



Deconstructing the DGAT1 enzyme: Binding sites and substrate interactions

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ABSTRACT

Diacylglycerol acyltransferase 1 (DGAT1) is a microsomal membrane enzyme responsible for the final step in the synthesis of triacylglycerides. Although DGATs from a wide range of organisms have nearly identical sequences, there is little structural information available for these enzymes. The substrate binding sites of DGAT1 are predicted to be in its large luminal extramembranous loop and to include common motifs with acyl-CoA cholesterol acyltransferase enzymes and the diacylglycerol binding domain found in protein kinases. In this study, synthetic peptides corresponding to the predicted binding sites of DGAT1 enzyme were examined using synchrotron radiation circular dichroism spectroscopy, fluorescence emission and adsorption onto lipid monolayers to determine their interactions with substrates associated with triacylglyceride synthesis (oleoyl-CoA and dioleoylglycerol). One of the peptides, Sit1, which includes the FYxDWWN motif common to both DGAT1 and acyl-CoA cholesterol acyltransferase, changes its conformation in the presence of both substrates, suggesting its capability to bind their acyl chains. The other peptide (Sit2), which includes the putative diacylglycerol binding domain HKWCIRHFYKP found in protein kinase C and diacylglycerol kinases, appears to interact with the charged headgroup region of the substrates. Moreover, in an extended-peptide which contains Sit1 and Sit2 sequences separated by a flexible linker, larger conformational changes were induced by both substrates, suggesting that the two binding sites may bring the substrates into close proximity within the membrane, thus catalyzing the formation of the triacylglyceride product.

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1. Introduction

The acyl-CoA:diacylglycerolacyltransferase 1 (DGAT1) is a microsomal enzyme located mainly in the endoplasmic reticulum (ER) membrane; it plays an essential role in lipid metabolism by catalyzing the production of triglycerides from acyl-CoA and diacylglycerol substrates [1,2]. Dysfunction in this stage of lipid metabolism can lead to serious disorders, such as obesity, which is an important risk factor for cardiovascular diseases, hypertension and diabetes [3,4]. Transgenic DGAT1-deficient mice have been shown to resist induced obesity even when fed on a rich fat-based diet, in addition to showing a remarkable reduction in the accumulation of triacylglycerol in their adipose tissues [5]. This suggests that DGAT1 enzymes could be potential therapeutic

targets for the inhibition of obesity [6–9]. In addition, DGAT1 is an important element in food production/agriculture as cattle milk-fat production can be greatly enhanced by a single point mutation in this enzyme [10].

Although DGAT orthologues from a wide variety of mammalian sources have nearly identical sequences (~90%), and even orthologues from distantly related eukaryotes have identities of >40%, there is as yet no crystal structure of any DGAT protein nor of any closely-related protein which might form a good template for modeling of its structure. Bioinformatics analyses indicate that it is an integral membrane protein [11], but that its enzymatic function lies in a large luminal loop in the C-terminal half of the protein. Two peptides in this loop are thought to comprise the binding sites for the two types of substrates: Sit1, which includes the motif FYxDWWN [12] that is highly conserved between the DGAT and acyl-CoA cholesterol acyltransferase (ACAT) enzymes [13,14] (³⁵⁶FGDREFYRDWWNSE³⁷⁰), and Sit2, which includes the putative diacylglycerol binding motif HKWCIRHFYKP found in protein kinase C and diacylglycerol kinases (³⁷⁹NIPVHKWSIRHFY³⁹¹). In this study, we have examined substrate interactions involving synthetic peptides corresponding to these two motifs, as well as with a third

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peptide, consisting of the central regions of Sit1 and Sit2 peptides joined by a short flexible linker. The Sit1 and Sit2 sequences are located in the same extramembranous loop as the proposed catalytic residue, His⁴¹⁶, albeit at some distance from it in the linear sequence. It has been suggested that the two proposed binding sites may be important for directing the substrates into an extended active site, making them available for reaction at the catalytic histidine. Hence, the interactions of the peptides with the oleoyl-CoA and dioleoylglycerol substrates were examined using synchrotron radiation circular dichroism (SRCD) and fluorescence spectroscopies, and adsorption onto lipid monolayers.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycerol (DOG) and coenzyme A (CoA) were purchased from Sigma Aldrich; oleoyl coenzyme A (OCoA) and the phospholipid 1-palmitoyl-2-oleoyl-sn-glycerol-3-phospho-rac-(1-glycerol) (POPG) were purchased from Avanti Polar Lipids.

The synthetic peptides were designed based on the primary structure of bovine DGAT1 (UniProt code [Q8MK44](#)). Sit1 (its location within the full length protein is noted in [Fig. 1](#)) includes the most conserved region present in DGAT1 and the ACAT family, the FYxDWWN segment. Sit2 is the putative diacylglycerol binding domain, and includes the sequence HKWCIRHFYKP, similar to that found in protein kinase C and the diacylglycerol kinases. A third peptide, Sit1&2, links together the most conserved residues of Sit1 and Sit2 with a flexible linker region with the sequence GSG (peptide sequences are in Suppl. Table 1).

