Synthesis of Ribose - Oleic Acid Esters in the Presence- and Absence of Candida antarctica Lipase B

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Abstract: D-ribose-oleic acid esters were produced with or without a biocatalyst, using in the same organic media, dimethyl sulfoxide (DMSO): tert-butanol (TBU) or 2-methyl-2-butanol (2M2B). The yield of the ester product was above 90% in both of the reactions. The biocatalyst used was lipase B of Candida antarctica. Molecular characterization was performed by using all the analytical methods available: IR, ¹H-NMR and ¹³C-NMR, HSQC, and ESI-MS.

Key words: biocatalyst, fatty acid, non-enzymatic esterification, pentose, sugar-fatty acid ester

1 Introduction

In the biosphere cell and species diversity are far beyond that of the building blocks, making up the macromolecules and other biomolecules. This could imply that the building blocks have been attained after a severe molecular selection before the emergence of the cell. For example, only one of the monosaccharides, the ribose, has entered the structure of nucleic acids and other nucleotide-containing biomolecules. Other sugars, hexoses, are usually found in more static entities such as the polymers of energy store, bacterial surface antigens, and prokaryotic cell wall. Biomolecule structures also appear to have been selected during the molecular evolution. For example, while an average human cell has about 50,000 different proteins, there are only three different secondary structures (motifs) available for them. The different combinations of these three secondary structures play a major role in determining the three-dimensional structure of a given protein. Three biopolymers, nucleic acids, proteins, and carbohydrates, which have been preferred in structure and function, are the main components of all living species. Fatty acid polymers do not exist, but they can make different combinations with other biomolecules. Thus, those combinations of biomolecules that have not been preferred by the cell can be a good source for the production of novel biologically active molecules.

There is only one research article suggesting that D-ribose has been specifically chosen in the synthesis of nucleic acids during molecular evolution. The model structures built for other sugar molecules including arabinose and xylose have been tried instead of ribose and it has been demonstrated that ribose was the most suitable unit for the physiological structure of nucleic acid polymer while other sugars caused steric hindrance. Hexose and disaccharide-fatty acid esters have been synthesized for the production of bio-surfactants. These ester molecules have been used in pharmaceutical and food industry as emulsifying agents. Information on the ribose-fatty acid esters, however, is available in the literature. By basing on the fact that ribose is involved in the structure of dynamic macromolecules, such as nucleic acids, it might be plausible to argue that this molecule might provide a relatively smoother surface area as the charged head region of the surfactant and that it
might also lead to a uniform micelle structure. Smooth surfaces may also interfere better with the adsorption of particulate substances by enabling a more homogenous dispersion of the solutes in a solution. In summary, it could be expected that a ribose head might enable surfactant molecules to exert relatively better dynamic structural properties.

Another outstanding feature of this study was that negative control reactions in which only the biocatalyst Candida antarctica lipase B was excluded, produced ribose-oleic acid esters with the same regio, and stereospecificity and with similar yields of the ester product. Therefore, in this work it was demonstrated for the first time that the organic reaction media, consisting of dimethyl sulfoxide: tert-butanol or dimethyl sulfoxide: 2-methyl-2-butanol (4:1, v/v) could support by itself in order to synthesize the ester bond between the fifth carbon of the ribose and the carbonyl group of the fatty acid.

2 Materials and Methods

2.1 Reagents

Merck: dimethyl sulphoxide (DMSO; ≥ 99.5%), oleic acid (≥ 99%). Sigma-Aldrich: immobilized lipase B of Candida antarctica, 2-methyl-2-butanol (≥ 99%), and tert-butanol (≥ 99.5%). AppliChem: D-ribose. Alfa-Aesar: molecular sieves.

2.2 Synthesis of ribose esters

The same esterification reaction conditions were employed in the presence (S) and in the absence (C) of a catalyst (Fig. 1). The reaction conditions were a slight modification of those employed by Deng and Zimmermann: 25 mL reaction volume included two organic media: 20% DMSO (v/v) and 80% 2-methyl-2-butanol (v/v) or 20% DMSO (v/v) 80% tert-butanol (v/v). Substrate molecules were D-ribose (0.2 M, 0.6 g) and oleic acid (0.2 M, 1.128 g). For the enzymatic synthesis 140 mg immobilised Candida antarctica lipase B were used. Water molecules produced by the esterification reactions were trapped in molecular sieves (2 g). The reaction time was 24 h, during which the samples were incubated in a shaker incubator at 200 rpm at 55°C. After this the samples were centrifuged at 5,000 rpm and supernatants were transferred into fresh tubes and then stored at −20°C.

2.3 Qualitative analysis of the ester products

Thin layer chromatography was used to visualise the esterification products (silica gel 60, Merck). Two microliter aliquots of the esterification samples were applied on top of the gel, leaving a 1 cm margin at the bottom. Running buffer consisted of chloroform (70 mL), methanol (20 mL), acetic acid (8 mL), and water (2 mL). The images of the bands were obtained by p-anisaldehyde/H2SO4 or by orcinol treatment. Resolution time was 10 min at 100°C. Rf values of the bands were found as described in Ref.35.

