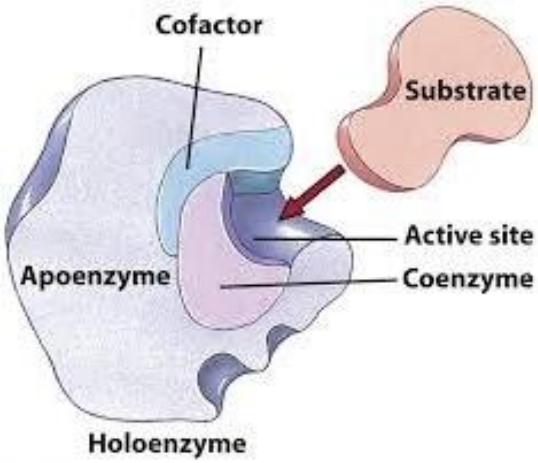
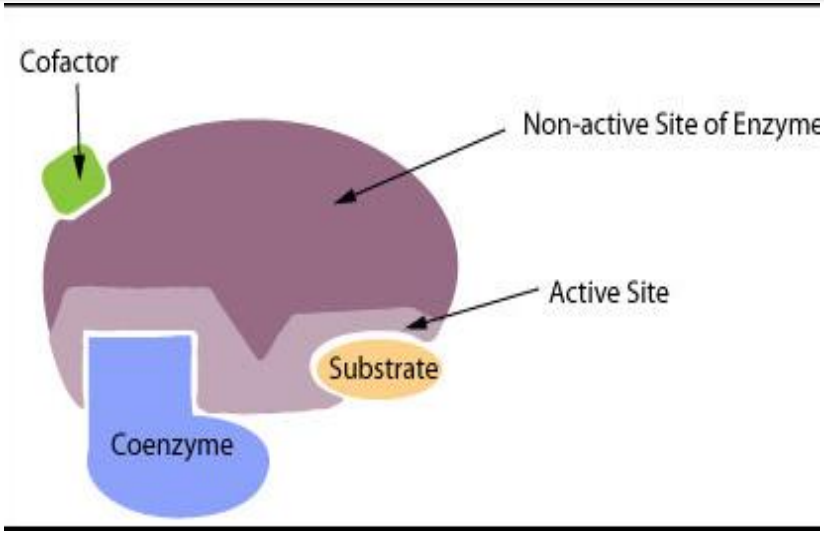


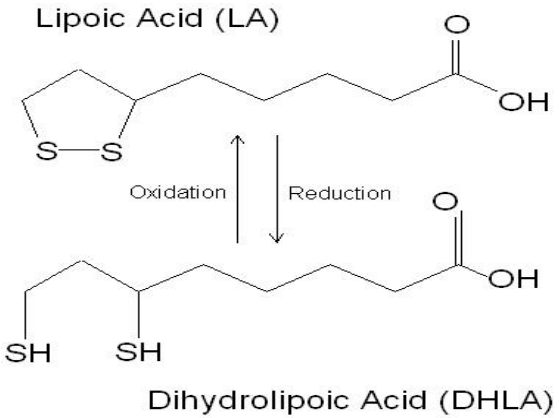
Coenzymes & Cofactor



LIPOATE

- ✓ **This was discovered in 1941 as a growth factor for the protozoan, *Tetrahymena*, and was called factor II. The substance was crystallized in 1951 by Reed, DeBusk, Gunsalus and Hornberger, who proposed the name 'α-lipoic acid', on account of its solubility in organic solvents.**

- ✓ **On chemical grounds, it was called thioactic acid. The name lipoic acid is now used most commonly for the oxidized form.**



- ✓ **One of the carbon atoms, is asymmetric, so two optical isomers exist. The natural isomer of oxidized form is dextrorotatory but gives rise on reduction, a levo-rotatory reduced form.**

- ✓ **Only the natural isomer active in pyruvate oxidsae system. The considerable portion of lipoate in the cells appear to exist bound to a lysyl group and can be removed by an enzyme from yeast and bacteria.**

