University of Jordan
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Biochemistry

● Sheet ○ Slides

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Title: Biosynthesis of PAG

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Biochemistry sheet #19

Biosynthesis of Triacylglycerol and Phosphoacylglycerol

Slide 1

- This slide shows the components of triacylglycerol (TAG) and phosphoacylglycerol.
- TAG → (Glycerol) Esterified to 3(Fatty Acids)
- Phosphoacylglycerol → Glycerol esterified to 2 fatty acids, and at carbon-3 it is esterified to a phosphate which in turn can form a diester bond with an alcohol and glycerol.
- The difference between TAG and phosphoacylglycerol structures is at the 3rd position on glycerol; where it may either bind a phosphate group or another fatty acyl group.
- TAGs are known as "neutral fat" because the negative charge of the fatty acid (at the carboxyl group) is lost upon esterification between it and glycerol.
- On the other hand, phosphoacylglycerol is a charged molecule because phosphate carries a negative charge.
- TAG and phosphoacylglycerol both have (glycerol esterified to 2 fatty acids) in their structure.

Slide 2

- A common intermediate in the biosynthesis of TAG and phosphoacylglycerol is phosphatidic acid.
- Phosphatidic acid is composed of (glycerol + 2 FA + phosphate), which can be considered as diacylglycerol phosphate (DAG-P).
- Phosphatidate → is the ionized form of phosphatidic acid.
- Phosphatidyl → is phosphatidic acid esterified to alcohol
- Whether TAG or phosphoacylglycerol is synthesized, phosphatidic acid will be synthesized during the pathway!

Slide 3

- Biosynthesis of TAG requires acyl CoA (the active form of FA) and glycerol phosphate (even though there is no phosphate group in the final structure of TAG, but it is required to be present on glycerol for the initiation of the process because this phosphate apparently has a role in binding to the active site of the enzyme).
- Why is the active form of FA needed?
  -Hydrolysis of TAG to DAG & FA is exergonic due to the fact that ANY HYDROLYSIS REACTION IS EXERGONIC ! (as illustrated in the first equation)
  -So if we invert the equation, meaning synthesis of TAG from DAG & FA, it will obviously be endergonic and we can't expect, even with the presence of an enzyme, for FA to be directly added to DAG to make TAG. (as illustrated in the second equation)
  -THERE SHOULD BE AN ACTIVE FORM, i.e. Acyl CoA !!!
  -When Acyl CoA is transferred to DAG, a high-energy thioester bond is broken which will supply enough energy to push the reaction in the forward direction towards the synthesis of TAG. (as illustrated in the third equation)
  -Conclusion: an acyl group can be transferred if and only if the donor is Acyl CoA (active form)!
  -For example, synthesis of acetylcholine occurs in a similar manner.

- IN THE SYNTHESIS OF ANY ESTER THE DONOR OF THE ACYL GROUP WILL BE THE CoA DERIVATIVE!!!
- From here they deduced the name of Coenzyme A (CoA) because they found it is required in **A**cylation reactions (adding an acyl group).

- Formation of Acyl CoA: FA + CoA + ATP → FA-CoA (acyl-CoA)+ AMP + PPi
- Remember formation of FA-CoA is the first reaction of beta-oxidation, equivalent to the consumption of 2 ATP, and it is catalyzed by fatty acyl CoA synthetase (thiokinase) !!

Slide 4

- Once you have acyl CoA all that is needed is to transfer the acyl group to glycerol phosphate.
- As you can see we have glycerol-3P and acyl CoA. The acyl group is transferred to position #1 by the enzyme acyltransferase forming 1-acyl-3-phosphoglycerol (aka. Lysophoshatidic acid) which will be mentioned in moments.
- The next step will be the transfer of another acyl group to position #2 which is also catalyzed by acyltranferase forming phosphatidic acid (the common intermediate of TAG & phosphoacylglycerol biosynthesis).

Slide 5

- Now, if we're making TAG, the next step will be removing the phosphate group from phosphatidic acid because it's not needed anymore forming DAG, and this step is catalyzed by phosphatase.
- The last step is the transfer of the last activated fatty acid (acyl CoA) by acyltransferase of course, forming TAG.
- Summary of TAG synthesis: add first then second activated FAs then remove the phosphate group then the last third acyl group.
- Note: addition is always at position #1 then #2 because of enzyme specificity for their substrates.

