Research Article

Properties of Surfactin C-15 Nanopeptide and Its Cytotoxic Effect on Human Cervix Cancer (HeLa) Cell Line

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1. Introduction

Surfactin is one of the most powerful biosurfactants that has been known so far. It is an acidic cyclic nonribosomal lipopeptide that is produced by Bacillus subtilis. In this presentation we investigated different properties of surfactin C-15. The nanomicelle forming ability of surfactin C-15 in different aqueous environments with various ionic strengths was studied by scanning electron microscope. Surfactin second structure was investigated by Far-UV CD spectrum. Its hemolytic activity and cytotoxicity were measured by hemolysis and MTT assays, respectively. Surfactin formed spherical nanomicelles in distilled water (pH = 7.4) and amorphous nanomicelles in PBS buffer (pH = 7.4). The hemolysis assay results indicated that HC50 of surfactin was 47 μM. Surfactin C-15 arrested growth of human cervix cancer HeLa cell line in a time- and dosage-dependent method, so that its IC50 at 16, 24, and 48h were 86.9, 73.1, and 50.2 μM, respectively.

2. Materials and Methods

2.1. Materials. Surfactin and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) were obtained from Sigma. RPMI-1640, fetal bovine serum (FBS), and Trypsin-EDTA 5x were purchased from Gibco, and HeLa cell line was obtained from Pastor Institute of Iran.
was measured by AVIV 215 spectropolarimeter in different concentration (20, 40, 60, 240, 500, and 766 μM) at 25°C. The wavelength ranged between 195 and 260 nm.

2.3. Physical Characterization of Surfactin Nanomicelles. For the preparation of nanomicelles by surfactin, 1 mg surfactin was sonicated in 1 mL distilled water (pH = 7.4) and 1 mL PBS (phosphate buffer saline, pH = 7.4) for 15 min at 30°C. The size and shape of nanomicelles were studied by scanning electron microscope (SEM, LEO1430VP).

2.4. Red Blood Cell Preparation. Human red blood cells (RBCs) were separated by centrifugation at 2000 × g. RBCs were then washed once in PBS-EDTA and three times in an isotonic buffer (10 mM Tris, 150 mM NaCl adjusted to pH 8.5 with HCl). RBCs were then suspended in this buffer at a cell density of 5 × 10⁸ cells/mL [16].

2.5. Hemolysis Assay. 850 μL of isotonic buffer was added to 50 μL of a PBS solution containing the copolymer. 100 μL of RBC suspension was added, and the reaction was performed at 25°C during 30 min. Unaltered RBCs were then removed by a 10000 × g centrifugation, and the absorbance of the supernatant at 540 nm was compared with two control samples in order to determine the percentage of hemolysis. The first one (100%) was totally hemolysed with distilled water [16], and the second one (0%) contained 900 μL PBS plus 100 μL of RBC suspension. Each test has been carried out at least twice.

2.6. Cell Culture. Human cervix cancer HeLa cell line was cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, 100 U/mL streptomycin, and 1% glutamine in a humidified cell incubator with an atmosphere of 5% CO₂ at 37°C [17].

2.7. MTT Assay. Cell viability was estimated by MTT assay. Briefly, 3 × 10⁶ cells/well was plated in 24-well culture plates. After overnight incubation, the cells were treated with different concentrations of surfactin (0, 20, 40, 60, and 80 μM) for 16, 24, and 48 hours. The cells were treated with 50 μL of 5 mg/mL MTT and the resulting formazan crystals were dissolved in DMSO (500 μL). The optical density (OD) of each well was measured at 570 nm. Each test was performed in triplicate experiments. The effect of surfactin and surfactin-loaded nanoparticles on cell viability was assessed as percentage cell viability compared to vehicle-treated control cells, which were arbitrarily assigned 100% viability [18, 19].

2.8. Statistical Analysis. All of the experiments were done in triplicates, and the averages of the data were compared with independent t-test. A P value of <0.05 was considered as a statistical significance.

3. Results

3.1. Surfactin Second Structure Study. Surfactin C-15 in PBS showed a CD spectrum dominated by a broad negative band centered at 225 nm and a maximum peak at 195 nm (Figure 1). Surfactin second structure percents were measured (Table 1).

3.2. Physical Characterization of Surfactin Nanomicelles. Micellization ability of surfactin C-15 was studied by scanning electron microscope. The results exhibited that the nanomicelles in distilled water were spherical in shape and their size was about 100–200 nm (Figure 2(a)), while the nanomicelles shape in PBS was amorphous and their size was 100–400 nm (Figure 2(b)).