Peptides were manually synthesized using Fmoc strategy on Rink Amide MBHA resin. Coupling reactions were performed with two equivalents of Fmoc-protected amino acids, N,N'-diisopropylcarbodiimide/N-hydroxybenzotriazole in dimethylformamide (DMF) (1:1, volume ratio) until reaction went to >95% completion, as checked by Kaiser assay [15], and deblockings were done with 20% piperidine in DMF. After the incorporation of all amino acid residues, the final cleavage of the peptide from the resin was performed with a mixture of trifluoroacetic acid (TFA):triisopropylsilane:ethanedithiol:H₂O (94:1:2.5:2.5, v/v) for 4 h. The crude peptides were precipitated with cold diethyl ether, centrifuged and lyophilized. The synthetic peptides were solubilized in 0.1% (v/v) TFA and the purification was carried out with semi-preparative HPLC using a reverse-phase C₁₈ column (Vydac, Hesperia, CA) equilibrated with 0.1% (v/v) TFA/water. The peptides were eluted from the column with a linear gradient from 5% to 95% of acetonitrile in water containing 0.1% TFA using a 2 mL/min flow rate, over a 30 min gradient and with absorbance monitored at 220 and 280 nm (Suppl. Fig. 1a). The sequences of the purified peptides were confirmed by mass spectrometry analyses using an ESI-microTOF-Q (Bruker Daltonics). Peptides were solubilized in formic acid 0.5% and analyzed in the 50–3000 m/z range (Suppl. Fig. 2).

Peptide concentrations were determined by measuring the absorbance at 280 nm, using their calculated molar extinction coefficients [16].

2.2. Prediction of secondary structure propensity and transmembrane segments

The secondary structure propensities of the peptides were calculated based on the sequences as found in the full length bovine DGAT1 enzyme and in the isolated peptides using the GOR [17] method. The prediction of transmembrane segments in DGAT1 used the SACS MEMSAT software [11] to produce [Fig. 1A](#).

2.3. Monolayer surface activity

The surface activities of the Sit1, Sit2 and Sit1&2 peptides were measured in a Kibron trough (Kibron, Finland) by injection of the peptides (10 µM) into the aqueous subphase and recording the surface pressure over 30 min. To measure the kinetics of the adsorption of these peptides onto monolayers formed by 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphoglycerol (POPG) and dioleoyl glycerol (DOG), each lipid was solubilized in a mixture of chloroform/methanol (4:1) and spread on the top of the water to achieve surface pressures ranging from 5 to 40 mN/m. After solvent evaporation, each of the DGAT1 peptides (10 µM) was injected in the subphase, and the surface pressure recorded as a function of time.

2.4. Synchrotron radiation circular dichroism spectroscopy in solution

SRCD spectra of each of the peptides in an aqueous solution (0.3 mM) and in the presence of oleoyl-CoA (OCoA) at 1:1, 1:2 and 1:4 peptide:lipid molar ratios were measured on beamline CD1 at the Institute for Storage Ring facilities (Aarhus, Denmark). Triplicate scans of samples and baselines (taken using the same exact condition of the cognate sample, containing all of the components in the sample, except the peptide) were obtained over the wavelength range from 280 to 176 nm in 1 nm increment using a 2 s dwell time in demountable Suprasil quartz cells (Hellma Analytics, UK) with 0.0050 cm pathlength at 25 °C. The sample and baseline scans were each averaged and the averaged baseline subtracted from the averaged sample spectrum, smoothed with a Savitzky–Golay filter, and calibrated with camphor-sulfonic acid using CDTTool processing software [18]. Replicate measurements were also done at the DISCO beamline of the Soleil Synchrotron (Paris, France) and at the CD12@ANKA beamline of the ANKA Synchrotron (Karlsruhe, Germany). Mean residue weights of 142, 141, and 141, respectively, were used to scale the spectra of the Sit1, Sit2 and Sit1&2 peptides to delta epsilon values.

Additionally, conventional CD spectroscopy was used to investigate the binding of the DGAT1 peptides (25 µM) to CoA at a 1:10 molar ratio. Measurements were obtained over the wavelength from 185 to 280 nm in 1 nm intervals using a Jasco J-715 spectropolarimeter as an average of 6 scans using a 0.1 cm pathlength quartz cuvette, at 25 °C. Baseline subtraction and data processing were performed using CDTTool software.

