2. REVIEW OF LITERATURE

Till date ACAT has not been isolated in its pure form. This has disallowed the application of crystallographic and computer aided drug design techniques in the design and synthesis of potent inhibitors.[16]

Further many groups confuse the Human Cytosolic Thiolase (CT) (PDB Code: 1WL5) as the Acyl-CoA cholesterol acyltransferase (ACAT/SOAT). CT catalyses the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA, which is the first reaction of the metabolic pathway leading to the synthesis of cholesterol whereas ACAT/SOAT converts cholesterol into cholesteryl esters. In addition to this, the CT is produced from the gene ACAT-2 so this name may be the cause of confusion between the two targets under study. The human CT has only around 10% sequence similarity with the human sequence of SOAT-1 and SOAT-2. The alignment sequence has been studied and shown in **Figure 3**.

Different studies have reported the existence of the ACAT family of enzymes, which are integral ER membrane proteins, in transmembrane domains (TMDs) as predicted by TMD algorithms. The topology analysis of the sequence is an important parameter for understanding the substrate-binding to the membrane enzymes.[152]

Till date many trials by various groups have proposed their investigated membrane topology. Initially in 1999, Lin *et al.* projected a model with 7-TMD for ACAT-1 based on the observation of outcomes of HA-tag insertion and subsequent immunofluorescence methods. Here, they proposed the two long hydrophobic polypeptide stretches and one long hydrophilic polypeptide stretch, situated in the ER lumen.[153]

In 2000, Joyce *et al.* proposed a model for ACAT-1with 5-TMD on the basis of their C-terminal truncation method.[152]

The 9 TMD model for ACAT-1 was reported by Guo *et al.* in 2005 on the basis of their cysteine scanning mutagenesis and subsequent cysteine-specific modification approach.[154] In this model, the long hydrophobic polypeptide stretches were observed to be embedded into the membrane bilayer. This model also reported that a long polypeptide stretch rich in conserved

hydrophobic and hydrophilic residues among TMD6 and TMD7 is positioned in the cytosol and this stretch may outline the binding site for acyl-coenzyme A.

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sp|P35610|SOAT1_HUMAN          MVGEEKMSLRNRLSKSRENPEEDEDQRNPAKESLETPSNGRIDIKQLIAK
sp|O75908|SOAT2_HUMAN          -MEPGGARLRLQRTEGLGGERERQPCGDGNTETHRAPDLVQWTRHMEAVK
1WL5_A|PDBID|CHAIN|SEQUENCE    ----------------------MNAGSDPVVIVSAARTIIGSFNGALAA
                                                    .      :.      :   .

sp|P35610|SOAT1_HUMAN          KIKLTAEAEELKPFFMKEVGSHFDDFVTNLIEKSASLDNGGCALTTFSVL
sp|O75908|SOAT2_HUMAN          AQLLEQAQGQLRELLDRAMREAIQSYPS-----------------QDKPL
1WL5_A|PDBID|CHAIN|SEQUENCE    VPVQDLGSTVIKEVLKRAT-----------------------------V
                                         :: .: :                              :

sp|P35610|SOAT1_HUMAN          EGEKNNHRAKDLRAPPEQGKIFIARRSLLDELLEVDHIRTIYHMFIALLI
sp|O75908|SOAT2_HUMAN          PPPPPGSLSRTQEPSLGKQKVFIIRKSLLDELMEVQHFRTIYHMFIAGLC
1WL5_A|PDBID|CHAIN|SEQUENCE    APEDVSEVIFGHVLAAGCGQNPVRQASVGAGIPYSVPAWSCQMICGSGLK
                                    .       .    :  : : *:   :       :   :  : *

sp|P35610|SOAT1_HUMAN          LFILSTLVVDYIDEGRLVLEFSLLSYAFGKFPTVVWTWWIMFLSTFSVPY
sp|O75908|SOAT2_HUMAN          VFIISTLAIDFIDEGRLLLEFDLLIFSFGQLPLALVTWVPMFLSTLLAPY
1WL5_A|PDBID|CHAIN|SEQUENCE    AVCLAVQSIGIGDSS---IVVAGGMENMSKAPHLAYLRTGVKIGEMPLTD
                                . ::.  :.  *..   : .     :.: *       : :. :  .

sp|P35610|SOAT1_HUMAN          FLFQHWATGYSKSSHPLIRSLFHGFLFMIFQIGVLGFGPTYVVLAYTLPP
sp|O75908|SOAT2_HUMAN          QALRLWARGTWTQATGLG------CALLAAHAVVLCALPVHVAVEHQLPP
1WL5_A|PDBID|CHAIN|SEQUENCE    SILCDGLT---------------DAFHNCHMGITAENVAKKWQVSREDQ
                                 :                     :   :  :    .