3.3. Hemolysis Assay. Surfactin C-15 was tested in different concentration for its hemolytic activity. The results were exhibited in Figure 3. It gives rise to a concentration-dependent hemolysis [10]. HC₅₀ which is defined as the
**Table 1: Surfactin second structure percents.**

<table>
<thead>
<tr>
<th>Material</th>
<th>α-helix</th>
<th>Antiparallel</th>
<th>Parallel</th>
<th>β-turn</th>
<th>Rndm.coil</th>
<th>Total sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Srf 20 μM</td>
<td>13.2</td>
<td>22.4</td>
<td>11.9</td>
<td>16.8</td>
<td>35.7</td>
<td>100</td>
</tr>
<tr>
<td>Srf 40 μM</td>
<td>10.8</td>
<td>22.7</td>
<td>12.7</td>
<td>16.1</td>
<td>37.7</td>
<td>100</td>
</tr>
<tr>
<td>Srf 60 μM</td>
<td>8.3</td>
<td>23.2</td>
<td>13.8</td>
<td>15.2</td>
<td>39.5</td>
<td>100</td>
</tr>
<tr>
<td>Srf 240 μM</td>
<td>11.1</td>
<td>22.4</td>
<td>12.6</td>
<td>16.1</td>
<td>37.8</td>
<td>100</td>
</tr>
<tr>
<td>Srf 500 μM</td>
<td>11.4</td>
<td>21.7</td>
<td>12.5</td>
<td>16.2</td>
<td>38.2</td>
<td>100</td>
</tr>
<tr>
<td>Srf 766 μM</td>
<td>12.3</td>
<td>21.9</td>
<td>12.3</td>
<td>16.4</td>
<td>37.1</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 2: Scanning electron microscope scan of surfactin nanomicelles in distilled water (a) and PBS (pH = 7.4) (b).

Figure 3: Surfactin hemolysis assay. (A) HC$_{50}$ of surfactin C-15 was estimated 47 μM; (B) the macroscopic figure of surfactin C-15 hemolysis assay in (a) negative control, (b) 20 μM, (c) 40 μM, (d) 60 μM, (e) 80 μM, and (f) positive control.

3.4. **MTT Assay.** To study the cytotoxic effects of surfactin C-15, HeLa cell line was treated with different concentration of surfactin C-15 for 16, 24, and 48 h, and then cell viability was determined by MTT assay. Surfactin C-15 arrested HeLa cell line growth in a dose- and time-dependent method (Figure 4), with IC$_{50}$ at 16, 24, and 48 h of 86.9, 73.1, and 50.2 μM, respectively.

**4. Discussion and Conclusion**

In this study, scanning electron microscope determined that surfactin C-15 exhibits different manners in different ionic aqueous solutions. Surfactin nanomicelles in PBS show the presence of large and amorphous aggregates. This is a similar result to that observed by Zou et al. [20]. The nanomicelles in distilled water are spherical in shape and smaller in size compared with the nanomicelles in PBA, and this caused different ionic strengths in distilled water and PBS. The study carried out by Li et al. showed that when the acyl chain length of the surfactin decreases, the hemolytic activity under hypotonic conditions decreases [10], and in this study the HC$_{50}$ of surfactin C-14 was recorded 300 μM. According to our study, HC$_{50}$ of surfactin C-15 is 47 μM that is consistent with Li et al. studies. Surfactins have been considered to be potential antitumoral agents. Recently, it is reported that the cytotoxic effects of surfactins on tumor cells are by inducing the apoptosis, which is related with...
the enzyme activities. In addition, several biological activities of some lipopeptides are also related with their effects on the enzyme activities. For instance, the selective inhibitory effect of surfactin on cytosolic phospholipase A2 contributes to its anti-inflammatory activities. Another study indicates that the inhibitory effect of surfactin on the alkaline phosphatase had been attributed to a chelating action of the free carboxyl groups of the Asp and Glu residues in the peptide moiety of surfactin [1]. Although the obtained results show the cytotoxic effect of surfactin C-15 on HeLa cell line in a dose- and time-dependent method, more studies are necessary to confirm its anticancer effects. Also more studies are needed to determine and clarify the mechanism of surfactin action and anti-proliferative effects on cancerous cell lines targeted delivery.

References


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