2.5. SRCD spectroscopy of films

Either DOG or POPG (the latter is not a substrate but examined as a control for specificity of the interactions) in chloroform/methanol (4:1) was incubated with each of the DGAT1 peptides at peptide:lipid molar ratios of 1:50. A film of the mixture was prepared by depositing 10 µl of the peptide/lipid mixture onto a circular Suprasil quartz plate (Hellma Analytics, UK), then allowing the evaporation of the solvent; the film was further dried in a desiccator under vacuum for 3 h, and then re-hydrated for 24 h in a specially designed sample chamber [19] at 97% relative humidity. SRCD measurements were obtained at 25 °C, over the wavelength range from 280 to 170 nm with 1 nm step size and 2 s dwell time. Measurements were made at four different rotational positions of the plate separated by 90° in the direction perpendicular to the beam in order to detect any signal due to linear dichroism. These four rotational repeats were averaged to produce the final spectrum. Baseline measurements (obtained with an empty cell) were subtracted from the sample spectra using CDTTool software.

Fig. 1. Transmembrane (TM) and secondary structure predictions for DGAT1 enzyme. A) Predicted membrane disposition of bovine DGAT1 and B) secondary structure predictions. The TM region is indicated in orange. In both cases the locations of the Sit1 and Sit2 peptides are in green and cyan overlay, respectively, and the putative catalytic histidine is in red. The secondary structure code is c, coil; e extended; and h, helix.

[illegible]

2.6. Steady state fluorescence spectroscopy

The fluorescence emission spectra of the DGAT1 peptides (25 μM) in an aqueous solution, alone and in the presence of OCoA at 1:1, 1:2 and 1:4 molar ratios were measured on an ISS K2 spectrofluorimeter (ISS Fluorescence, Analytical and Biomedical Instruments, Illinois, USA) using a 1 cm path length quartz cuvette, at 25 °C. Samples were excited at 295 nm and the emission spectra were recorded from 305 to 450 nm. Reference spectra of the solution were recorded and subtracted for each sample.

The fluorescence emission spectra of the DGAT peptides (25 μM) were also measured in the presence of CoA at 1:10 molar ratio, using the same conditions described above.

3. Results and discussion

3.1. Bioinformatics predictions

Sequence-based prediction of the transmembrane (TM) segment disposition of the DGAT1 protein suggests that it consists of 7 TM segments plus a large luminal extramembranous domain. The Sit1 (residues 356–370) and Sit2 (residues 379–391) peptides fall within the extramembranous domain, as does the proposed catalytic histidine, H⁴¹⁶ (Fig. 1A–B). This raises the question of the nature of the extended binding site, and if one or both of the peptides could interact with one or both of the substrates. Secondary structure predictions indicate that in the intact protein, the Sit1 peptide will be ~80% extended beta-strand structure and 20% unordered (or at least not adopting one of the canonical types of secondary structure). Not surprisingly, similar calculations on the isolated peptide sequences produce different proportions of extended (~50%) and unordered structures (~50%). On the other hand, the Sit2 sequence in either context is predicted to be ~40% extended strands and ~60% disordered (Suppl. Table 2). None of the secondary structure predictions suggest that either peptide will be helical.

3.2. Monolayer interactions

Surface activity experiments for the peptides at a water interface (Suppl. Fig. 1b) indicated that both Sit1 and Sit2 peptides presented a low surface activity, although the hybrid Sit1&2 peptide promoted a considerable change in surface pressure ($\Delta\pi \sim 8 \text{ mN m}^{-1}$). The higher surface activity of Sit1&2 is probably due to the enhancement of the hydrophobic character of the peptide, promoted by its increased relative proportion of hydrophobic/non-polar and aromatic residues, which become exposed at the air–water interface. The isolated peptides (Sit1 and Sit2) did exhibit more surface activity on the lipid monolayers than at the water interface.

Exclusion surface pressure (π_{exc}) (also known as a critical surface pressure) is a parameter that determines the ability of a molecule solubilized in the aqueous subphase to interact with, and penetrate into a preformed lipid monolayer at the interface. In order to evaluate this for the Sit1, Sit2 and Sit1&2 peptides, Langmuir monolayers [20] composed of either DOG (a substrate for the enzyme) or POPG (not a substrate, but used as a control to examine the specificity of the interactions for actual substrates) were used to determine exclusion surface pressure values.

The Sit1 peptide promoted larger changes in surface pressure values than Sit2 (Fig. 2A) on the DOG monolayers. Sit2 essentially did not interact with the DOG monolayer, except for minor changes at low initial surface pressure values. This was contrary to expectations, as

