stirring while cooling down the mixture at room temperature. The stirring was continued until the complete dissolution of cellulose within a few hours.

2.5. Synthesis of Tosyl Cellulose. Tosyl cellulose was prepared according to literature [26].

In brief, 2 mol eq. of TEA (43.12 mmol, 5.82 mL) diluted in 10 mL of DMAc was added to the cellulose/DMAc/LiCl solution with stirring at room temperature. Afterwards, the reaction temperature was adjusted to $8^\circ$C and a solution of tosyl chloride ($p$-TsCl) (7 mol eq., 21.56 mmol, 4.12 g) in 25 mL of DMAc was added dropwise over 45 min. The reaction mixture was kept stirring for additional 24 h at $8^\circ$C. The

Figure 1: Infrared spectrum of MCC (a), tosyl cellulose (b), and cellulose-L-methionine (c).
mixture was poured slowly into 500 mL of ice-cold water. The precipitate was filtered off, washed with about 500 mL of distilled water, and then washed with 500 mL of ethanol. The obtained product was dried at 40°C in oven for 12 h (yield: 81%; elemental analysis for tosyl cellulose: C = 45.23, H = 5.11, N = 0.038, and S = 10.22). The degree of substitution (DS ≈ 1) in tosyl cellulose was determined from the sulfur content determined by the elemental analysis [27].

FTIR (KBr, ν/cm⁻¹) is as follows: 3459 (νOH), 2924 (νC-H), 1500, 1456 (νC-Arom), 1370 (ςSO₂), 1172 (ςSO₂), 1116 (C-O-C), and 813 (νC-H-Arom) cm⁻¹ (see Figure 1).

¹H-NMR (DMSO-d₆) is as follows: δ 2.43 (p-CH₃), δ 3.3–5 (cellulose backbone), and δ 7.12–7.82 ppm (tosyl cellulose) (see Figure 2).

2.6. Synthesis of Cellulose-L-Methionine. 200 mg of tosyl cellulose (0.63 mmol) with DS ≈ 1 was dissolved in 3 mL of hot DMF (S₁). Then, 3 mol eq. (1.89 mmol, 282 mg) of the L-methionine amino acid was dissolved in 3 mL of potassium hydroxide solution (10%) and added dropwise over 60 min to S₁. The reaction mixture was then stirred and heated to 80°C for 24 h. The product was isolated by precipitation of
the reaction mixture into 100 mL of ethanol. The crude solid product was filtered off and washed several times with 100 mL of ethanol. The obtained product was oven dried at 40°C.

Elemental analysis for cellulose-L-methionine is as follows: C = 47.31, H = 6.18, N = 3.23, and S = 5.79.

FTIR (KBr, v/cm⁻¹) is as follows: 3333 (vOH), 2920 (vC-H), 1654 (vC=O), 1574 (vC-Nstr), and 1023 (C-Ostr) cm⁻¹ (see Figure 1).

1H-NMR (D₂O) is as follows: δ 2.06, 2.51, and 2.67 ppm (CH₃, CH₂, and CH of L-methionine amino acid, respectively) and δ3.3–5 ppm (protons of cellulose backbone) (see Figure 2).

2.7. Agar Disc Diffusion Method. The agar disc diffusion method (ADD) was employed for the determination of antibacterial activities of the tested products as described previously. The principle of this technique is to estimate the bacteriostatic activity of antibacterial agents by measuring the growth inhibition zone of germs around wells. It is mostly used in a preliminary step to further study because it provides access to essentially qualitative results. The test samples were first dissolved in distilled water (DW), which did not affect the microbial growth.

Briefly, the test was performed in sterile petri plates containing medium agar. 30 mL of sterilized medium was poured into sterile petri plates. After solidification, 100 μL of fresh cultures of bacteria species (one microorganism per petri plate). Sterile filter paper disc (6 mm in diameter) was impregnated with 6 μL of the test samples (40 mg/mL). All plates were sealed with sterile laboratory films to avoid eventual evaporation of the test samples and then incubated at 37°C for 24 h. The diameter of inhibition zone was measured in millimeters. In addition, the antibacterial activity of the cellulose-L-methionine sample on Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus fasciens bacteria was compared with the commercially available antibiotics. The antibiotic discs such as ampicillin and chloramphenicol were placed on the surface of the plates. Distilled water was used as a negative control. The plates were incubated at 37°C for 24 h after incubation. The diameter of inhibition zone was measured in mm and was recorded [28, 29].

2.8. Determination of the Minimum Inhibition Concentration (MIC). We tested six (6) serial concentrations of the high active products at concentrations 40, 20, 10, 5, 2.5, and 1.25 mg/mL, diluted in BHI broth. For MIC assessed, 5 mL of culture medium was inoculated with 0.1 mL of bacteria species. The MIC is the lowest concentration of samples, for which no growth was detected for 24 h at 37°C.

3. Results and Discussion

3.1. Nucleophilic Substitution of Tosyl Cellulose with L-Methionine Amino Acid. The tosyl cellulose derivative was synthesized by esterification of the hydroxyl groups of the microcrystalline cellulose chains with tosylic acid. The reaction was carried out homogeneously in DMAc/LiCl (8%) solvent system at 8°C for 24 h in the presence of a strong base such as triethylamine (TEA). The tosyl cellulose with DS ≈ 1 so obtained was used as an intermediate to introduce additional amino acid into C-6. This new cellulose derivative was prepared by nucleophilic substitution (SN) reaction of the tosyl group; we have chosen DS ≈ 1 to avoid double steric effects of both the tosyl groups at C-2 and C-3 and the
glucose ring against nucleophilic attack of the tosyl group. The reaction process for the synthesis of cellulose-L-methionine is shown in Figure 3.

