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Data Article

Data on Orphan tear lipid analogs, synthesis and binding to tear lipocalin

Ben J. Glasgow*, Adil R. Abduragimov

Departments of Ophthalmology, Pathology and Laboratory Medicine, Jules Stein Eye Institute, UCLA, United States

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ABSTRACT

Data found in this article include the structures of the orphan tear lipids and their analogs that are binding candidates to tear lipocalin, the mass spectrum of products of collision induced dissociation of putative synthesized compounds of synthesized (O-oleoyl)-16 hydroxypalmitic acid. These data and analyses support the research article "Interaction of ceramides and tear lipocalin" Glasgow et al. (2018) [1].

Specifications Table

Subject area	*Chemistry, Biology,*
More specific subject area	*Tear Lipids, Lipid binding proteins, Tear lipocalin*
Type of data	*Graph, Figure, Image*
How data was acquired	*Mass spectrometry, Computer generating docking from Swiss Dock, UV-Visible Absorption Spectrophotometry*
Data format	*Analyzed*
Experimental factors	*Chemical incubation of reactants for synthesis, Incubation of protein with ligands*
Experimental features	*Oleoyl chloride and 16-hydroxypalmitc acid were reacted in acidic conditions for 36* h.

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* Corresponding author.
E-mail address: bglasgow@mednet.ucla.edu (B.J. Glasgown).

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	PDB files of tear lipocalin and stearic acid and sphingosine were uploaded to Swissdock for analysis.
Data source location	*Los Angeles, CA, USA.*
Data accessibility	*Data is with this article.*

Value of the data

- Structures of orphan lipids of the tear film are shown.
- Data show mass spectrum of products of synthesis of (O-acyl)-omega-hydroxy fatty acids.
- Data show docking of tear lipocalin to stearic acid, a native ligands of tear lipocalin.
- Data show docking sphingosine to tear lipocalin indicating that both the fatty acid and sphingosine moieties of ceramide may be inserted into the cavity of tear lipocalin.

1. Data

The data here is gathered for recently described lipids in the tear film that are candidates to bind tear lipocalin. Because native (O-acyl)-omega-hydroxy fatty acids (OAHFA's) cannot be obtained commercially, (O-oleoyl)-16 hydroxypalmitic acid was synthesized and confirmed by the mass spectrometric data shown here. For other lipids, NBD labeled analogs were utilized and the data validating their use is shown including that NDB alone does not bind tear lipocalin. The spectra validating the use of spectra of NBD analogs for linear spectral summation are shown. The data showing docking of native ligands, stearic acid and sphingosine, to tear lipocalin are featured through Swissdock. (http://www.swissdock.ch/#)

2. Experimental design, materials and methods

The lipids used in this study are shown in Fig. 1.

3. Method of synthesis of (*O*-oleoyl)-16- hydroxypalmitic acid

Because native OAHFA's are not commercially available, a member of this class, (*O*-oleoyl)-16-hydroxypalmitic acid was synthesized using the previous published methods with minor modifications [2,3].

In brief, equimolar (0.8 mmol) amounts of oleoyl chloride and 16-hydroxypalmitic acid, dissolved in chloroform were combined at room temperature with 4 µl of sulfuric acid and reacted for about 36 hours. The reaction was monitored to completion with thin layer chromatography using chloroform: methanol 90:10 v:v or hexane:diethyl ether:acetic acid (80:20:4). The products were dried in a rotary evaporator, dissolved in 30 mL chloroform, washed with water (3×15 mL) and saturated sodium chloride (15 mL). The organic phase was separated and evaporated in rotary evaporator. The residue was dissolved in hexane, and applied to 160 mL of a silica gel (Purasil 60A, 320–400 mesh) chromatography column previously equilibrated in hexane. After washing the column in hexane, progressively more polar solvents were added to the column. The final product 130 mg, yield = 30%). The purity of the product dissolved in methanol was confirmed by direct infusion into a mass spectrometry (Advion CMSL) in both positive and negative ion modes. Instrument settings included ESI source 3000 V, capillary 150 V, capillary temperature at 250 °C. Collisional induced dissociation was produced by adjusting the source offset voltage in increments of 5 V.