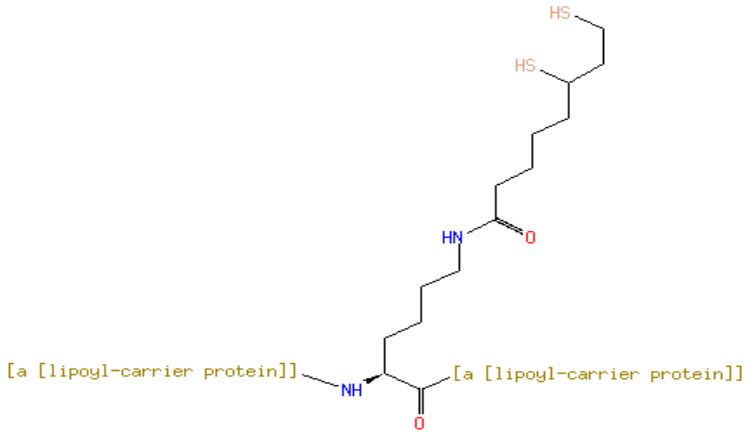
- ✓ **The oxidized form is yellowish with an absorption band at about 335 nm which is due to the five membered ring.**

- **Powerful reducing agents, like Zn + HCl (not borohydride) opens the ring by the reduction of disulfide bond. It is reduced by pyruvate and 2-oxoglutaratein the presence of their respective dehydrogenases and thiamine pyrophosphate (TPP), in these cases the reduction is accompanied by the transfer of an acyl group from the substrate through thiamine to the lipoate; giving acetyl/ acylhydrolipoate and succinylhydrolipoate, respectively.**

- **The acetyl group remains attached to the thiol in the sixth position.**

- **The reduced lipoate, after removal of any attached acyl group, is oxidized by NAD^+ in the presence of dihydrolipoamide dehydrogenase (NAD^+).**

- **It may also be oxidized by mild chemical oxidizing agents, such as iodine.**

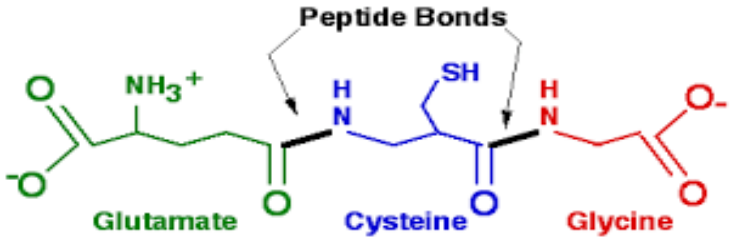


E-L-lipoyl lysine also called lipoamide

iv. GLUTATHIONE

- This widely distributed sulfur containing peptide was discovered in 1921 by Hopkins. The reduced form of glutathione was first isolated in the non-crystalline state and believed to be a dipeptide of cys and glu.

- Later it was found to be a tripeptide of cys, gly and gly. The structure was first established as ϒ-glutamyl-L-cysteinyl-glycineby synthesis by Harington (1955).



