Egg Components

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Structure of egg

- White: 60% of the total egg weight.
  - Chalaziferous, inner thin, outer thick, and outer thin albumen
  - Total solids content of albumen: 11-12%
- Yolk: 30-33% of the total egg weight
  - Composed of vitelline membrane and yolk
  - Total solids content of yolk is about 50-52%
- Shell: 9-12% the total egg weight
  - Largely consists of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%), and organic matters (4%)

New use of eggs

- Antibody source
- Phospholipids
- Lysozyme
- Ovotransferrin
- Avidin

Non-Food Uses of Eggs

- Avidin: Application in Avidin-Biotin Technology
- Egg white Lysozyme: A preservative for foods
- Sialiac acid: yolk and shell membranes
- Antibody: IgY
- Fat source: Yolk
- Phospholipids: Yolk lipids
- Cholesterol: Yolk lipids
- Protein: albumen and yolk protein
- Modified Eggs as a source for special nutrients: w-3 fatty acids, CLA, Iodine
- Ovotransferrin: Iron binder
- Phosvitin: Iron binder

Lipid and Protein Components of Egg Yolk

Lipids (31%)	Proteins (17%)
Neutral Lipids (65%)	Lipovitellins (a- and b-): 69%
Phospholipids (30%)	- a- Lipovitellins: 58%
- PC (83%)	- b- Lipovitelins: 11%
- PE (14%)	Livetins: 12%
- Sphingomyelin (2.5%)	- a- livetin (serum albumin): 4%
- Phosphatidylinositol (0.5%)	- b- livetin (glycoprotein): 5%
Cholesterol (5%)	- g- livetin (g-globulin): 2%
Carotenoids (carotenes)	Phosvitin: 7%
Xantophylls (lutein, zeaxanthin)	apo Low-density lipoproteins: 12%

Value-Added Components from Egg - Yolk (50% solids)

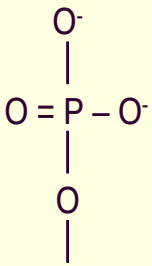
- Neutral Lipids: 21% of yolk
- Phospholipids: 10% of yolk
- Cholesterol: 1.55% of yolk
- Phosvitin: 1.25% of yolk
- IgY: 0.4% of yolk
- Lutein: 7.5-22 mg/g yolk

Phosvitin

- Phosvitin is a principal phosphoprotein present in egg yolk (approximately 16% of egg yolk proteins)
- Contains ~10% phosphorus. Thus, phosvitin has an excellent metal (iron and calcium) binding capacity.
- The calcium binding properties of phosvitin is influenced by pH.
- The calcium binding capacities of native phosvitin were 20 mol Ca++/mol of phosvitin at pH 3.6 and 148 mol Ca++/mol phosvitin at pH 7.0 .
- Phosvitin was capable of inhibiting lipid oxidation in phosphotidylcholine liposomes, muscle homogenates, and ground pork.

Amino Acid Sequence of Egg Yolk Phosvitin

Phosvitin is a principal phosphoprotein present in egg yolk with molecular mass of ~35 kD. Phosvitin contains ~10% phosphorus.