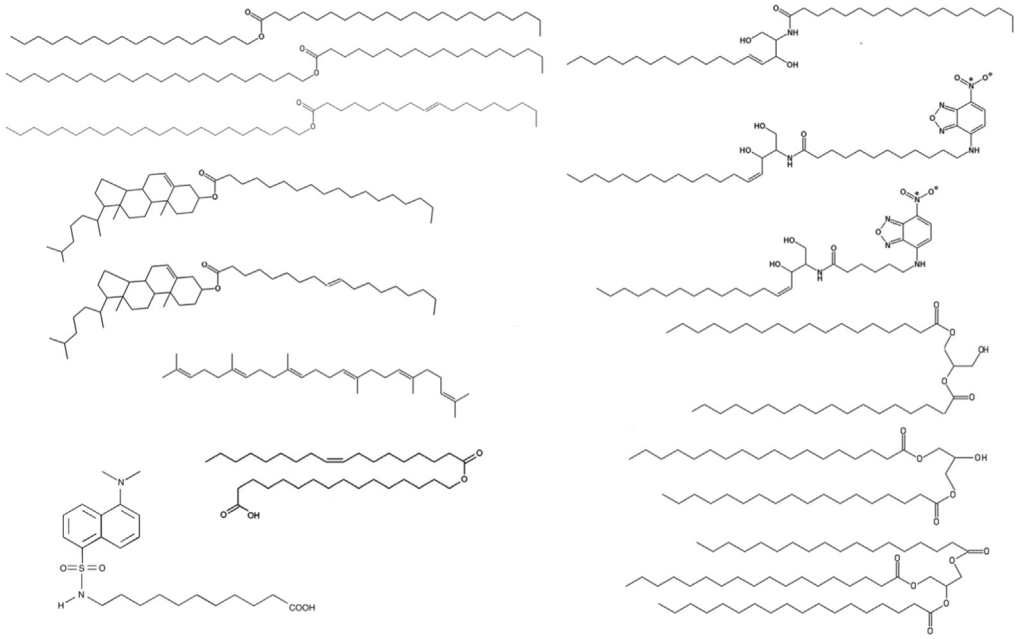

Fig. 1. Two dimensional diagrams depict the chemical structures of the main lipid molecules interrogated for lipocalin binding. From top to bottom in left column: stearyl behenate, behenyl stearate, behenyl oleate, cholesteryl stearate, cholesteryl oleate, squalene, (*O*-oleoyl)-16-hydroxypalmitic acid, and DAUDA. From top to bottom right column: C18-ceramide, C12-NBD ceramide, C6-NBD ceramide, 1,2-distearin, 1,3-distearin, tristearin.

4. Results of synthesis (*O*-oleoyl)-16- hydroxypalmitic acid

Thin layer chromatography of the product revealed a unique spot corresponding to (*O*-oleoyl)-16-hydroxypalmitic acid, which separated from the starting reagents. The product was purified over silica gel chromatography and revealed a single spot by thin layer chromatography. The purified product corresponded to a mass of 535.5 Da in negative ion mode (Fig. 2) and 537.5 Da in positive ion mode in mass spectrometry. This is the expected mass of (*O*-oleoyl)-16- hydroxypalmitic acid. Some minor peaks of the expected intensity accounted for by C13 isotopes (Fig. 2). Collision induced dissociation products of m/z 281 and 271 in negative ion mode matched the expected masses for the product anions of oleic acid and 16-hydroxypalmitic acid (Fig. 2).

5. NBD binding to tear lipocalin by membrane separation

NBD-Cl alone easily passes through a centrifugal membrane filter. As a control binding of NBD-Cl to tear lipocalin was tested by separation of the mixture of 0.5 mL of tear lipocalin 10 μM in 10 mM sodium phosphate (pH 7.3) that had been incubated with 10 μM NBD-Cl at 25 °C for 30 min. The mixture was added to the sample reservoir of a 10,000 MW cutoff centrifugal membrane filter, Millipore Corp, (Billerica MA) and centrifuged at 14,000 g for 30 minutes. The retentate was washed 3 times by repeating the procedure with 0.5 mL of the buffer added to the reservoir. The presence of NBD-Cl in both the retentate or filtrate was quantified by UV–vis spectra and reconstituted or normalized to the original volume.