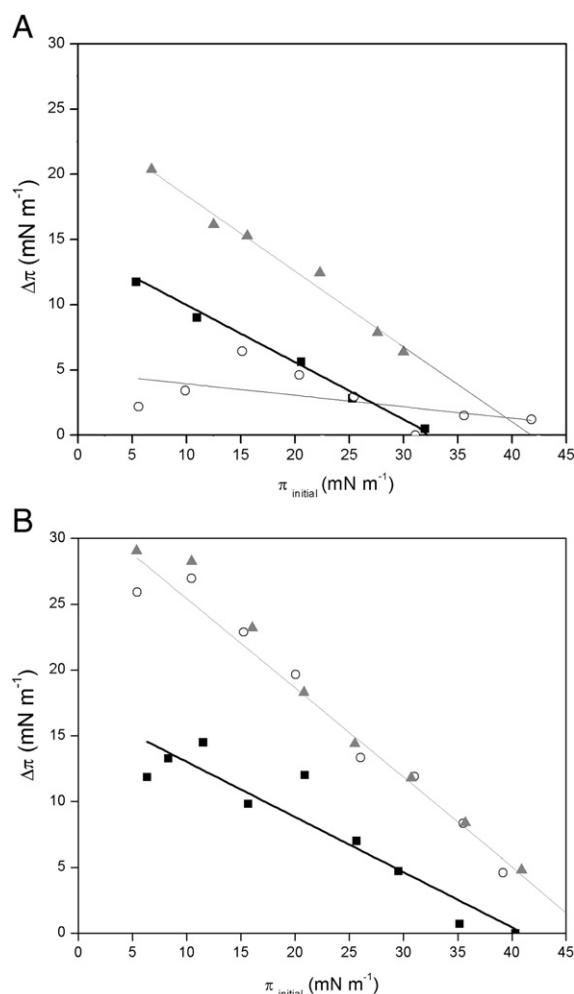


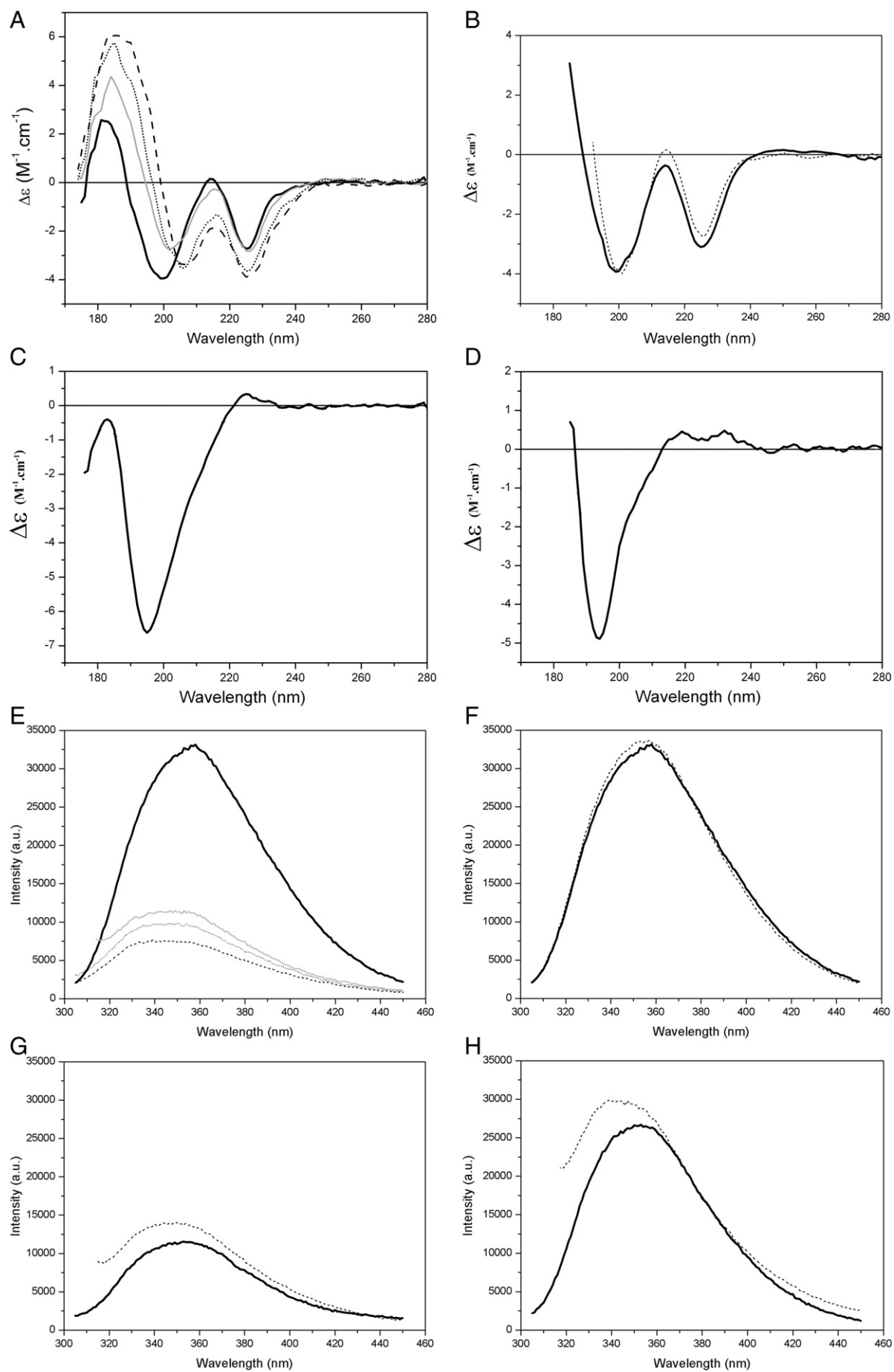
Fig. 2. Behavior on monolayers. Initial pressure (π_{initial}) versus the surface pressure change ($\Delta\pi$) for the adsorption of Sit1 (black square), Sit2 (white circle) and Sit1&2 (gray triangle) on A) DOG and B) POPG monolayers formed on the surface of water with initial surface pressures ranging from 5 to 40 mN/m. Linear fits were produced using Origin 8.5 software.