sp|P35610|SOAT1_HUMAN          ASRFIIIFEQIRFVMKAHSFVRENVPRVLNSAKEKSSTVPIPTVNQYLYF
sp|O75908|SOAT2_HUMAN          ASRCVLVFEQVRFLMKSYSFLREAVPGTLRARRGEG--IQAPSFSSYLYF
1WL5_A|PDBID|CHAIN|SEQUENCE    DKVAVLSQNRTENAQKAGHFDKEIVPVLVSTR---KGLIEVKTDEFPRHG
                                . ::  :: .   *:  * :* **  : :      :   : .   :

sp|P35610|SOAT1_HUMAN          LFAPTLIYRDSYPRNPTVRWGYVAMKFAQVFGCFFYVYYIFERLCAPLFR
sp|O75908|SOAT2_HUMAN          LFCPTLIYRETYPRTPYVRWNYVAKNFAQALGCVLYACFILGRLCVPVFA
1WL5_A|PDBID|CHAIN|SEQUENCE    SNIEAMSKLKPYFLTDGTGTVTPANASGINDGAAAVVLMKKSEADKRGLT
                                   ::   ..*  .  .     *   .  *.   .     .     :

sp|P35610|SOAT1_HUMAN          NIKQEPFSARVLVLCVFNSILPGVLILFLTFFAFLHCWLNAFAEMLRFGD
sp|O75908|SOAT2_HUMAN          NMSREPFSTRALVLSILHATLPGIFMLLLIFFAFLHCWLNAFAEMLRFGD
1WL5_A|PDBID|CHAIN|SEQUENCE    PLARIVSWSQVGVEPSIMGIGP----------------------IPAIK
                                : :    ::. *   : .  *                       :   .

sp|P35610|SOAT1_HUMAN          RMFYKDWWNSTSYSNYYRTWNVVVHDWLYYYAYKDFLWFFSKRFKSAAML
sp|O75908|SOAT2_HUMAN          RMFYRDWWNSTSFSNYYRTWNVVVHDWLYSYVYQDGLRLLGARARGVAML
1WL5_A|PDBID|CHAIN|SEQUENCE    QAVTKAGWSLEDVDIFEINEAFAAVSAAIVKELGLNPEKVNIEGGAIALG
                               : . :  *.  . . :  .  ... .           .. .  . *:

sp|P35610|SOAT1_HUMAN          AVFAVSAVVHEYALAVCLSFFYPVLFVLFMFFGMAFNFIVNDSRKKPIWN
sp|O75908|SOAT2_HUMAN          GVFLVSAVAHEYIFCFVLGFFYPVMLILFLVIGGMLNFMMHDQRTGPAWN
1WL5_A|PDBID|CHAIN|SEQUENCE    HPLGASGCRILVTLLHTLERMGRSRGVAALCIGGGMGIAMCVQRE-----
                                 : .*.      :   *  :    :  : :*  :.: :  .*

sp|P35610|SOAT1_HUMAN          VLMWTSLFLGNGVLLCFYSQEWYARQHCPLKNPTFLDYVRPRSWTCRYVF
sp|O75908|SOAT2_HUMAN          VLMWTMLFLGQGIQVSLYCQEWYARRHCPLPQATFWGLVTPRSWSCHT--
1WL5_A|PDBID|CHAIN|SEQUENCE    --------------------------------------------------
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Figure 3: Alignment of human sequence of ACAT-1, ACAT-2 and Cytosolic Thiolase.

This 9-TMD model also suggests the assumed active site H-E-Y (460-462) including His-460 of ACAT-1 positioned at the TMD7. This location appears to be responsible for the catalytic activity.

In the case of ACAT-2/SOAT2 the membrane topology has also been studied by different methods. The HA-tag insertion and subsequent immunofluorescence observation method proposed a 2-TMD model,[155] whereas the C terminal truncation method proposed a 5-TMD model.[152]

The sequence analysis study of SOAT-1 and SOAT-2 suggests that they have almost 55% sequence identity, therefore SOAT-2 may also contain nine TMDs. In the proposed 9-TMD model of SOAT-2, all long hydrophobic peptide stretches are observed to be in the membrane bilayer; the possible acyl-coenzyme-A binding site between TMD6 and TMD7 is observed in the cytosol, with proposed active site H-E-Y (434-436) including His-434. For ACAT-family of enzymes, the conserved His-460 for ACAT-1 and His-434 for ACAT-2 are anticipated to be the active sites.[156]