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  1  AEFGTEPDAKTSSSSSSASSTATSSSSSSASSPNRKKPMDEEENDQVKQA
 51  RNKDASSSSRSSKSSNSSKRSSSKSSNSSKRSSSSSSSSSSSSRSSSSSS
101  SSSSNSKSSSSSSKSSSSSSRSRSSSKSSSSSSSSSSSSSKSSSSRSSS
151  SSSKSSSHHSHSHHSGHLNGSSSSSSSSRSVSHHSHEHHSGHLEDDSSSS
201  SSSSVLSKIWGRHEIYQ
```

123 phophoserines

↓

Metal Chelating Capability

Phosphorous Source

Use of Phosvitin

- Phosvitin is an excellent source for phosphopeptide production
- Phosphopeptides, an enzymatic hydrolysate of milk protein: help calcium absorption, calcium retention, and bone calcification.
- Phosphopeptides from egg milk protein casein: has only 1 to 13 phosphoserine residues to stabilize amorphous calcium phosphate whereas a molecule of phosvitin has ~120 phosphoserine residues, implying higher calcium binding capacity

Use of Phosphopeptides

- Calcium supplement
  - Soluble calcium phosphate formation
- Iron supplement
  - Soluble iron phosphate formation
- Antioxidant
  - Iron chelating effect

Functional Bioactive Peptides

- **Definition**: small protein fragments that have biological effects once they are released during gastrointestinal digestion *in vivo* (e.g., Phosphopeptides, ACE-inhibiting factor)
- **Functions:**
  - Chelating metals
  - Calcium binding: calcium supplement
  - Antimicrobial: Food Safety
  - Antioxidant
  - Antihypertensive

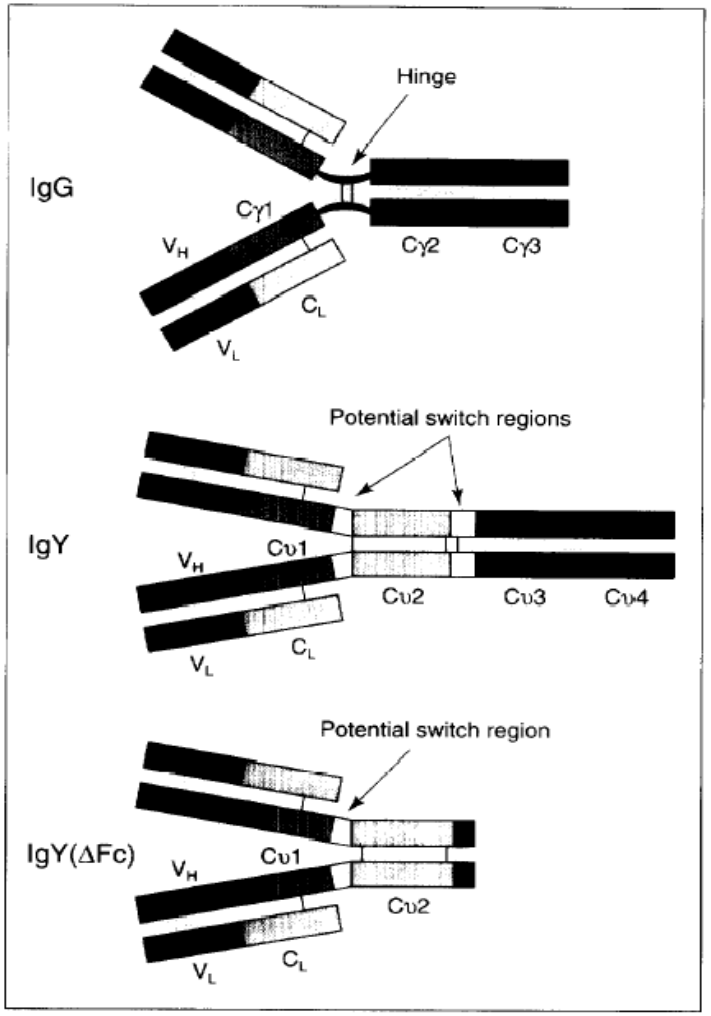
Phosphopeptides

- Usually contain clusters of phosphoserines, which can chelate various bivalent or trivalent metal ions.
- They may effectively bind calcium and iron
- Inhibit formation of insoluble calcium phosphates
- Resulting in increased calcium bioavailability
- Prevent lipid oxidation in foods.

General roles of Antibodies

- Neutralization of toxin and virus
- Opsonization
- Agglutination (clumping)
- Precipitation (immune complex)

Comparision of Mammal IgG and IgY



- Different M.W (180 kDa), PI, and binding behavior with complement
- Limited flexibility of IgY due to lack of hinge region
- Less binding activity of IgY with Fc receptor on the cell surface

Immunology today, 16 (8) :393

Neutralization

Virus, Toxin or Bacteria

↓ Binding

Cell receptor

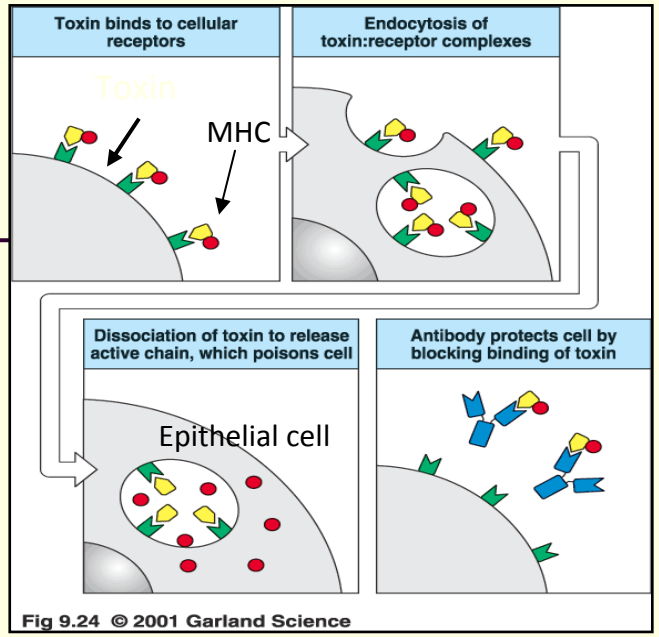
(sialic acid on glcoproteins of RBC or Epithelial cells)

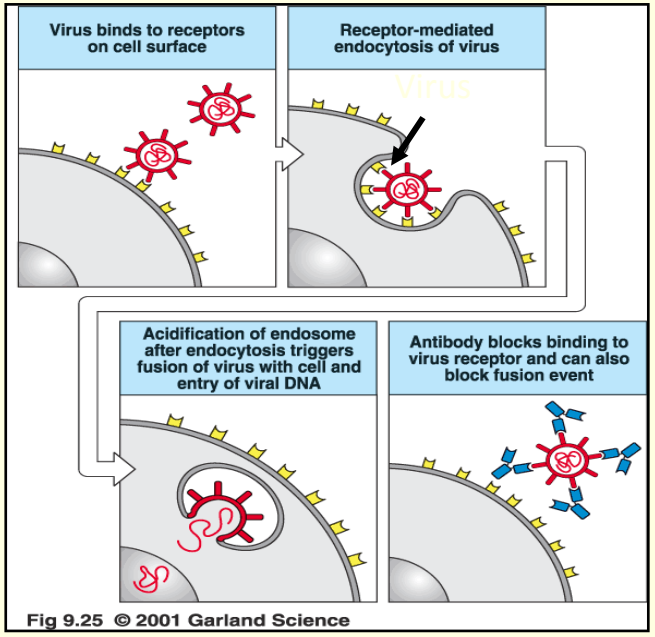
↓ Endocytosis

Infection or colonization

IgY binding

Immunobiology, Janeway et al, 2001





Advantages of Bird IgY

http://www.affiland.com/affinity/eggyolk.htm

Mammalian IgG	**COMPARISON**	***Chicken IgY***
Sometimes very poor avidity	**Mammalian antigens/ response**	High avidity
Yes	**Protein A & Protein G / binding**	No
Yes Common Fc receptor	**Mammalian IgG / recognition**	No
Yes	**Mammalian complements / recognition**	No
From 60th day in rabbits	**Antibody response**	From 30th day
50-70 ml sera / 90 days	**Antibody production capacity**	0.7egg per day (100~200mg/egg)
Antisera: expensive work*	**Antibody source collection**	Cheaper work
RIA[a], IRMA[b], ELISA, Conjugate, Enzymatic digestion	**Applications**	RIA, IRMA, ELISA, Conjugate

* : Bleeding, centrifugation, conservation at -20˚C or lower temperature)

a : RIA = Radioimmunoassay b : IRMA = Immuno-radio-metric assay

Egg Lipids

Neutral Lipids (65%)

Phospholipids (30%)

- PC (25%)
- PE (4.2%)
- Sphingomyelin (0.8%)
- Phosphatidylinositol (0.15%)

Cholesterol (5%)

Carotenoids (carotenes)

Xantophylls (lutein, zeaxanthin)

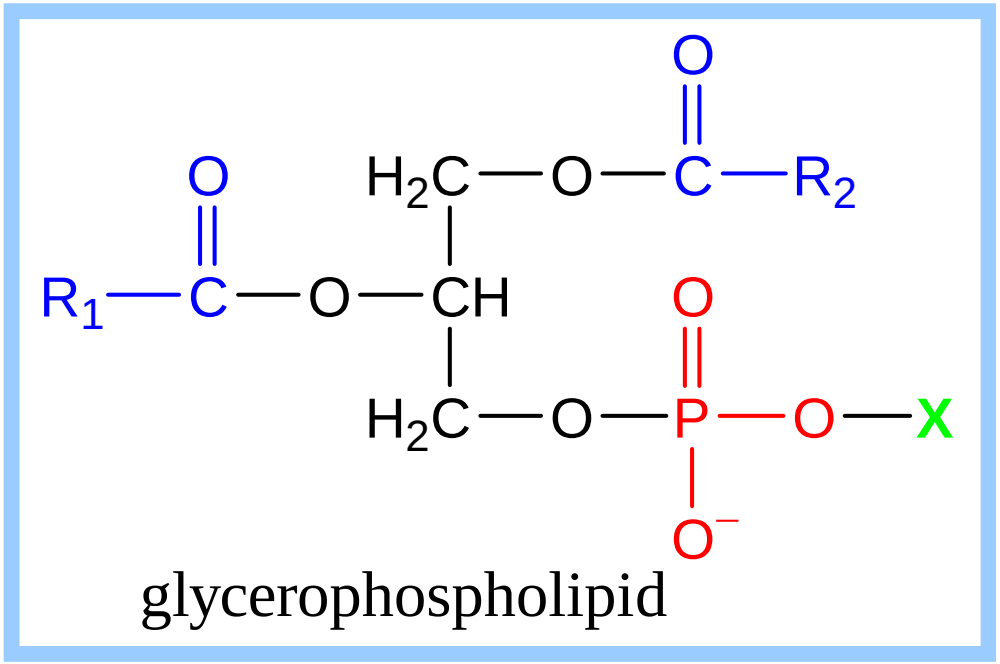
Phospholipids

- Phospholipids
  - Are composed of