The data for the control NBD-CL alone are shown in Fig. 3. As a control to exclude the binding of the fluorophore label, NBD-Cl alone was incubated with tear lipocalin for 30 minutes followed by centrifugation. The absorbance spectrum of the retentate matched the spectrum of lipocalin alone albeit with slightly lower intensity. Exiguous absorbance was observed in the retentate between 320–

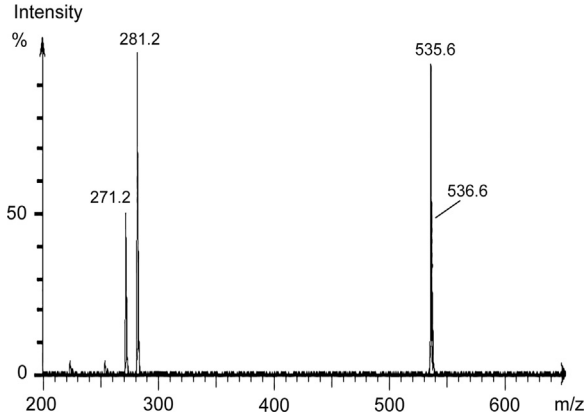

Fig. 2. Mass spectrometry of synthesized (*O*-oleoyl)-16- hydroxypalmitic acid The synthetic product was dissolved in ethanol and directly infused in negative ion mode. The peaks at 535.6 and 536.6 m/z fit the predicted MH- species and corresponding C13 isotype. The peaks of m/z 271.2 and 281.2 appeared when the source offset was increased to 30 V and correspond to the predicted peaks from the collision induced dissociation of the parent molecule.

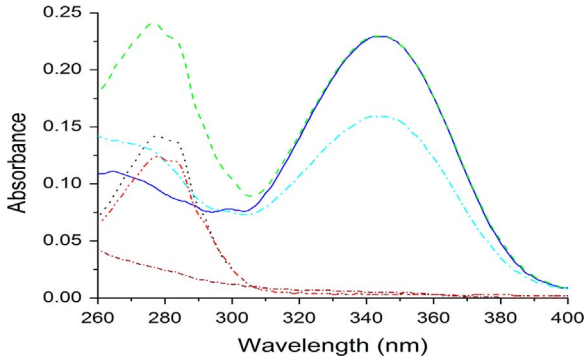

Fig. 3. Centrifugal cellulose membrane separation (Amicon 10,000 MW cutoff) to exclude complexation of NBD-Cl and tear lipocalin. Absorption spectra of NBD-Cl, and tear lipocalin incubated with NBD-Cl. Absorption spectra of tear lipocalin alone (▪▪▪▪), NBD-Cl alone (—), tear lipocalin mixed with NBD-CL (- - -), normalized spectra of the filtrate of mixture, (-●-●-), volume reconstituted retentate of mixture (-●●-●●-), 2% DMSO filtrate (- - -).

380 nm, corresponding to NBD-Cl whereas the filtrate showed marked spectral absorption in this range.

6. Assessment of spectral peak separation of C6-NBD ceramide and C12-NBD ceramide

In order to effectively use linear spectral summation there must be separation of the spectral peaks of free and bound ligand by about 7–10 nm. UV–vis absorption spectra for both ligands were tested (Fig. 4).

C6-NBD ceramide has distinctive peak spectra of bound versus free ligand, whereas C12-NBD showed broad spectra that gave inadequate separation between the free and bound spectral peaks.