Sit2 was designed based on homology with protein kinase C and the diacylglycerol kinases (Suppl. Fig. 4), which have the DOG substrates in common. However, the Sit1&2 peptide was able to penetrate into the DOG monolayer even at dense lipid packing. Therefore, synergism seen for the combination peptide is suggested to be the result of interactions of DOG with both parts of the Sit1&2 peptide.

The incorporation of the peptides into the POPG monolayers (Fig. 2B) showed that as the initial surface pressure increased, changes in the π value were smaller due to reduced incorporation of the peptides in the monolayers. The induced surface activity observed for Sit2 was higher than for Sit1, which may be simply due to the interaction between the negatively charged lipid and the positively charged peptide.

The exclusion surface pressure observed for Sit1 is around 35 mN m^{-1} , which is nearly that of lipid packing in cell membrane (25–30 mN m^{-1}) [21]. However, for Sit2 and Sit1&2, exclusion surface pressure is much higher, close to 50 mN m^{-1} , which means that those peptides are incorporated into the POPG monolayer, interacting with the hydrophobic tails of the lipid.

Fig. 3. Binding to oleoyl-CoA and CoA. SRCD spectra of the peptides A and B) Sit1, C) Sit2, and D) Sit1&2 in an aqueous solution (black solid line). Fluorescence emission spectra of E, F) Sit1, G) Sit2, and H) Sit1&2 in an aqueous solution (black solid line). The peptide Sit1 was titrated with 0.3 mM OCoA (gray), 0.6 mM OCoA (dot), and 1.2 mM OCoA (dash) in A) and E); and also, Sit1 was incubated with 0.25 mM of CoA (dash) in B) and F). The Sit2 and Sit1&2 peptides were incubated with 0.25 mM of CoA (dash) in G) and H), respectively.



3.3. SRCD spectroscopy of solutions of peptide/substrate interactions in solution

SRCD spectroscopy was used to examine if any structural changes occur in the DGAT1 peptides in the presence of the substrates. SRCD spectroscopy, as opposed to conventional CD spectroscopy, was employed since it provides a better signal-to-noise ratio and access to lower wavelength, both of which are especially important for examining peptides under the conditions used in this study, which include high non-chiral absorption of the substrates at low UV-wavelengths [22,23].

The SRCD spectra of the Sit1 and Sit2 peptides both exhibited very low per-residue magnitudes (i.e. delta epsilon values) relative to those found in proteins; this is common for peptides in solution which exhibit conformational flexibility, as their net spectra arise from the weighted sum of the many conformations present. For Sit1, in the absence of substrate, the spectrum is characterized by a small positive peak at ~184 nm and a larger negative peak at ~198 nm; the latter is often characteristic of disordered structures (Fig. 3A). It has no negative peaks in the region from 207 to 224 nm, which are typically characteristic for ordered secondary structures, but it does have a negative peak at ~228 nm, which could arise from exciton interactions of the adjacent Trp aromatic residues in this peptide [24,25]. Interestingly, upon binding OCoA, the 198 nm peak disappears and is replaced by a peak at ~207 nm, and the 184 nm peak red-shifts and increases in magnitude; both of these changes suggest an increase in ordering (or decrease in disorder), perhaps due to the formation of a small beta-strand-like structure. The 228 nm peak does not shift, but does increase in magnitude; again suggestive of an increased proportion of order (or decreased flexibility) producing increased aromatic side chain interactions. These results are consistent with there being a significant interaction between this peptide and the acyl-CoA substrate. Furthermore the changes seen tend to mimic the types of changes seen for the peptide when it is titrated with increasing amounts of the order-inducing solvent trifluoroethanol (Suppl. Fig. 3a). In both cases the changes are consistent with increases in ordered structures given the small size and flexibility of the peptides. When binding the substrate, however, the spectral change appears to be more like that associated with beta-strand formation (as suggested by the sequence-based prediction) rather than with formation of a helical structure.

Additionally, in order to investigate whether the CoA portion of the substrate alone could interact with Sit1, the peptide was incubated with CoA molecules (Fig. 3B). In this case, virtually no alteration in the CD spectrum was observed, suggesting that the peptide–substrate binding is likely to be due primarily to an interaction with the acyl-chain of the substrate (OCoA). Hence, the solution SRCD studies are consistent with Sit1 being a potential site of interaction for the acyl-CoA substrate, as had been suggested by the sequence homology with the acyl-CoA cholesterol acyltransferase (ACAT) enzymes which have this substrate in common with DGAT, and by mutational studies in this region for yeast ACAT1 [26].