    - Glycerol backbone
    - 2 fatty acids
    - Phosphate
  - Are soluble in water
  - Are manufactured in our bodies so they are not required in our diet

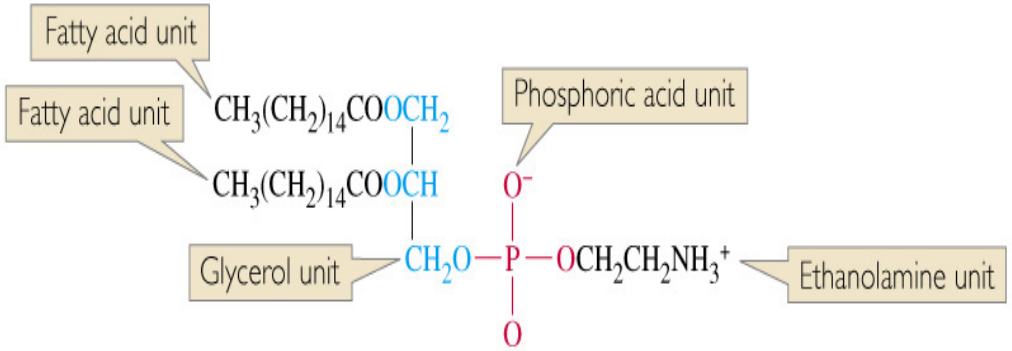
Phospholipids

Each glycerophospholipid includes

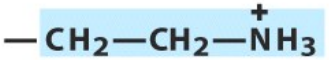
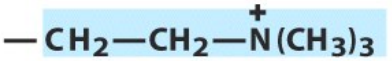
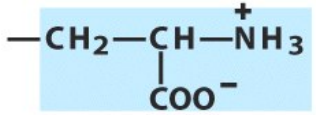
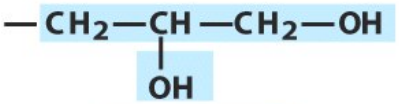
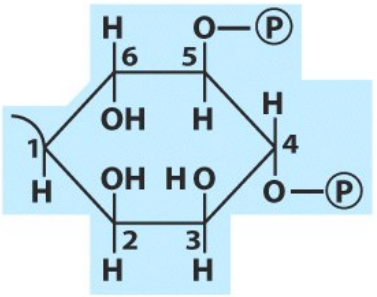
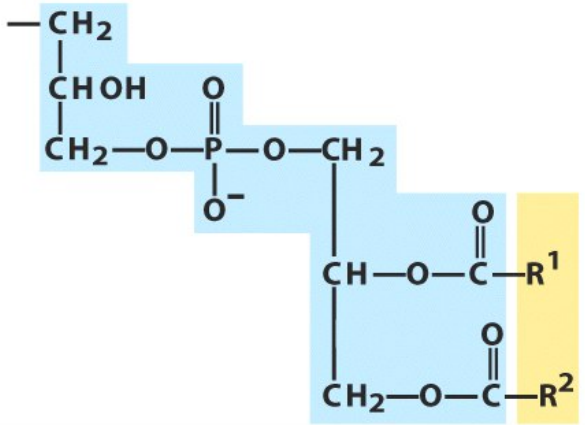
- a **polar** region: **glycerol**, **carbonyl O** of fatty acids, **P_i**, & the polar head group (**X**)
- **non-polar** hydrocarbon tails of fatty acids (**R_1**, **R_2**).



glycerophospholipid



Phosphatidylethanolamine (a cephalin)

Name of glycerophospholipid	Name of X	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—		−1
Phosphatidylethanolamine	Ethanolamine		0
Phosphatidylcholine	Choline		0
Phosphatidylserine	Serine		−1
Phosphatidylglycerol	Glycerol		−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4
Cardiolipin	Phosphatidyl-glycerol		−2

Sphingolipids

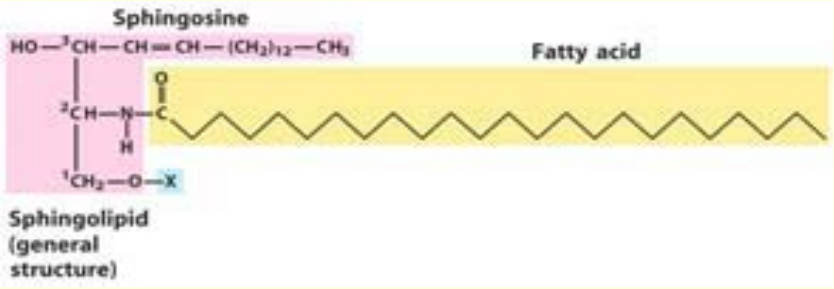
- No known nutritional requirement for sphingolipids
- Hydrolysed throughout the gastrointestinal tract to the same categories of metabolites that are used by cells to regulate growth, differentiation, apoptosis and other cellular functions.
- Feeding sphingolipids inhibits colon carcinogenesis, reduces serum LDL cholesterol and elevates HDL
- suggesting that sphingolipids represent a functional constituent of food.

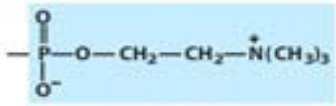
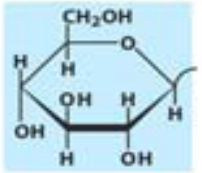
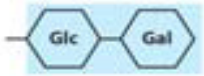
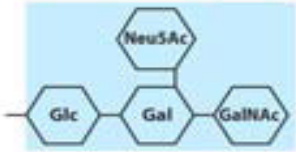
Sphingolipids

Sphingolipids (SPLs) also have a polar head group and two non-polar tails but do not contain glycerol

- Instead, the backbone is **sphingosine, a long-chain amino alcohol**
- Some derivatives are Ceramide, Sphingomyelin, and Glycosphingolipids

Sphingosine
is a fatty amine, a glycerol molecule
is never seen!



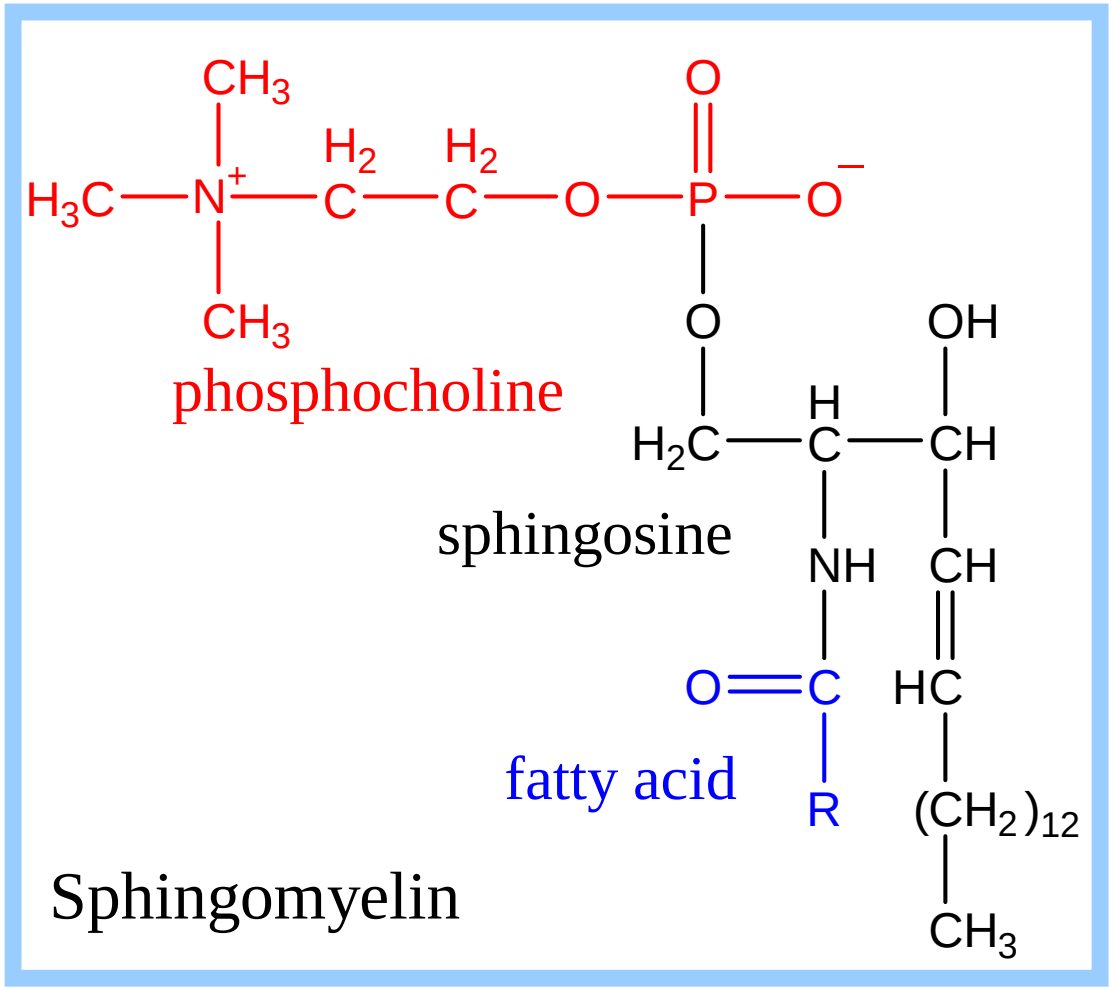
Name of sphingolipid	Name of X	Formula of X
Ceramide	—	— H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Sphingomyelin

- Important for the formation and maintenance of lipid rafts.
- Lipid rafts are involved in various signalings, including immunological responses and transportation of specific materials
- Play important roles in the expression of specific cellular functions, such as intracellular information transmission and maintenance of the membrane structure.
- Despite containing long fatty acid chains, sphingomyelin is uniquely suitable for the formation of liposomes.
- Concerning yolk-derived sphingomyelin, palmitic acid accounts for about 80% of the fatty acid chains bound by amide bonds

Sphingomyelin has a phosphocholine or phosphoethanolamine head group.

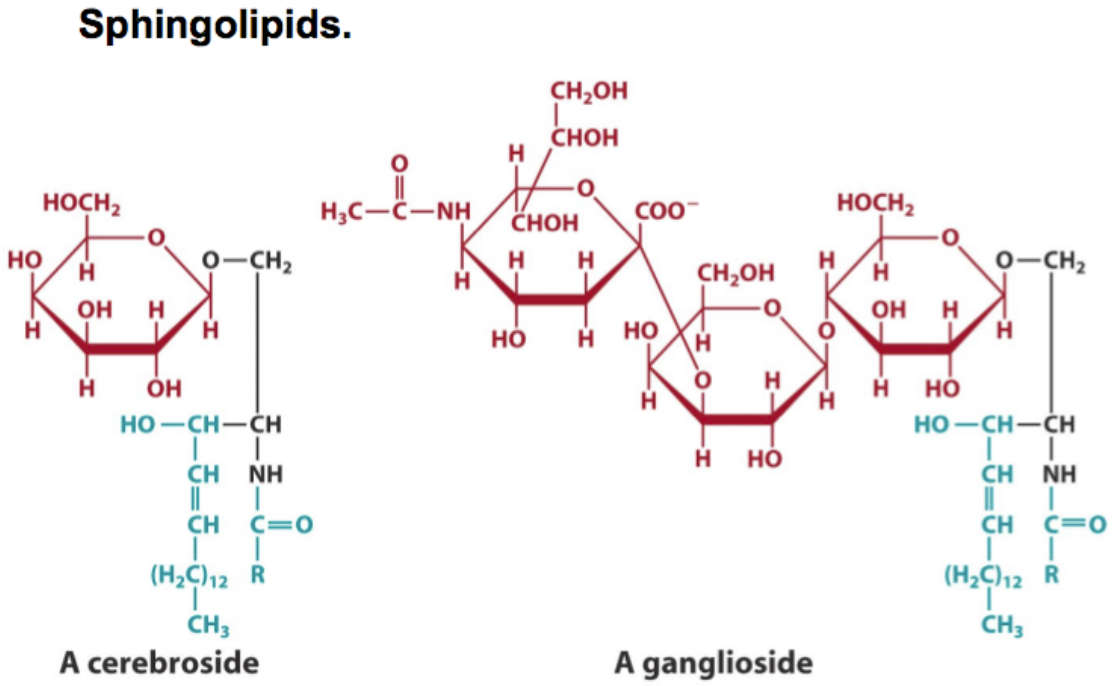
Sphingomyelins are common constituent of plasma membranes



Sphingomyelin, with a phosphocholine head group, is similar in size and shape to the glycerophospholipid phosphatidyl choline.

Glycosylated sphingolipids

A **cerebroside** is a sphingolipid (ceramide) with a **monosaccharide** such as glucose or galactose as polar head group.

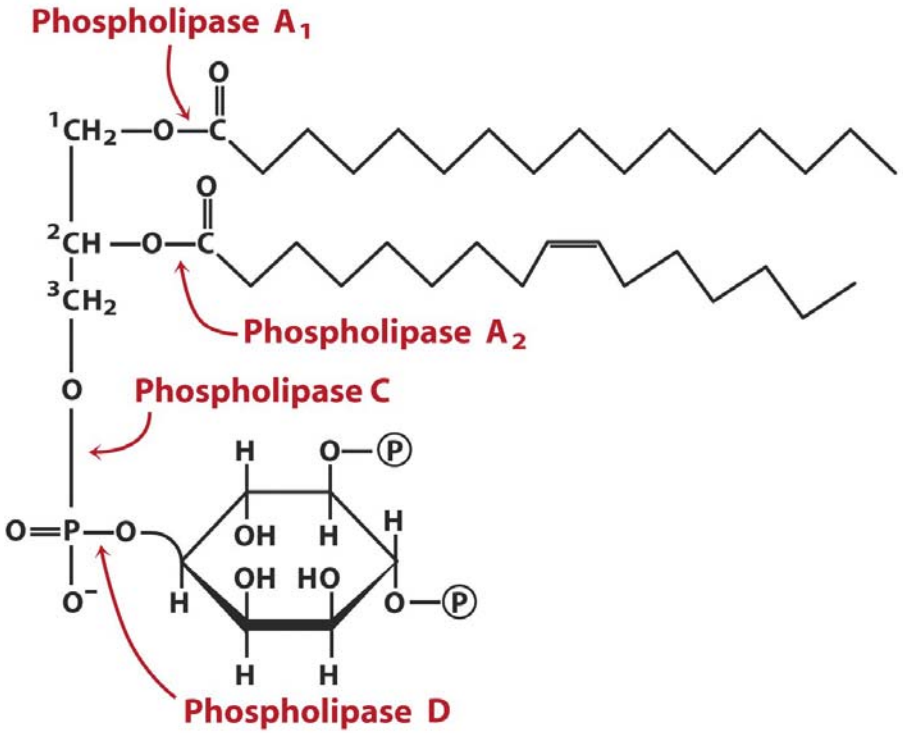


A **ganglioside** is a ceramide with a polar head group that is a **complex oligosaccharide**, including the acidic sugar derivative sialic acid.