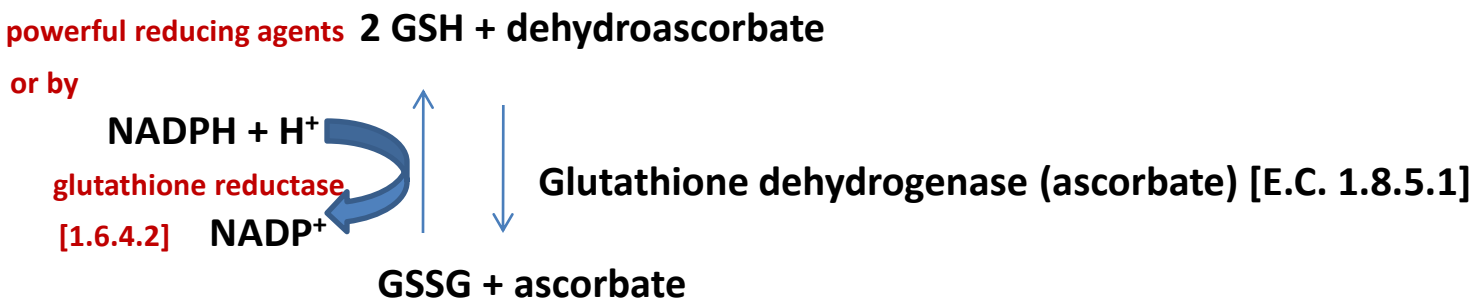
- Reduced gluatthione, GSH

- The functional group in the molecule is thiol group. Reduced GSH is oxidized to the disulfide by mild oxidizing agents eg iodine or ferricyanide:

$$2\,GSH \longrightarrow GSSG + 2H$$

- It is also oxidized by molecular oxygen under suitable conditions in the presence of traces of catalytic metals and cyt c.

- It is oxidized enzymatically by dehydroascorbate in the presence of glutathione dehydrogenase:



- Since glutathione can undergo enzymatic oxidation and reduction, it can act as biological H-carrier, and it was first such carrier to be discovered.

- Thiol disulfide systems come fairly readily in oxidation – reduction equilibrium with one another in the absence of enzymes; glutathione and cysteine (CSH) are thus in equilibrium in the way:

$$2\,GSH + CSSC = GSSG + 2\,CSH$$

- One such reaction is catalyzed by an enzyme- glutathione-homocysteine trnashydrogenase

glutathione homocysteine transhydrogenase (E.C. 1.8.4.1)

$$2\,GSH + \text{homocystine} = GSSG + 2\,\text{homocysteine}$$

- The functions of glutathione are still somewhat obscure in spite of a very extensive literature. It can undoubtedly act as a biological hydrogen carrier.

- It can provide a path for the oxidation of the coenzyme through ascorbate and either ascorbate oxidase in plants or cytochrome oxidase in animals.

- Glutathione also acts as specific coenzyme for glyoxalate system, for maleylacetoacetate and maleylpyruvate isomerases and for formaldehyde dehydrogenae.

- Many enzymes are –SH enzymes and are active only in the thiol state, and it has been suggested that a very imp. function of glutathione is to keep these enzymes in the educed form.

- Glutathione takes imp. part in combating oxidative stress:

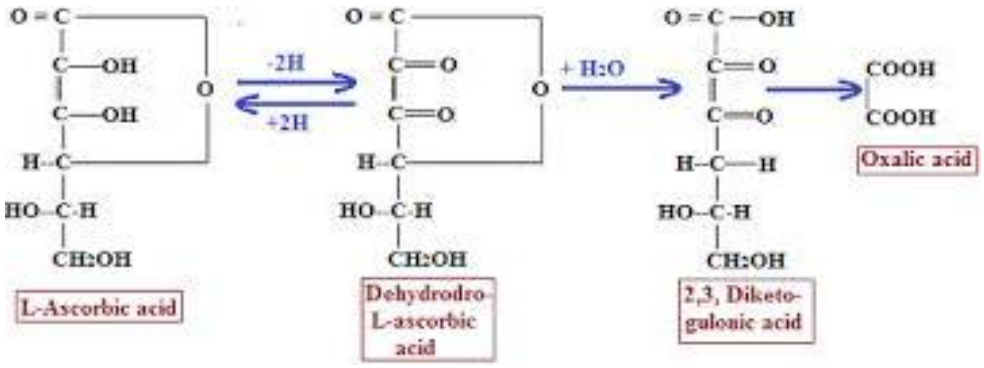
$$2\ GSH + H_2O_2 = GSSG + 2\ H_2O$$

- Glutathione maintains the redox state of the cell.

ASCORBIC ACID

- L-ascorbic acid (vit C) previously partially purified by several workers, was crystallized by Szent-Gyongyi (1928).