In contrast, the spectra of Sit2 and Sit1&2 alone include a large negative peak at around 195 nm, and no positive peaks at either low or high wavelengths, which suggests that they are almost entirely unstructured (Fig. 3C and D). When even small amounts of OCoA are introduced, the solution became turbid, accompanied by a large decrease in the SRCD high tension voltage (HT) signal (which reflects the peptide concentration in solution), suggesting that aggregation had occurred. This prevented the measurement of both the SRCD and fluorescence spectra with this substrate.

3.4. Fluorescence spectroscopy

The fluorescence emission spectra of the DGAT1 peptides in an aqueous solution (Fig. 3E, G, H) were in agreement with them being in a disordered state, with the Trp residues exposed to the solvent,

producing a maximum fluorescence emission (λ_{\max}) centered at 355 nm [27–29].

When Sit1 peptide was incubated with the OCoA (Fig. 3E), a blue shift to ~340 nm was observed. Upon titration with this substrate, the fluorescence intensity of Sit1 gradually decreased, in agreement with the interaction of this peptide as seen by SRCD spectroscopy. As a control to eliminate the possibility that substrate-induced fluorescence quenching of the peptide was responsible for the changes, oleoyl-CoA was added to the peptide analogue N-acetyl-tryptophan (data not shown) and shown to have no effect.

Moreover, as with the SRCD studies, the fluorescence emission spectra of Sit1 did not change in the presence of CoA group alone (Fig. 3F). In contrast, the fluorescence emission spectra of Sit2 and Sit1&2 in the presence of the CoA group were blue shifted of ~15 nm and increased in intensity by ~15%. These effects were in agreement with an ordering promoted on the peptide's structure due to the interactions between peptides with the charged CoA group (Fig. 3G and H).

3.5. SRCD spectroscopy of films

The DOG substrate is not water-soluble, hence preventing solution binding studies from being done. Therefore, SRCD spectroscopy [30, 31] was used to examine the interactions between the peptides with both the substrate (DOG) and the non-substrate analogue (POPG) prepared as films.

Fig. 4 shows the oriented SRCD (oSRCD) spectra of the DGAT peptides in the presence of hydrated films of DOG and POPG. In general,

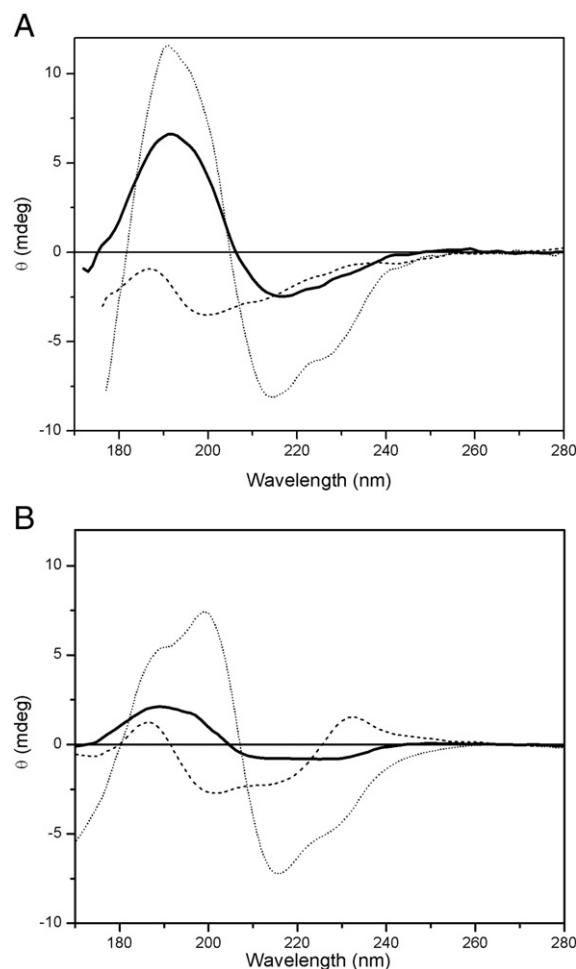


Fig. 4. SRCD spectra of films formed from substrates and non-substrates. Sit1 (solid), Sit2 (dash), Sit1&2 (dot) peptides in the presence of lipid films composed of A) DOG (substrate) and B) POPG (non-substrate).

the peptides look to have more ordered structures in the films than in solution, as is often the case, even if there are no specific interactions with the film molecules. There is some suggestion from the shapes of the curves and positions of the peaks associated with the differently oriented transitions, that they lie along the film surface rather than being incorporated perpendicularly into the films (but not as helices). Again, in this case we have prudently not attributed specific types of spectra with specific types of structure, especially as exemplar spectra for differently oriented beta sheets have not yet been defined. However, it is notable that the spectra for Sit1 are quite similar on both types of films suggesting that their interactions are not compound-specific. On the other hand, Sit2 clearly produces different spectra on the two types of lipids, suggesting specificity of interaction. The Sit1&2 oSRCD spectra are similar on both cognate and non-cognate substrates, with one exception: there is an extra peak at ~195 nm in the POPG film, perhaps indicative of differential interactions of this peptide also.