Cerebrosides and gangliosides, collectively called **glycosphingolipids**, are commonly found in the outer leaflet of the plasma membrane bilayer, with their sugar chains extending out from the cell surface.

Activities of Phospholipases

- Determined by analysis of products from enzyme-catalyzed hydrolysis
- Phospholipases
- Cleave specific bonds
- Enzymes are small
  - Only section being cleaved fits in active site
  - Remainder of lipid in non-aqueous environment or stabilized by nonpolar AA side chains

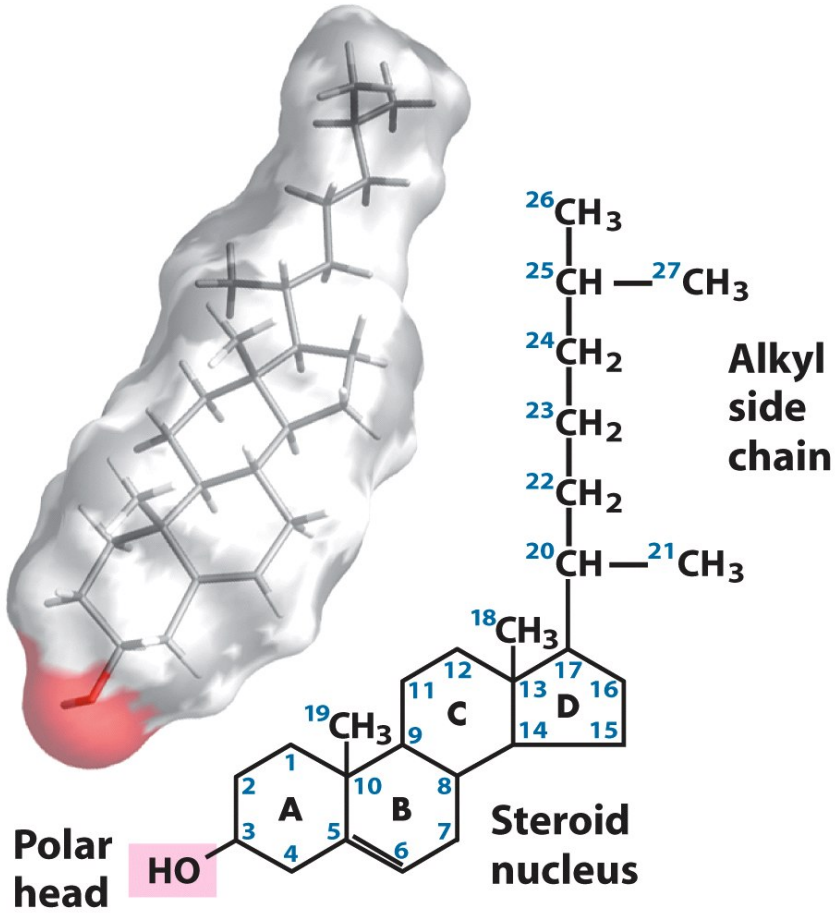


Sterols

- Compounds containing 4 carbon ring structure with any of a variety of side chains
- Many important body compounds are sterols
  - cell membranes, bile acids, sex & adrenal hormones, vit D & cholesterol.
- Sterols are found in plant & animal foods
  - Manufactured in bodies so non-essential

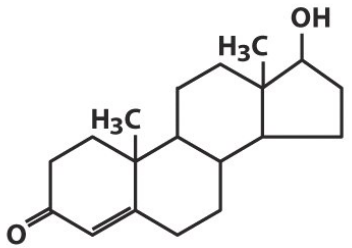
Sterols

- Sterol
  - Four fused rings
    - Greater rigidity than other membrane lipids
  - One or more hydroxyl groups
    - Gives amphipathic character
  - Hydrocarbon side chain
    - Length of C_{16} FA
- Cholesterol
  - Most abundant sterol in animals
  - Produced by liver; supplied by diet
  - High levels lead to gallstones and deposits on arteries (plaque)

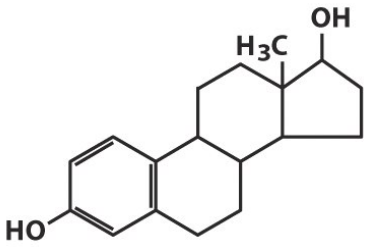


Sterols

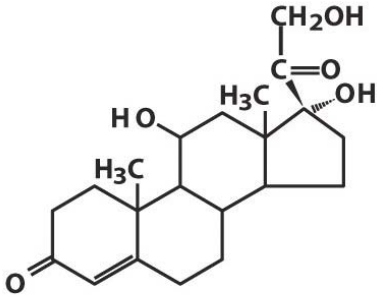
- Metabolic precursors of steroid hormones
  - Regulate physiological functions
  - Androgens (testosterone)
  - Estrogens (β-estradiol)
  - Glucocorticoids (cortisol)
- Insoluble in water
- Bind to proteins for transport to target tissue



Testosterone



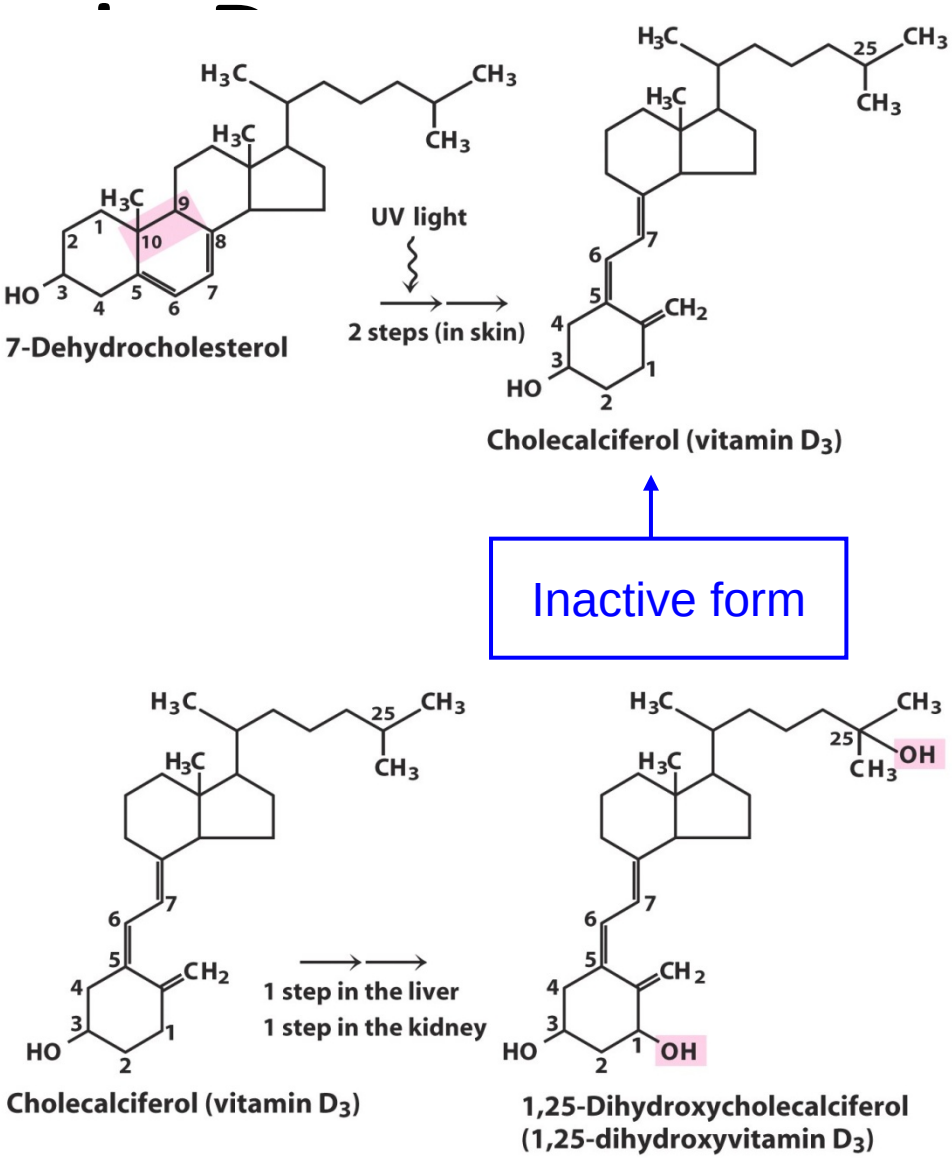
Estradiol



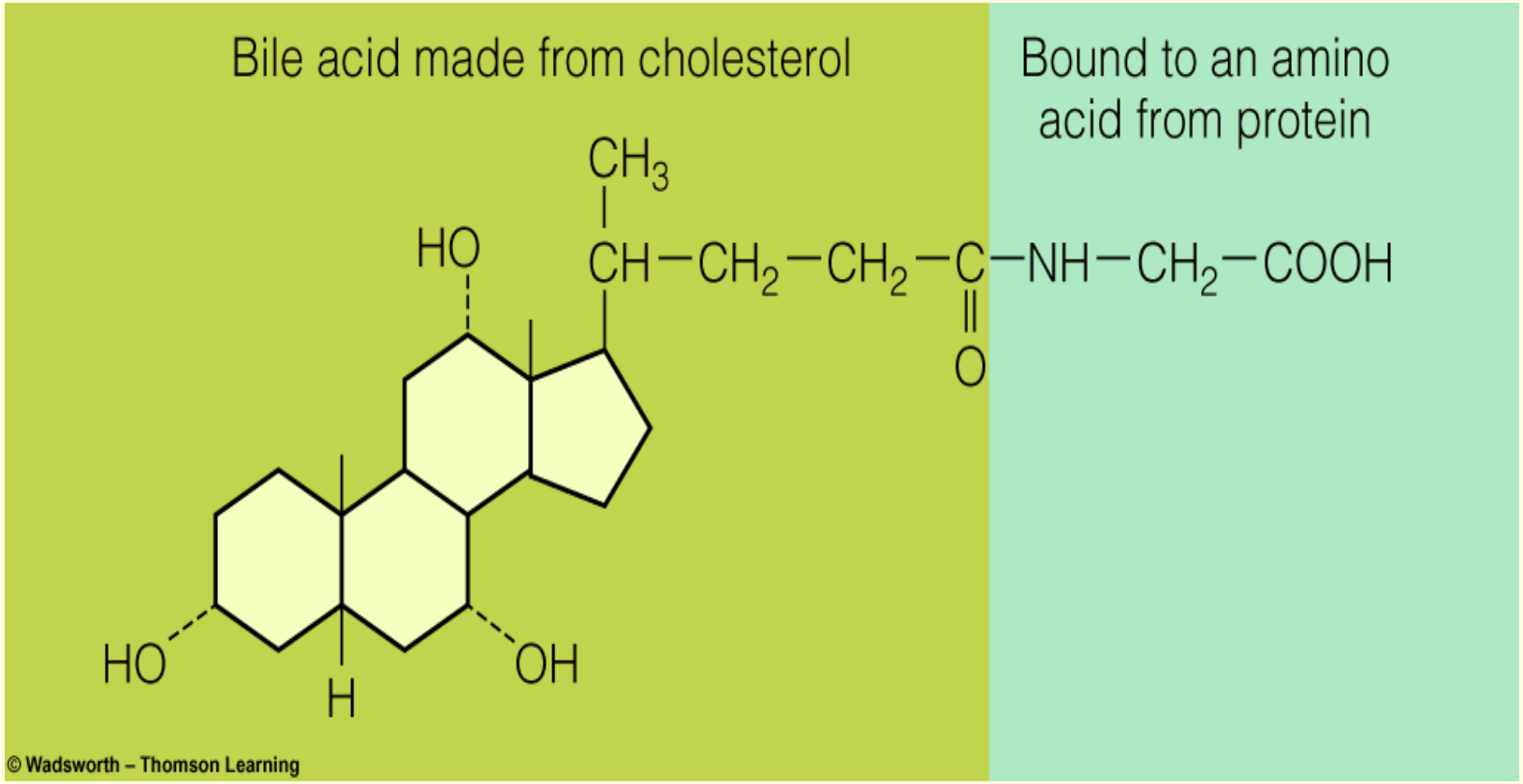
Cortisol

Vita

- Sterol derivatives
  - Open B rings
- Function
  - Regulate Ca and P absorption during bone growth
- Sources
  - Diet: D_2 (milk additive, plant sources) and D_3 (animal sources)
  - Precursor: intermediate in cholesterol synthesis
  - Formed in skin non-enzymatically from 7-dehydrocholesterol
- Deficiency
  - Soft bones, impaired growth and skeletal deformities in children



Bile Acid


Bile acid made from cholesterol
Bound to an amino acid from protein
CH3
HO
CH—CH2—CH2—C—NH—CH2—COOH
O
HO
OH
H
© Wadsworth – Thomson Learning

Sialyl-oligosaccharides

- Likely to play important role in the defence mechanisms against diseases caused by pathogenic microorganisms including pneumonia, diarrhoea, gastritis and ulcers.
- Sialic acid derivatives (gangliosides) are involved in brain function
- Important in protecting infants from various diseases.
- Sialic acid and sialyloligosaccharides have potential biological functions.
- Potential to be used in infant formula, health foods and nutritional supplements.

Yolk Pigments

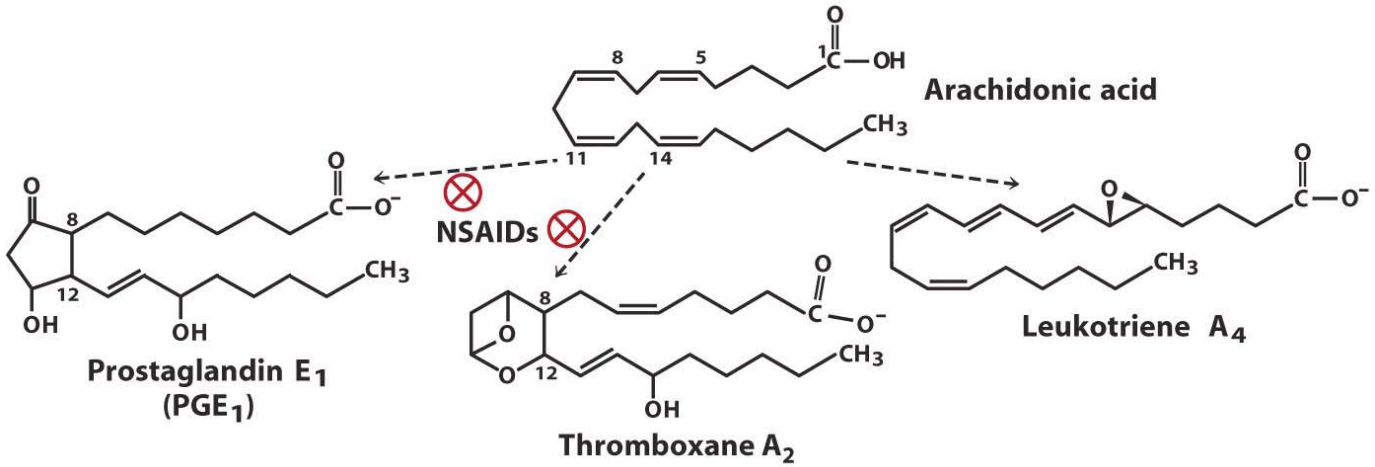
- Include carotenes and riboflavin and 0.02% based on the dry weight
- Carotenes are responsible for the color of the yolk, cannot be synthesized by the hen.
- The hen's feed is responsible for carotene content and the color of the egg yolk.
- Egg yolk carotenes are classified as xanthophils and carotenes.
- Lutein, zeaxanthin and cryptoxanthin belong to the xanthophil group

Carotenoid Composition of Egg Yolk

Pigments	Content (%)
Carotene	
a-carotene	Trace
b-carotene	0.03
Xanthophil	
Cryptoxanthin	0.03
Lutein	0.1
Zeaxanthin	0.2

Eicosanoids

- Hormones involved in production of pain, fever, inflammatory reactions
  - Prostaglandins
  - Thromboxanes
  - Leukotrienes
- Metabolites of arachidonic acid (a polyunsatruated FA)
- Synthesis inhibited by NSAIDs
  - e.g. acetylsalicylic acid (aspirin)