- The compounds can exist in oxidized and reduced form, the name ascorbic acid is given to the reduced form only and the oxidized form is ≡ dehydroascorbic acid.



active active inactive

- Ascorbate is an effective reducing agent, rapidly reduces many dyes and rapidly reducing even neutral silver nitrate to metallic silver. It is also oxidized by usual oxidizing agents (eg iodine) and by oxygen in the presence of traces of catalytic metals.

- In plants, a specific copper containing ascorbate oxidase brings this reaction about. This enzyme is not present in animal tissues, where ascorbate may be oxidized through a cytochrome system:

$$2\ \text{L-ascorbate} + O_2 = 2\ \text{dehydroascorbate} + 2H_2O$$

- Both forms of a.a. are lactones. The reduced form is acidic because of the ionization of enediol structure, while the oxidized form is neutral.

- The lactone ring is stable in a.a. but readily hydrolyzes in dehydroascorbic acid to give an open chain acid.

- ✓ **The lactone form of dehydroacorbic acid can readily be reduced to a.a. by H2S or by GSH in the presence of glutathione dehydrogenase (ascorbate) [E.C. 1.8.5.1].**

- ✓ **The open chain form i.e. diketogulonc acid, is not reduced back to ascorbic acid by H_2S, this form undergoes further reversible changes in solution, so that the oxidation of a.a. becomes effectively irreversible if the product is not reduced within a short time.**

- ✓ **An intermediate, which is the oxidized form of a.a., appears which is rapidly converted into dehydroascorbic acid, can bring about oxidation of NADH and NADPH in the presence of monodehyroascorbate reductase:**

monodehydroascorbate reductase (NADH) [E.C. 1.6.5.1]

NADH + 2-monodehydroascorbate ==== NAD^+ + 2-ascorbate

- ✓ **The oxidized form is produced at least transiently in the action of ascorbate oxidase. Thus ascorbate can act as biological hydrogen carrier although its significance in this respect is yet not entirely clear.**

- ✓ **In addition ascorbate is an essential part of the hydroxylase system of subgroup 1.14.11 and 1.14.17 eg.**

dopamine β-monooxygenase [E.C. 1.14.17.1]

3,4- dihydroxyphenylethylamine + asorbate + O_2 ===== nor adrenalin + dehydroascorbate + H_2O

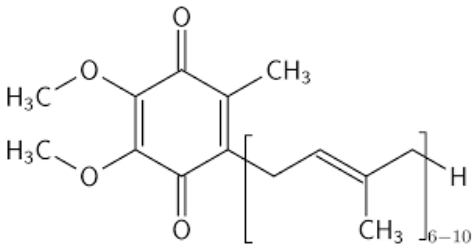
- **The wide distribution of ascorbate in animal tissues and other organisms despite the fact that it is an essential vitamin only in the case of primates and the guinea pigs, shows that it is probably plays an imp. role in animal metabolism.**

- **Being a good reducing agent, ascorbate like glutathione may play a part in maintaining the activity of –SH enzymes.**

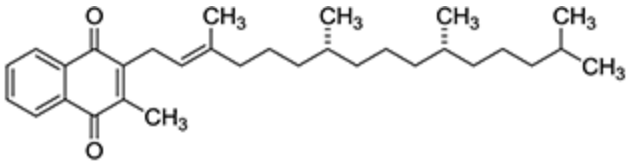
- ***In vitro,* it has been shown to have such an action in a no. of cases.**

QUINONES

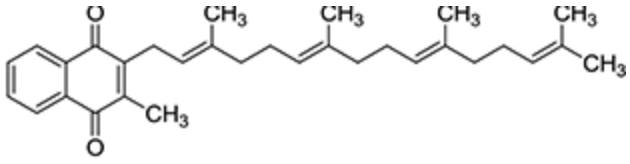
- **Quinones are widespread in living cells, it has been shown that some quinones play an important part as intermediate hydrogen carrier in the respiratory system, especially methylated quinones with polyisoprenoid side chain.**