2.4 Structural analyses of the ester products

IR analyses were performed at CUTAM, Sivas Cumhuriyet University, Turkey, and at Research Laboratory Centre, Erciyes University, Kayseri, Turkey. High-Performance Liquid Chromatography, and arbon and proton NMRs were performed at Research Laboratory Centre, Erciyes University, Kayseri, Turkey. Mass Spectrometry analyses were performed at Research Laboratory Centre, Istanbul University, Istanbul, Turkey. HPLC (Agilent 1260) was used for assessing the conversion percentage of sugar monomers into sugar-fatty acid ester using an Agilent ZORBAX Carbohydrate Analysis Column. IR (Perkin Elmer 400), Nuclear Magnetic Resonance (1H-NMR, Varian UNITY INOVA 500 MHz; 13C-NMR Bruker spectrometer, 125 MHz), and Mass Spectrometry (Thermo Finnigan LCQ LC-MS/MS Spectrometer) were used for the structural analysis of the ester products.

3 Results and Discussion

3.1 IR (ATR, cm⁻¹)

All of the four IR spectra were presented in one Figure in order to present a comparable image of them (Fig. 2).

3.1.1 CIS

This ester product was synthesized in the DMSO: tert-butanol reaction media in the presence of biocatalyst Candida antarctica lipase B (Table 1). The major peaks observed were as follows: the stretching vibration of the ester functional group (C = O), at 1717; a C-O stretching vibration, at 1198; an aliphatic C-H symmetric stretching vibration, at 2866; two asymmetric C-H aliphatic stretching vibrations at 2933 and 2969; and an O-H stretching vibration, at 3367. The symmetric C-H aliphatic stretching vibrations generally peaks at 2924-2970, while the asymmetric C-H
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This ester product was synthesized in the DMSO: tert-butanol reaction media in the absence of biocatalyst Candida antarctica lipase B (Table 1). Some of the peaks observed in the IR spectra were as follows: the stretching vibrations of the ester functional group (C = O), at 1714; a C-O stretching vibrations, at 1197; an aliphatic C-H symmetric stretching vibration, at 2881; two aliphatic asymmetric C-H stretching vibrations, at 2860 and 2926; and an O-H stretching vibration at 3371.

This ester product was synthesized in the DMSO: 2-methyl-2-butanol reaction media in the presence of biocatalyst Candida antarctica lipase B (Table 1). The most important peaks could be summarized as follows: the stretching vibration of the ester functional group (C = O), at 1718; two C-O stretching vibrations, at 1167 and 1187; two aliphatic C-H symmetric stretching vibrations, at 2860 and 2881; two aliphatic asymmetric C-H stretching vibrations, at 2925 and 2968; and an O-H stretching vibration at 3371.

This ester product was synthesized in the DMSO: 2-methyl-2-butanol reaction media in the absence of biocatalyst Candida antarctica lipase B (Table 1). The stretching vibrations of the ester functional group (C = O), at 1717; two C-O stretching vibrations, at 1167 and 1187; an aliphatic C-H symmetric stretching vibration, at 2881; two aliphatic asymmetric C-H stretching vibrations, at 2925 and 2968; and an O-H stretching vibration at 3383.

### Table 1

| Entry | Molar ratio Ribose/oleic acid | D-Ribose (g) | Solvent          | Enzyme          | Sugar conv. % |
|-------|-------------------------------|--------------|------------------|----------------|---------------|
| C1C   | 1:1                           | 0.72         | TBU/DMSO         |                |               |
| C1S   | 1:1                           | 0.72         | TBU/DMSO         |                | 90.30         |
| C2C   | 1:1                           | 0.72         | 2M2B/DMSO        |                |               |
| C2S   | 1:1                           | 0.72         | 2M2B/DMSO        |                |               |

5-O-ribose oleate (C1S)

$^{13}$C-NMR (125 MHz, DMSO-d$_6$/TMS) $\delta$ 14.29 (CH$_3$), 22.52, 24.92 (oleic acid C3), 26.99 (oleic acid C8 and C11), 28.95, 29.09, 29.13, 29.29, 29.48, 29.54 (oleic acid C4, 5, 6, 13, 14, 15, 17), 31.63 (oleic acid C7, 12, 16), 34.09 (oleic acid C2), 63.68 (open chain ribose C5), 67.18, 67.38, 67.57, 68.98 (open chain ribose C3 and C4), 75.97 (cyclic ribose C5), 83.32 (cyclic ribose C2 and C3/open chain ribose C2), 94.13, 94.91, 101.86 (cyclic ribose C1), 129.92 (oleic acid C9 and C10), 174.79 (CH$_2$COO).

$^{3}$H-NMR (500 MHz, DMSO-d$_6$/TMS) $\delta$ 0.84, 0.98 (t and s, 3H, J = 6.83 Hz, CH$_3$), 1.10 (s, 12H, oleic acid H-5, 7, 12, 15, 16, 17), 1.23 (s, 8H, oleic acid H-4, 6, 13, 14), 1.48 (t, 2H, J = 6.83 Hz, oleic acid H-3), 1.97 (q, 4H, J = 6.86 Hz, oleic acid H-8, 11), 2.16 (t, 2H, J = 7.32 Hz, oleic acid H-2), 2.49 (q, ribose H-2, 3, 4), 3.38 (s, 2H, ribose H-6), 4.18 (s, 3H, OH), 4.72 (d, J = 5.37 Hz, ribose H-5), 5.31 (t, 2H, J = 4.93 Hz, oleic acid H-9, 10).