  - Acylate Ser residue, preventing access to active site



Carotenoids

- Sweet yellow corn, egg yolk, kiwi fruit, pumpkin, zucchini, spinach, squash, grapes and peas are also rich in carotenoids
- The food matrix in which carotenoids are found affects their bioavailability.
- Egg yolk is a highly bioavailable source of lutein and zeaxanthin

Lutein

- Lutein, zeaxanthin and Vitamin E protect eyes against macular degeneration.
- Of the estimated 600 carotenoids occurring in nature, only lutein and zeaxanthin are selectively incorporated into the macula.
- These carotenoids absorb blue light, which is damaging to retinal membranes.
- Lutein and zeaxanthin are antioxidants quenching free radicals induced by oxidative stress.

Potential Value-Added Components in Egg White (10-11% solids)

- Ovoalbumin: 6.5% of egg white
- Ovotransferrin: 1.5% of egg white
- Lysozyme: 0.4% of egg white
- Ovomucin: 0.2% of egg white
  - Inhibits haemagglutination by viruses
  - Have a cytotoxic effect on cultured tumour cells
- Avidin: Binds biotin
- Cystastatin:
  - Antimicrobial, antiviral and insecticidal effects
  - Prevention of cerebral haemorrhage
  - Control of cancer cell metastasis
- Ovoinhinitor:
  - A trypsin inhibitor that inhibits bacterial and fungal serine proteinases and chymotrypsin
- Avidin
- Growth factor:

Proteins of Egg White

Protein	Amount (%)	M Wt (KDa)	pI	Characteristics
Ovalbumin	54	45	4.5	
Ovotransferrin	12-13	77.7	6.0	Binds iron and other metal ions
Ovomucoid	11	28	4.1	Inhibits serine proteinases
Lysozyme	3.4-3.5	14.3	10.7	Lysis of bacterial cell walls
Ovomucin	1.5-3.5	220-270000	4.5-5.0	Interacts with lysozyme
G2 ovoglobulin	1.0	47	4.9-5.3	
G2 ovoglobulin	1.0	50	4.8	
Ovoflavoprotein	0.8	32	4.0	Binds riboflavin
Ovostatin	0.5	760-900	4.5-4.7	
Cystatin	0.05	12	5.1	Inhibits cysteine proteinases
Avidin	0.05	68.3	10.0	Binds biotin
Thiamine-binding protein	-	38	-	Binds thiamine
Glutamyl aminopeptidase	-	320	4.2	
Minor glycoprotein 1	-	52	5.7	
Minor glycoprotein 2	-	52	5.7	

(from Awade, 1996)

Ovalbumen

- The predominant protein in albumen (54% of albumen).
- Classified as a phosphoglycoprotein since carbohydrate and phosphate moieties are attached to the polypeptide.
- MW of about 45,000, and made up of 3 components, A1, A2, and A3, which differ in phosphorous content.
- Ovoalbumen A1 has two phosphate per molecule, A2 has one, and A3 has none.
- Ovoalbumen in solution is readily denatured and coagulated by exposure to new surfaces (e.g., shaking) but is resistant to thermal denaturation (84.5C).

Ovotransferrin

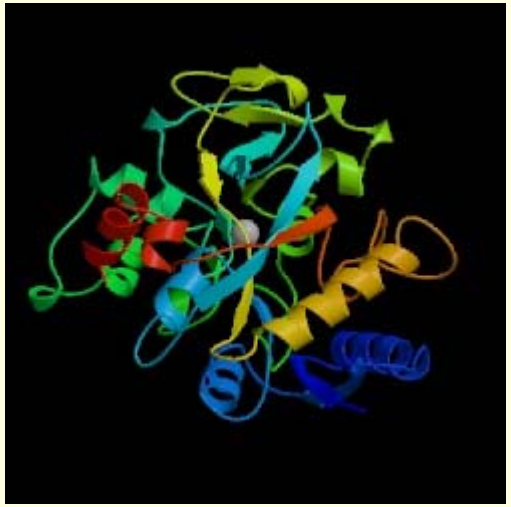
- A major avian egg white protein (12% of total egg white protein)
- A monomeric glycoprotein consists of a 686-residue single polypeptide chain. Molecular mass: ~ 78 kDa
- pI: 6.73 for apo-form (iron-free), 5.78 for holo-form
- Possess antiviral and antibacterial activities
- Transport of iron in a soluble form to target cells
- Iron binding protein (reversible, two iron atoms/molecule, as Fe^{3+}).
- The most heat liable in white: forming aggregation by heating at 60 C, resulting in milky white gel
- The release of Fe3+ from ferric transferrin requires the presence of a simple anion such as pyrophosphate, sulfate, and chloride

Characteristics of Ovotransferrin

- Metal-free (apo) form is easily destroyed by physical and chemical treatments, while holo-form (iron bound) is a salmon-pink colored
- Iron-complex is stable to proteolytic hydrolysis and thermal denaturation.