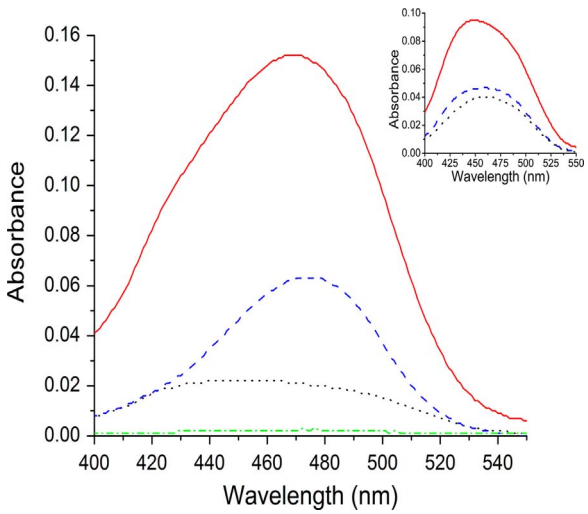

Fig. 4. Absorption spectral changes with C6-NBD ceramide and tear lipocalin complex. Unbound C6-NBD ceramide (3 μM) (·····), C6-NBD ceramide (3 μM) complexed with tear lipocalin (10 μM) (- - -), composite spectrum of C6-NBD ceramide (12 μM) with tear lipocalin (10 μM) (—). Negative control for non-specific binding: supernatant after incubation of lysozyme (10 μM) with C6-NBD ceramide (16 μM) followed by centrifugation (-●-●-). Inset, insufficient spectral separation of bound and unbound C12-NBD ceramide. Unbound C12-NBD ceramide (3 μM) (- - -), C12-NBD ceramide (2 μM complexed with tear lipocalin (10 μM) (—), composite spectrum of C12-NBD ceramide (8 μM) with tear lipocalin (10 μM) (—).

Fig. 5. Docking pose of stearic acid in the tear lipocalin cavity ($\Delta G = -7.3$ kcal/mole).

7. Docking studies of stearic acid and sphingosine

To test the possible orientations of the ceramides in the binding complex docking studies were performed. Docking studies for C18 ceramide (Figure 7, Glasgow et al. (2018) [1].) were accompanied by controls (shown here) for ligands of known orientation. Stearic acid shows the expected pose with the extended alkyl chain in the cavity and carboxylic moiety near the mouth of the calyx (Fig. 5). The

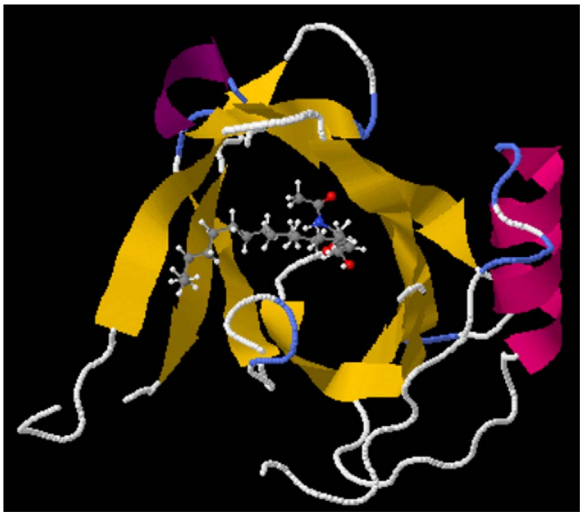

Fig. 6. Docking studies show sphingosine posed near the mouth ($\Delta G = -8.21$ kcal/mole) in the lipocalin complex.

free energy of binding ($\Delta G = -7.30$ kcal/mole) is less that seen from the C18 ceramide. These data match the greater dissociation constant (less binding) observed for stearic acid than ceramide with the tear lipocalin complex.

Since ceramide is composed of two long alkyl chains, it is conceivable that either chain is capable of entering the lipocalin cavity. Docking studies were therefore performed with sphingosine alone Fig. 6.

The docking studies show sphingosine or the fatty acid alkyl chain to be posed in the cavity. Ligands of lipocalin both nitroxide labeled and native fatty acids have been shown to occupy many intracavitary positions by site directed tryptophan fluorescence suggesting a binding energy landscape [4].

Acknowledgements

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Transparency document. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2018.03.102.

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