Ubiquinone



vitamin K_1 (phylloquinone)



vitamin K_2 (menatetrenone, MK-4)

- **The ubiquinone, differing only in the value of n nd vit K2 are each a group of closely related compounds , differing only in the value of n. For the ubiquinone occurring in animals and higher plant tissues, n =10, but in lower organisms homologues with value of n 6 to 9 have been identified.**

- **For K2, n may 6, 7 or 9.depends on it.**

- **Ubiquinone was first discovered by Morton and his group in 1953.**

- **Ubiquinone is also ≡ coenzyme Q. Like other quinones, ubiquinone undergoes reduction to a hydroxyquinone form, and its main physiological function . It occurs in mitochondria and forms and essential constituent of the mitochondrial respiratory chain.**

- **It is insoluble in water, so that the reaction with which it is concerned take place *in vivo,* in a non-aqueous phase.**

- **Plant chloroplasts contain a group of closely related quinones ≡ plastaquinones. Their function is not yet precisely known, but it has been established that they form one of the links of the photosynthetic chain in the green plants.**

VITAMIN K

- **Although it has been suggested that vit K acts as carrier in respiratory chain, there is little convincing evidence for it.**

- **However, via K, especially menadione, can undergo reduction and oxidation in mitochondrial suspensions and a very active menadione reductase exist, which brings about the reduction of menadione [but not of vit K1 and k2] by NAD(P)H.**

- **This enzyme is inhibited by minute conc of dicoumarol which has no effect on either O_2 uptake or phosphorylation in mitochondria.**

- **It is therefore, this enzyme is involved in respiratory chain, there must be an alternative pathway which comes into operation when the inhibitor is added.**

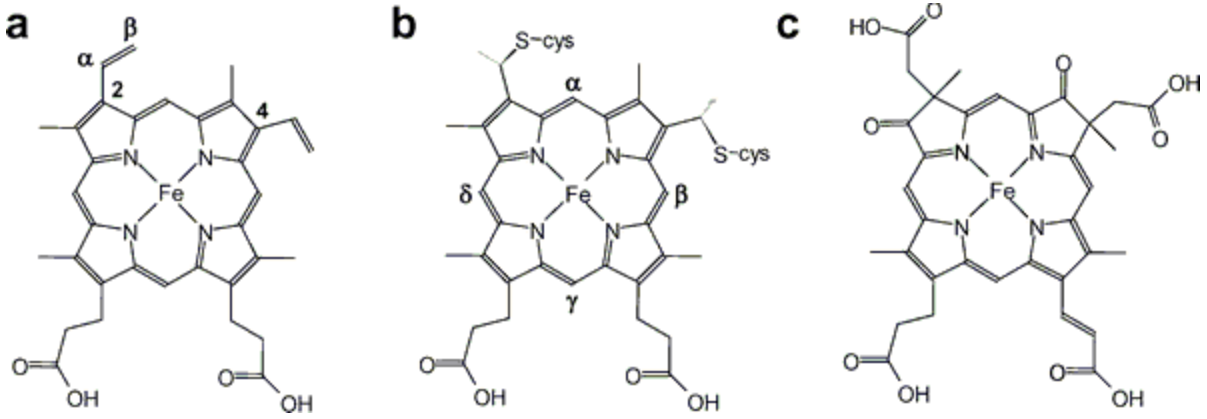
CYTOCHROMES

- **The name cytochrome is given by Keilin (1925) to a group of of intracellular hemoproteins which in the reduced form show a marked absorption spectrum in the visible region.**