- The two iron-binding sites are located within the interdomain cleft of each lobe.
- At acidic pH, the N-lobe of ovotransferrin displays less binding stability and more accelerated release of Fe^{3+} than the C-lobe.

Structure of Ovotransferrin

- **N-terminal lobe, Holo form**



N-TERMINAL LOBE, APO FORM

- Covalent dimer protein, composed of an N- and a C-terminal domain,
- Each one binds one atom of transition metal (Fe [III], Cu [III], Al [III]) very tightly.
- Although the N and C lobes assume very similar overall conformation, each binding properties are markedly different,

http://www.rcsb.org/pdb/explore.do?structureId=1IEJ

Natural Substances Approved for Microbial Reduction in Foods

Ingredient	Purpose/products	Amount	Classification
Activated Lactoferrin	Meat and poultry carcass and products	Up to 2% of water-based spray	Direct food additive (GRAS)
Nisin	Sauces and fully cooked meat or poultry	No more than 690 ppm nisin in finished products	Approved as GRAS, 2000 USDA

http://www.meatami.com/Content/TradeShow/ConferenceMaterials/2004Mirc/Benji%20Mikel.pdf

Significance of using Ovotransferrin as an Antimicrobial Agent

- A natural antimicrobial agent for meat or meat products

- Increase the value and use of egg

- Use of ovotransferrin is cheaper antibacterial agent than lactoferrin

- High possibility of becoming a GRAS material like lactoferrin

Antimicrobial Mechanisms of Lactoferrin

- **Iron binding**: Chelating iron induces an iron-deficient environment, resulting in suppression of microbial growth (*Rev. Infect. Dis. 1983. 5: S759-S777)*

- **Direct bactericidal activity**: specific binding against outer membrane proteins leads to inhibition of various cellular functions and deregulation of adhesion/fimbrial synthesis on the bacterial surface (*Infect. Immun. 1986. 51:373-377, and Food Technology 2002. 56: No. 3)*

Ovomucoid

- Best known for its trypsin inhibitory activity
- Hen ovomucoid has nine disulfides and no free sulfhydryl groups
- Heat resistant glycoprotein
- MW is about 28,000, and 11% of total albumen
- Ovomucoid can be heated at 100 C under acidic conditions for long periods without any apparent changes in its physical or chemical properties

Ovomucoid

- Ovomucoid may play an important role in the pathogenesis of allergic reactions to egg white than other egg white proteins
- Water-soluble glycoprotein which is antigenic even in boiled shell eggs
- Low ovomucoid egg white preparation
  - Applicable as a new processed food for ovomucoid-sensitive patients.
  - A chemically-altered ovomucoid with enhanced digestibility and lower allergenicity
  - Ethanol precipitation
- The disulfide bonds play a significant role in the digestive resistance of ovomucoid

Lysozyme

- ~~Lysozyme constitutes approximately 3.5% of hen egg white~~
- The name lysozyme was originally used to describe an enzyme which had lytic action against bacterial cells
- Also known as muramidase and N-acetylmuramic-hydrolase and is one of the oldest egg components to be utilised commercially
- A bacteriolytic enzyme commonly found in nature and is present in almost all secreted body fluids and tissues of humans and animals.

Lysozyme

- ~~Lysozyme is one of the simplest ubiquitous enzymes. Also~~ been isolated from some plants, bacteria and bacteriophages
- The chicken egg white is a rich and easily available source of lysozyme
- The lysozyme content of a laying hen's blood is 10-fold higher than in mammals because it is being transferred to the egg white

Lysozyme