4. Conclusions

The interactions of DGAT1 peptides that had been proposed to incorporate binding sites for different substrates and non-substrates of the triacylglycerol catalysis reaction have been examined using spectroscopic and monolayer methods. Such studies are experimental evidence for the interaction of the luminal extramembranous domain of the DGAT1 enzymes binding the specific substrates for triacylglyceride synthesis. Knowledge of the nature of the interactions involved in the binding of the substrates to their binding sites may be useful in the search for, and the design of, potent inhibitors for the DGAT1 enzymes [32].

Sit1 appears to specifically bind the OCoA substrate but not the non-substrate analogue CoA in an aqueous solution, suggesting that indeed it does incorporate a binding site for this substrate, while Sit2 displays differential types of interaction with the other substrate (DOG) versus the non-substrate (POPG) lipid in the films.

This study has indicated that while the Sit2 peptide (predicted DAG binding site) is located in a large extra-membranous loop rather than in a transmembrane segment, it is capable of interacting with the membrane-bound substrate DAG. This somewhat unexpected finding of the binding site for the membrane bound substrate being localized within the aqueous compartment suggests that this interaction may create an optimal motif and juxtaposition enabling molecular recognition.

Moreover, the combination peptide Sit1&2 exhibited enhanced interactions with substrates, as shown in the SRCD film changes with the DOG. The two binding sites may act in concert using a combination of hydrophobic and electrostatic interactions. We therefore propose that the predicted putative binding sites might work synergistically to bring the triacylglycerol substrates into close proximity near membrane, so that the proposed active site histidine, also located in the same extramembranous loop, can catalyze the formation of the triacylglyceride product.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbame.2014.08.017>.