$^{13}$C-NMR (125 MHz, DMSO-d$_6$/TMS) $\delta$ 14.29 (CH$_3$), 22.52, 24.92 (oleic acid C3), 26.99 (oleic acid C8 and C11), 28.95, 29.09, 29.13, 29.29, 29.48, 29.54 (oleic acid C4, 5, 6, 13, 14, 15, 17), 31.63 (oleic acid C7, 12, 16), 34.09 (oleic acid C2), 63.68 (open chain ribose C5), 67.18, 67.38, 67.57, 68.98 (open chain ribose C3 and C4), 72.34 (cyclic ribose C5/open chain ribose C3 and C4), 75.97 (cyclic ribose C4), 83.32 (cyclic ribose C2 and C3/open chain ribose C2), 94.13, 94.91, 101.86 (cyclic ribose C1), 129.96 (oleic acid C9 and C10), 174.86 (CH$_2$COO).
67.61, 69.08, 69.29, 69.47 (open chain ribose C3 and C4), 71.06, 71.59, 72.36 (cyclic ribose C5/open chain ribose C3 and C4), 75.60, 75.97 (cyclic ribose C4), 82.33 (cyclic ribose C2 and C3/open chain ribose C2), 94.15, 94.93, 101.8 (cyclic ribose C1), 129.96 (oleic acid C9 and C10), 174.79 (CH₂COO).

5-O-ribose oleate (C2C)

$^{13}$C-NMR (125 MHz, DMSO-d$_6$/TMS) δ 9.03, 14.33 (CH$_3$), 22.43, 22.55, 24.94 (oleic acid C3), 26.99 (oleic acid C8 and C11), 28.95, 29.09, 29.29, 29.54 (oleic acid C4, 5, 6, 13, 14, 15, 17), 31.36, 31.74 (oleic acid 7, 12, 16), 34.13, 36.17, 36.35, 36.52 (oleic acid C2), 63.49, 63.68 (open chain ribose C5), 68.36, 68.91, 69.15, 69.35, 69.53 (open chain ribose C3 and C4), 71.08, 72.32 (cyclic ribose C5/open chain ribose C3 and C4), 75.94 (cyclic ribose C4), 83.28 (cyclic ribose C2 and C3/open chain ribose C2), 94.89, 101.84 (cyclic ribose C1), 128.15, 130.01 (oleic acid C9 and C10), 174.93 (CH₂COO).

As can be seen above, the chemical shift values (ppm) of protons and carbons belonging to both the open chain and the cyclic structure of the ribose structure were determined both in $^1$H-NMR and $^{13}$C-NMR spectra. In oleic acid the carbon atom of the COOH group structure is observed at 180.58 ppm (source: SDBS, no: 1035). In this study, in the esterification products, the peaks of the carbon in the COOR group were found at the 174.79-174.93 ppm (Fig. 3). This down shift could be taken as the main evidence indicating the formation of an ester bond between ribose and oleic acid. Literally, the carbon atoms belonging to the ester group are expected within the 165-175 ppm range. A further proof for the existence of the ester bond was provided by the spectra of $^1$H-NMR where the proton of the alcohol group disappeared as a result of the formation of the ester bond (Fig. 4).

In solution carbohydrates can be found both in cyclic and open chain form. In the produced ester molecules, the ribose head forming rotational isomers in the open chain configuration and this could explain why one carbon atom is represented by more than one peak.

3.3 ESI-MS analysis of 5-O-ribose oleate (C1C)

The molecular mass of the control sample C1C (ribose-oleic acid, synthesized in the absence of the biocatalyst) was determined by ESI-MS. In this technology because the ionization method used was relatively softer, only major ionization peaks could be registered. Two of the prominent peaks, 431.1 ([M + H]$^+$), 100 and 429.1 ([M + H]$^+$), 12.53 indicated the unionized state- and the ionized state of the ester molecule, respectively (Fig. 5).

4 Conclusion

The analytical analyses performed revealed that D-ribose could form ester bonds with oleic acid in the organic reaction media which has been optimised for the enzymatic
The ester yield was found to be similar to that of the enzymatic synthesis. An attempt was made to explain its chemical mechanism as follows (Fig. 6): free electrons on the oxygen of the primary alcohol attacked on the carbonyl oxygen of the acid through the SN2 mechanism. The organic media used encouraged the release of the proton (H+) and a Fischer esterification took place. This proton in turn attacked the free electrons of the carbonyl oxygen of the fatty acid and then led to the production of a water molecule. The enzymatic synthesis could have also followed this Fischer esterification[6] as the same reaction procedure was applied.

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Fig. 5 ESI-MS spectrum of 5-O-Ribose oleate.

Fig. 6 The Mechanism of acid catalyzed esterification.

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