- Egg white lysozyme consists of 129 amino acid residues with a MW of 14.4 kDa
- Binds to ovomucin, transferrin or ovalbumin in egg white
- Highly stable in acidic solution and heating at 100 C for 1-2 minutes
- The thermal stability of lysozyme is partly due to its four disulfide bonds
- Catalyzes the hydrolysis of the (1-4)-glycosidic linkage between N-acetylmuraminic acid and N-acetylglucosamine in the polysaccharides of certain bacterial cell walls

Lysozyme

- In nature, found mainly as a monomer but also exist as a reversible dimer
- The dimeric form of lysozyme exhibits therapeutic, antiviral and anti-inflammatory properties
- Induces the activity of phagocytizing cells
- Influences immunological processes by stimulating immunoglobin synthesis
- Promotes interferon synthesis and modulates tumour necrosis factor generation

Lysozyme

- Demonstrates antimicrobial activity against a limited spectrum of bacteria and fungi
- Its enzyme activity can be enhanced by certain substances including
  - EDTA
  - Butylparaben
  - Tripolyphosphate
  - other naturally occurring antimicrobial agents

Other biological functions of lysozyme

- Anti-viral action by forming an insoluble complex with acidic viruses
- Enhanced antibiotic effects
- Anti-inflammatory
- Anti-histaminic actions
- Direct activation of immune cells
- Anti-tumour action

Use of Lysozyme

- Pharmaceutical industry: against bacterial, viral or inflammatory diseases such as dental caries and spray for nasal tissue protection
- Therapeutic creams: protection of the skin and soft tissues (*e.g.* burns, viral diseases).
- Oral administration: shown to have an antihistaminic effect.
- In cheese making: prevent off-flavours and late blowing in some cheeses (*e.g.* Swiss Cheese, Parmesan, Edam, Gouda and Cheddar).

Use of Lysozyme

- Acceleration of cheese ripening: lysis of starter bacteria releases cytoplasmic enzymes, which play a key role in proteolysis during cheese ripening
- Brewing: control of lactic acid bacteria in beer.
- Sulfur dioxide (SO2) is commonly used to inhibit spoilage bacteria and yeasts in wines, but may cause allergic reactions in sensitive individuals.
- The high affinity binding of lysozyme to bacterial lipopolysaccharide results in reversible inactivation of its enzymatic activity

Lysozyme

- Lipophilization broadened the bactericidal action of lysozyme to Gram-negative bacteria with little loss of enzymic activity
- Glycosylation produces more stable proteins, with improved conformational stability, protease resistance, charge effects and water-binding capacity
- Lysozyme-dextran conjugate: retains good emulsifying properties and heat stability
- Heat denaturation of lysozyme results in the progressive loss of enzymatic activity, but a greatly improved antimicrobial action towards Gram-negative bacteria.

Why lysozyme has been used as an antimicrobial agent in various foods

- Heat stable
- Active in a broad range of temperatures (from 1 C to nearly 100 C)
- Withstands boiling for 1-2 min
- Stable in freeze-drying and thermal drying
- Not inactivated by solvents,
- Maintains its activity when redissolved in water
- Has optimum activity at pH 5.3 to 6.4 (*i.e.* typical for low-acidic food)
- The presence of other proteins in food, however, can reduce its stability by the formation of sulfide bridges

Ovomucin

- Ovomucin comprises 1.5-3.5% of the total egg white solids
- Consists of an a-subunit (220 kDa containing 10-15% carbohydrate) and a β-subunit (400 kDa containing 50-65% carbohydrate), which are bound by disulfide bonds