Slide 6

- Production of G3P is done by two ways.
- First way: glycerol-3P is produced by transferring the phosphate group of ATP to glycerol at position #3 (the peripheral position so to speak) by the catalysis of glycerol kinase.
- Glycerol kinase is NOT found in adipose tissue, instead it's expressed in liver tissue!!!
- The absence of this enzyme from AT is extremely important. (the reason will be mentioned later on)
- Second way: using the intermediates of glycolysis, i.e. reduction of DHAP to G3P by GP (glycerol phosphate) dehydrogenase accompanied with the oxidation of NADH to NAD+. (as illustrated in the equation)

Slide 7

- In the liver,G3P production can be done by both ways.
- In adipose tissue, it's done only by the second way because it lacks glycerol kinase.

- The significance of this pattern of G3P production has to do with the regulation of TAG synthesis.

Slide 8

- Imagine this is an adipocyte, where TAG is stored, comprising 90% of its total volume.
- Upon stimulation of hormone-sensitive lipase (HLS) by low blood glucose level, TAG is hydrolyzed to 3 fatty acids and glycerol.
- When there is a need for the synthesis of TAG (i.e. high blood glucose, high insulin, increased expression of GLUT-4 on adipocyte membrane), it starts from G3P and FA-CoA, which are both formed by the aforementioned pathways.
- Now, if there was a glycerol kinase in this adipocyte, a futile cycle of TAG synthesis & TAG degradation will occur.
- The production of one TAG from glycerol and 3 FAs costs seven ATP (1 TAG needs 3 FA-CoA, each FA-CoA requires 2 ATP. Also, 1 TAG needs 1 G3P which requires 1 ATP to be made from glycerol).
- So this futile cycle will act as a sucker of the ATP pool of the cytosol (7 ATP/cycle) and that itself SUCKS FOR THE ADIPOCYTE!
- The resolution for this problem is to avoid expression of glycerol kinase thus avoiding the futile cycle.
- So the glycerol resulting from TAG hydrolysis will have only one fate which is to GO TO THE LIVER.
- Synthesis of TAG can occur only when there are high levels of blood glucose thus insulin, leading to more transporters at the membrane, as mentioned earlier, and entrance of glucose to the adipocyte. While at low levels, synthesis of TAG will be greatly decreased.
- This is why people with uncontrolled diabetes suffer from profound decrease in body weight, i.e. loss of weight is a symptom of diabetes!

Slide 9

- Moving on to the biosynthesis phosphoacylglycerol (aka. phosphoglyceride or glycerophospholipid).
- Refer to chapter 17 of Lippincott's concerning this subject knowing that the chapter is NOT completely included. مش كله مطلوب

Slide 10

- Reminding you once again with the structure and components of phosphoacylglycerol, which is a glycerol backbone esterified to two fatty acids and to a phosphate group which is further esterified to an alcohol.

Slide 11

- Remember: phoshatidic acid is an intermediate of phosphoacylglycerol and TAG biosynthesis.
- Due to the fact that PHOSPHATE CAN FORM TWO ESTER BONDS (i.e. PHOSPHODIESTER BOND), phosphatidic acid can form an ester linkage with two different groups of compounds namely (serine or ethanolamine or choline) or (inositol or glycerol).

- The difference between these two groups is that the first one (to the left on the slide) contains an amine group in addition to the alcohol group which is needed to form the ester bond with phosphate, i.e. aminoalcohols. While the second one only contains the alcohol group as its functional group of course.
- When phosphatidic acid (or any acid) forms an ester bond we change the name from phosphatid**ic** to phosphatid**yl** (e.g. phosphatidylserine, phosphatidylinositol… etc)

Slide 12

- To know the relationship between these compounds we'll consider their structures.
- Ethanol structure is shown for comparison NOT to implicate that these compounds originate from it!!!
- Difference between ethanol and ethanolamine is the presence of an amino group at the beta-carbon (ethanol is NOT converted to ethanolamine).
- Difference between ethanolamine and serine is that serine is amino acid , i.e. carboxyl group is present along with the amino group.
- Serine can be converted to ethanolamine by a DECARBOXYLATION reaction.
- Ethanolamine can be converted to choline by the REPLACEMENT of the 3 hydrogen atoms with the methyl groups at the nitrogen atom.
- It's very important to understand and memorize the structures!