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References

- [1] Q. Liu, R.M. Siloto, R. Lehner, S.J. Stone, R.J. Weselake, Acyl-CoA:diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology, *Prog. Lipid Res.* 51 (2012) 350–377.
- [2] D. Cheng, R.L. Meegalla, B. He, D.A. Cromley, J.T. Billheimer, P.R. Young, Human acyl-CoA:diacylglycerol acyltransferase is a tetrameric protein, *Biochem. J.* 359 (2001) 707–714.
- [3] A. Subauste, C.F. Burant, DGAT: novel therapeutic target for obesity and type 2 diabetes mellitus, *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 3 (2003) 263–270.
- [4] W. McCoull, M.S. Addie, A.M. Birch, S. Birtles, L.K. Buckett, R.J. Butlin, S.S. Bowker, S. Boyd, S. Chapman, R.D. Davies, C.S. Donald, C.P. Green, C. Jenner, P.D. Kemmitt, A.G. Leach, G.C. Moody, P.M. Gutierrez, N.J. Newcombe, T. Nowak, M.J. Packer, A.T. Plowright, J. Revill, P. Schofield, C. Sheldon, S. Stokes, A.V. Turnbull, S.J. Wang, D.P. Whalley, J.M. Wood, Identification, optimisation and in vivo evaluation of oxadiazole DGAT-1 inhibitors for the treatment of obesity and diabetes, *Bioorg. Med. Chem. Lett.* 22 (2012) 3873–3878.
- [5] C.L.E. Yen, S.J. Stone, S. Koliwad, C. Harris, R.V. Farese Jr., Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis, *J. Lipid Res.* 49 (2008) 2283–2301.
- [6] H.C. Chen, R.V. Farese Jr., DGAT and triglyceride synthesis: a new target for obesity treatment? *Trends Cardiovasc. Med.* 10 (2000) 188–192.
- [7] D. Matsuda, H. Tomoda, DGAT inhibitors for obesity, *Curr. Opin. Investig. Drugs* 8 (2007) 836–841.
- [8] H. Denison, C. Nilsson, M. Kujacic, L. Löfgren, C. Karlsson, M. Knutsson, J.W. Eriksson, Proof of mechanism for the DGAT1 inhibitor AZD7687: results from a first-time-in-human single-dose study, *Diabetes Obes. Metab.* 15 (2013) 136–143.
- [9] P. Mougnot, C. Namane, E. Fatt, F. Camy, R. Dadji-Faihun, G. Langot, C. Monseau, B. Onofri, F. Pacquet, C. Pascal, O. Crespin, M. Ben-Hassine, J.L. Ragot, T. Van-Pham, C. Philippo, F. Chatelain-Egger, P. Péron, J.C. Le Bail, E. Guillot, P. Chamot-Clerc, M.A. Chabanaud, M.P. Pruniaux, F. Schmidt, O. Venier, E. Nicolai, F. Viviani, Thiadiazoles as new inhibitors of diacylglycerol acyltransferase type 1, *Bioorg. Med. Chem. Lett.* 22 (2012) 2497–2502.
- [10] B. Grisart, W. Coppieters, F. Famir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, R. Snell, Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition, *Genome Res.* 12 (2002) 222–231.
- [11] D.T. Jones, W.R. Taylor, J.M. Thornton, A model recognition approach to the prediction of all-helical membrane protein structure and topology, *Biochemistry* 33 (1994) 3038–3049.
- [12] R.A. Coleman, D.P. Lee, Enzymes of triacylglycerol synthesis and their regulation, *Prog. Lipid Res.* 43 (2001) 134–176.
- [13] S. Cases, S.J. Smith, Y.W. Zheng, H.M. Myers, S.R. Lear, E. Sande, S. Novak, C. Collins, C.B. Welch, A.J. Lusis, S.K. Erickson, Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 13018–13023.
- [14] K.F. Buhman, M. Accad, R.V. Farese Jr., Mammalian acyl-CoA:cholesterol acyltransferases, *Biochim. Biophys. Acta* 1529 (2000) 142–154.
- [15] E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides, *Anal. Biochem.* 34 (1970) 595–598.
- [16] M.R. Wilkins, E. Gasteiger, A. Bairoch, J.C. Sanchez, K.L. Williams, R.D. Appel, D.F. Hochstrasser, Protein identification and analysis tools on the ExPASy Server, *Methods Mol. Biol.* 112 (1999) 531–552.
- [17] J. Garnier, J.F. Gibrat, B. Robson, GOR secondary structure prediction method version IV, *Methods Enzymol.* 266 (1996) 540–553.
- [18] J.G. Lees, B. Smith, F. Wien, A. Miles, B.A. Wallace, CDtool—an integrated software package for circular dichroism spectroscopic data processing, analysis, and archiving, *Anal. Biochem.* 332 (2004) 285–289.
- [19] F. Wien, B.A. Wallace, Calcium fluoride micro cells for synchrotron radiation circular dichroism spectroscopy, *Appl. Spectrosc.* 59 (2005) 1109–1113.
- [20] I. Langmuir, The constitution and fundamental properties of solids and liquids. II. Liquids, *J. Am. Chem. Soc.* 39 (1934) 1848–1906.
- [21] D. Marsh, Lateral pressure in biomembranes, *Biochim. Biophys. Acta* 1286 (1996) 183–223.
- [22] B.A. Wallace, R.W. Janes, Synchrotron radiation circular dichroism (SRCD) spectroscopy: an enhanced method for examining protein conformations and protein interactions, *Biochem. Soc. Trans.* 38 (2010) 861–873.
- [23] B.A. Wallace, Protein characterisation by synchrotron radiation circular dichroism spectroscopy, *Q. Rev. Biophys.* 42 (2009) 317–370.
- [24] R.W. Woody, Contributions of tryptophan side chains to the far-ultraviolet circular dichroism of proteins, *Eur. Biophys. J.* 23 (1994) 253–262.
- [25] I.B. Grishina, R.W. Woody, Contributions of tryptophan side chains to the circular dichroism of globular proteins: exciton couplets and coupled oscillators, *Faraday Discuss.* 99 (1994) 245–262.
- [26] Z. Guo, D. Cromley, J.T. Billheimer, S.L. Sturley, Identification of potential substrate-binding sites in yeast and human acyl-CoA sterol acyltransferases by mutagenesis of conserved sequences, *J. Lipid Res.* 42 (2001) 1282–1291.
- [27] Y. Chen, M.D. Barkley, Toward understanding tryptophan fluorescence in proteins, *Biochemistry* 37 (1998) 9976–9982.
- [28] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Second ed. Plenum Publishers, New York, 1999.

- [29] E. Crusca Jr., A.A. Rezende, R. Marchetto, M.J.S. Mendes-Giannini, W. Fontes, M.S. Castro, E.M. Cilli, Influence of N-terminus modifications on the biological activity, membrane interaction, and secondary structure of the antimicrobial peptide hylin-a1, *Biopolymers* 96 (2011) 41–48.
- [30] J. Burck, S. Roth, P. Wadhwani, S. Afonin, N. Kanithasen, E. Strandberg, A.S. Ulrich, Conformation and membrane orientation of amphiphilic helical peptides by oriented circular dichroism, *Biophys. J.* 95 (2008) 3872–3881.
- [31] Y. Wu, H.W. Huang, G.A. Olah, Method of oriented circular dichroism, *Biophys. J.* 57 (1990) 797–806.
- [32] J. Cao, Y. Zhou, H. Peng, X. Huang, S. Stahler, V. Suri, A. Qadri, T. Gareski, J. Jones, S. Hahm, M. Perreault, J. McKew, M. Shi, X. Xu, J.F. Tobin, R.E. Gimeno, Targeting acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) with small molecule inhibitors for the treatment of metabolic diseases, *J. Biol. Chem.* 286 (2011) 41838–41851.