  - The β-subunit from ovomucin was shown to have a cytotoxic effect on the cultured tumour cells through scanning electron microscopy
  - Both fragments of highly glycosylated peptide fragments (220 and 120 kDa) separated from pronase-treated hen egg white ovomucin derived from the β-subunit inhibited the growth of tumours

Ovomucin

- A glycoprotein that contributes the gel-like structure of thick white
- Heat resistant
- The amount in thick albumen is 4x higher than that in thin white
- Thinning of thick albumen is caused partly by the interaction of ovomucin with lysozyme when the pH rises to around 9.0.
- The formation of ovomucin with lysozyme complex causes the loss of lysozyme activity.
- Inhibits hemagglutination by virus

Avidin

- Avidin is a trace component (0.05%) of egg white
- A tetrameric, strongly basic glycoprotein protein
- Composed of subunits of identical amino acid composition and sequence (15.6 kDa and 128 amino acids each)
- Combines with biotin to form a stable complex, which is incapable of absorption by the intestinal tracts of animals.
- Avidin binds with 4 biotin molecules.

Avidin

- Avidin is irreversibly denatured at 70 C, but the avidin-biotin complex is stable to 100 C
- The binding between biotin and avidin is so strong that separation requires heating the complex at 120 C for 15 minutes.
- The high affinity constant of avidin for biotin has been widely used in molecular biology
  - Affinity chromatography
  - Molecular recognition and labelling
  - Enzyme Linked ImmunoSorbent Assay (ELISA)
  - Histochemistry and cytochemistry

Ovoglobulins and Ovomacroglobulin

- **Ovoglobulins**
- Excellent foaming agents in egg white
- Composed of ovoglobulins G2 and G3, which have molecular weights of 36 and 45 kDa, respectively.

- **Ovomacroglobulin**
- Ovomacroglobulin is the second largest egg glycoprotein after ovomucin
- Molecular weight is 760-900 kDa.
- Has the ability to inhibit hemagglutination

Ovoinhibitor

- A proteolytic enzyme inhibitor
- Functions as a multi-headed inhibitor and inhibits bacterial serine proteinase, fungal serine proteinase and chymotrypsin

Flavoprotein

- All riboflavin in egg albumen is bound in the flavoprotein in a 1:1 ratio
- Ensure transfer of the riboflavin from the blood serum to the albumen in the egg white bound to an apoprotein called flavoprotein.
- The apoprotein is acidic with a molecular weight of 32-36 kDa, and contains a carbohydrate moiety (14%) made up of mannose, galactose and glucosamines, 7-8 phosphate groups and 8 disulfide bonds.
- One mole of apoprotein binds one mole of riboflavin, but this binding ability is lost when the protein is exposed to a pH below its isoelectric pH

Cystatin

- A family of cysteine protease inhibitors with homology to chicken cystatin.
- Cystatins comprise about 115 amino acids (12.7 kDa), are largely acidic, contain four conserved cysteine residues known to form two disulfide bonds with high thermal stability
- A proteinase inhibitor in egg white (also called ficin-papain inhibitor).
- Inhibits sulphydryl proteinases activity
- Potential application: antimicrobial, antiviral and insecticidal agent, prevention of cerebral haemorrhage, control of cancer cell metastasis.
- Less severe side effects than synthetic protease inhibitors
- The greatest problem: high cost (US$140 egg cystatin/mg).

Growth Factor

- Egg white contains growth factors
- Research is in primitive stage
- Potential to separate from embryo