Slide 13

- Inositol is a six-membered ring (a cyclohexanol ring).
- It is not a monosaccharide nor is it a derivative of a monosaccharide such glucose or galactose. Notice the upper right angle of the ring doesn't contain an oxygen atom.
- Inositol contains six hydroxyl groups.
- Inositol exists in nine possible stereoisomers, of which the most prominent form, widely occurring in nature is myo-inositol. (the doctor didn't say this sentence but just to let you know the difference)

Slide 14

- This is the structure of phosphatidylcholine.
- Phosphatidylcholine is known by its common name "Lecithin".
- Lecithin is artificially added to chocolate and powder milk.
- The reason of this addition will be mentioned in moments.

Slide 15

- This is the space-filling model of phosphatidylcholine (lecithin).
- This model aids in identifying different properties of the represented compound by visualizing the 3D structure.
- Notice that lecithin contains two long hydrocarbon chains (one of them is unsaturated causing the kink) and a hydrophilic head (composed of phosphate and choline)
- The blue ball represents the nitrogen atom of choline which is positively charged.

- The red balls represent oxygen atoms which are polar (one of the oxygen atoms at the phosphate group bears a negative charge).
- The yellow ball is phosphate obviously.
- If there are two regions of different polarity in the same molecule we name it an amphipathic molecule.
- Lecithin (phosphatidylcholine) is an amphipathic molecule.

Slide 16

- Amphipathic molecules can form:

1. Micelles (shown to the left), formed to avoid contact with water at the nonpolar side chains.
2. Lipid bilayer (shown in the middle)
3. Liposome {the cell membrane} (shown to the right).

- Back to lecithin → it works as an emulsifying agent (mixing water-insoluble substances with water). That's why it's put in chocolate and powder milk, to allow the mixing of fat present in these substances with the watery medium of our gastrointestinal tract. Else, dissolving will at occur with extreme difficulties (like in the old days when the doctor was a child :P).

Slide 17

- The upper structure is phosphatidylethanolamine.
- The lower structure is phosphatidylserine.
- Both are characterized by two long hydrocarbon chains and polar head (bearing + & - charges), i.e. amphipathic molecules.

Slide 18

Phosphatidylinositol → 2 hydrocarbon chains & a polar head (with a – charge at the phosphate and no charges on the alcohol inositol).
There are 5 free hydroxyl groups at the inositol that can interact with water.

Slide 19

Phosphatidylglycerol → samething

Slide 20

Normally phosphatidylglycerol isn't present as such but a part of a molecule called cardiolipin.
Cardiolipin: two molecules of phosphatidic acid connected through glycerol.
Cardiolipin is found in membranes (especially the inner mitochondrial membrane IMM).

Slide 21

Showing that the different phospholipids are part of the plasma membrane.

Slide 22

- Degradation of phospholipids.
- There are four different ester bonds.
- Each bond is hydrolyzed by a specific enzyme.
- These enzymes are called phospholipases accordingly:

1. Phospholipase A1 → hydrolyzes the 1st ester bond
2. Phospholipase A2 → hydrolyzes the 2nd ester bond (note that this enzyme does NOT act after A1 as the name may suggest, it actually acts on an intact molecule)
3. Phospholipase C → hydrolyzes the 3rd ester bond (between glycerol and phosphate)
4. Phospholipase D → hydrolyzes the 4th ester bond (between phosphate and the alcohol)

Slide 23

- Why did we skip phospholipase B? → to answer this question notice this molecule of lysophosphatidylcholine which is the product of phospholipase A2-mediated hydrolysis.
- Phopholipase B acts on the product of phospholiase A2 (lysophosphatidyl"something") by removing the first fatty acid.
- Phospholipase B is more correctly named as lysophospholipase (it does NOT act on an intact molecule).
- Lysophosphatidylcholine (LPC) causes lyses of the membranes, that's why it's a **lyso**phospholipid.
- LPC acts as a surface tension-reducing agent.