3.2. Characterization of Samples

3.2.1. FTIR Spectroscopy. FTIR spectra of the MCC (a), tosyl cellulose (b), and cellulose-L-methionine (c) are shown in Figure 1. It can be seen that spectrum of tosyl cellulose provides a clear evidence of tosylation by showing the presence of some important peaks at 813 cm\(^{-1}\) for aromatic ring (C-H) stretching, 1116 cm\(^{-1}\) for (C-O-C) asymmetric stretching, and ring asymmetric stretching for cellulose. The absorption peaks at 1172 and 1370 cm\(^{-1}\) correspond, respectively, to (SO\(_2\)) group symmetric and asymmetric stretching, 1500 and 1456 cm\(^{-1}\) for aromatic (C-C) stretching, 3459 cm\(^{-1}\) for group (OH) stretching of cellulose, and 2924 cm\(^{-1}\) for (C-H) cellulose. As expected, the infrared spectrum (c) of the new product, cellulose-L-methionine, showed neither asymmetric nor the symmetric valence vibrations of the (SO\(_2\)) group at 1172 cm\(^{-1}\) and at 1370 cm\(^{-1}\), which suggests its complete displacement by the L-methionine amino acid derivative. Furthermore, this disappearance of the tosyl group occurs with a concomitant appearance of a new strong absorption band around 1654 and 1574 cm\(^{-1}\) characteristic of the carboxylic group (C=O) and C-N\(_{str}\) bend, respectively. Based on the FTIR results, it can be concluded that the reactions of nucleophilic substitution of tosyl cellulose with L-methionine amino acid have been successful.

3.2.2. Elemental Analysis. The elemental composition of tosyl cellulose (TC) and cellulose-L-methionine was determined by microanalytical elemental analyses. The elemental analysis revealed that the S% in tosyl cellulose was 10.22 and it was negligible in MCC, but it was 5.79 in cellulose-L-methionine. Similarly, the N% in both MCC and TC were 0.006 and 0.038 which increase upon the coupling of the amino acid to be 3.23% in cellulose-L-methionine.

3.2.3. NMR Spectroscopy. Comparing the \(^1\)H-NMR spectra of the synthesized samples, tosyl cellulose (a) and cellulose-L-methionine derivative (b) in Figure 2 gave a clear-cut
confirmation of the nucleophilic substitution reaction. The
1H-NMR results clearly suggest the success of tosylation
reaction of microcrystalline cellulose by existence of phenyl
protons at δ 7.12–7.82 ppm, methyl protons (p-CH₃) of the
tosyl group at δ 2.43 ppm, and protons of cellulose backbone
at δ 3.3–5 ppm [15]. Meanwhile, the 1H-NMR spectrum of
cellulose-L-methionine revealed the presence of new signals
related to the L-methionine amino acid moieties substituted
C-6 of cellulose, such as the signals at δ 2.06, 2.51, and
2.67 ppm for CH₃, CH₂, and CH, respectively. It is evident
that the complete replacement of the tosyl group was con-
firmed by the disappearance of the signals ascribed to the
tosyl group.

3.2.4. Scanning Electron Microscopy (SEM). Figure 4 displays
the morphological structure of MCC (a), tosyl cellulose (b),
and cellulose-L-methionine derivative (c). The SEM images
of the surface of (a), (b), and (c) show clear differences
between them. The MCC is mainly composed of platelet-
like cellulose microfibrils, shaped into a spherical agglomera-
tion. However, the surface structure of tosyl cellulose (b) is
compact, the cellulose-L-methionine (c) had much greater
porosity, and the surface roughness of cellulose derivative
increased more than unmodified MCC. Interruption of the
backbone of tosyl cellulose polymer can explain this observa-
tion as a result of reaction of the hydroxyl group (-OH) of
MCC with p-tosyl chloride and probably due to breaking of
hydrogen bonds present in the MCC and interaction between
the newly introduced hydrophobic phenyl groups. Thus,
nucleophilic substitution reaction of tosyl cellulose with L-
methionine amino acid leads to very significant changes in
the morphology of the unmodified MCC.

3.3. In Vitro Antibacterial Activity. The development of gen-
erations of new strains of bacteria is potentially harmful and
stresses the importance of developing materials to fight
human pandemics; for that, the natural antibacterial agent
has been one of the hot topics in scientific research to develop

**Figure 5**: Antibacterial activity of cellulose-L-methionine (C-methionine) and commercial antibiotics ampicillin and chloramphenicol against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus fasciens*. 

![Graph of antibacterial activity](image-url)
Table 1: Minimal inhibitory concentration (MIC) of the cellulose-L-methionine.

<table>
<thead>
<tr>
<th>Samples</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>S. fasciens</th>
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<td>10</td>
<td>20</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>methionine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6.25</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

The tosyl cellulose with degree of substitution DS = 1 was successfully synthesized by an esterification reaction of microcrystalline cellulose in DMAC/LiCl (8%) solvent system in the presence of triethylamine (TEA) at 8°C for 24 h. The nucleophilic displacement (SN2) reaction of the tosyl group with L-methionine amino acid could be efficiently carried out leading to water-soluble methionine-substituted cellulose derivative. The characterization by means of NMR and FTIR spectroscopy, elemental analysis, and SEM indicates a uniform structure of the tosyl cellulose and cellulose-L-methionine. The antibacterial test of cellulose-L-methionine shows significant activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus fasciens strains of bacteria. In our future research, we intend to focus on explaining the exact mechanism of cellulose-L-methionine that induces bacterial cytotoxicity. This work encourages us to develop cellulose-based antibacterial materials for use in different biomedical and pharmaceutical applications.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References


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