Slide 24

- Phospholipase A2:

1. Present in many mammalian tissues and pancreatic juice, snake and bee venoms. So when your bitten by a snake or a bee PLA2 will reach the phospholipids of the plasma membrane hydrolyzing them to lysophospholipids which will cause the lyses of the cell, i.e. degeneration of the cells of the tissue is the result of the injected PLA2 that produces a substance that causes further lyses تهنك الأنسجة

2. Acts on phosphotidylinositol, releasing arachidonic acid AA (the precursor of prostaglandins). Note that the second position is usually occupied by an unsaturated FA (i.e. arachidonic acid).
3. Pancreatic secretions are rich in PLA2. (Proenzyme)
4. PLA2 is inhibited by glucocorticoids. (e.g. cortisol)

- Phospholipase C:

1. Found in liver lysosomes and the alpha-toxin of clostridia and other bacilli.
2. Membrane-bound PLC is activated by the PIP2 system (i.e. plays a role in producing second messengers IP3 & DAG).

Slide 25

- Biosynthesis of phospholipids.
- Remember: phosphatidic acid is a common intermediate.
- To understand the strategy of phospholipid synthesis we have three prime components:

1. Alcohol [1] → it is DAG
2. Phosphate → linking alcohol[1] with alcohol[2]
3. alcohol[2] → an aminoalcohol or just alcohol

- There are two ways (each working in a specific situation) to synthesize the phospholipid:
1. Transfer of ~(phosphate-Alcohol¹) to Alcohol².
2. Transfer of ~(phosphate-Alcohol²) to Alcohol¹.
- Note that ~ means the activated form.
- ACTIVATED FORMS ARE A MUST because we're FORMING AN ESTER BOND (i.e. synthesizing = anabolism).

Slide 26

- Synthesis of phosphatidylinositol
- ➢ Done by the transfer of CDP-DAG [~(phosphate-alcohol¹)] to inositol [alcohol²].
- CDP → Cytidine diphosphate
- CDP-DAG = Phosphatidic acid

Slide 27

- Synthesis of phosphatidylcholine.
- ➢ Done by the transfer of CDP-Choline [~(phosphate-alcohol²)] to DAG [alcohol¹].
- Synthesis of phosphatidylethanolamine is similar to that of phosphatidylcholine.
- SUMMARY:
- ✓ Phosphatidylinositol → the 1st way of phospholipid synthesis
- ✓ Phosphatidylcholine & Phosphatidylethanolamine → the 2nd way of phospholipid synthesis

Slide 28

- A representation of what is mentioned in slides 26 & 27 from the book.

Slide 29

- Formation of Activated Carrier.
- Alcohol-P (either phosphatidic acid or phosphatidylcholine) reacts with CTP to result in CDP-Alcohol and PPi.
- Notice the pattern in which the bonds are cleaved and formed.
- The ΔG for this reaction is near zero because we're cleaving a high energy bond and forming another one. So in order to push the reaction in the forward direction rapid hydrolysis of PPi is required.
- All in all, CDP-Alcohol (the activated carrier) is formed by an exergonic reaction.
- This activated carrier is then transferred to an alcohol (as mentioned in the previous two mechanisms) to result in the formation of the phospholipid and CMP.

Slide 30

- The polar head of the phospholipid phosphatidylethanolamine can be **exchanged**.
- ➢ Phosphatidylethanolamine + Serine → Phosphatidylserine + Ethanolamine
- Note: serine is readily available in the cell because it's a nonessential amino acid.

Slide 31

- Alteration of the polar head of phosphatidylserine does NOT occur by exchange as with ethanolamine instead by decarboxylation.
  - Phosphatidylserine → Phosphatidylethanolamine + CO_2
- Notice that phosphatidylserine cannot be synthesized by the any of the 2 ways of phospholipid synthesis, only by the EXCHANGE process (mentioned in slide 30).

Good Luck!

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