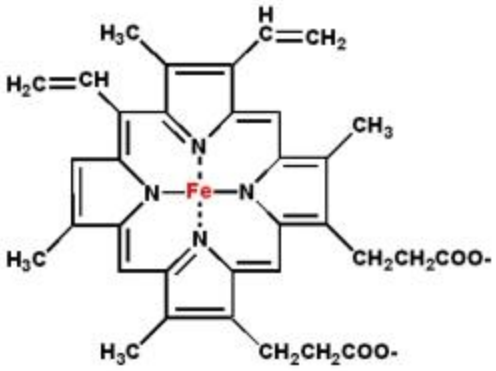
- **This name as at present used appears to include all intracellular hemopropteins with the exception of hemoglobin, myoglobin, peroxidase and catalase.**

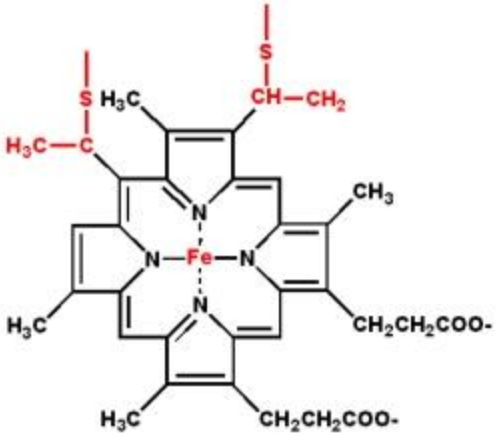
- **The group include substances with many different functions. The functions of no. of cytochromes are unknown, but they all appear to act by undergoing oxidation and reduction.**

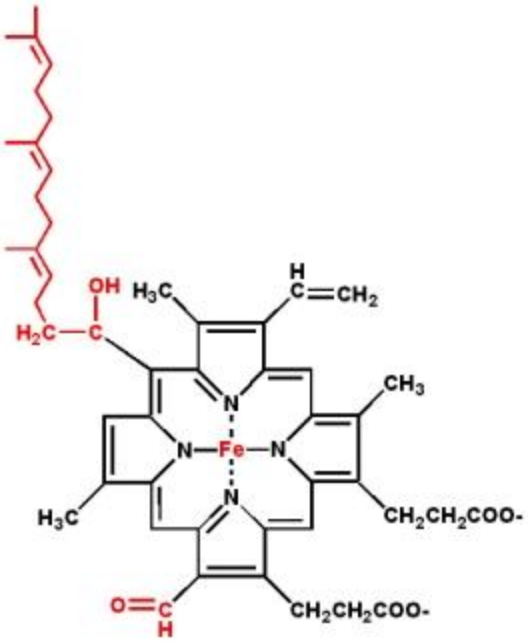
- **Some of those whose functions are known are enzymes while others are simply redoc carriers.**



Images of cytochromes







- **Cytochromes fall into four groups differing in the nature of the heme prosthetic group . The four heme types a,b,c abd d are characterized by the side chains of their porphyrins and the corresponding cyt types are defined as**

  -cyt a: heme gp contains formyl side chain

  -cyt b: protoheme as prosdthetic gp, not covalerntly bound to the protein

  -cyt c: covalent linkage between heme side chain and the protein

  -cyt d: heme gp contains dihydroporphyrin

MECHAMISM

- **For many years, the reduction and oxidation of cyt c was regarded as a simple addition or removal of an e^- to or from the iron atom by analogy with simple ferric and ferrous ions. This is not the case.**

- **In cyts iron atom is not directly accessible, but is at the center of the complex coordination structure which is buried deep in the protein molecule.**

- **Many amino acids take part in the transfer of e^- . The amino acids are:**

  -Tyr-74

  -Trp-59

  -Tyr-67

- **The sequence of events is**

1. **One e^- is transferred from the reductase complex To Tyr-74 and from there to Tyr-59 by 'π e^- cloud overlap' .**

   Since the e^- is placed on Tyr by reductase, it will migrate spontaneously To Trp, since the longer delocalized ring system of the latter group leads to a lower lying first antibonding orbital for the e^- to occupy.

   e^- flow from residue 74 to 59 will be downhill.

2. **Another e^- is transferred from the Tyr-67 to the heme ring, leaving Tyr-67 with an e^- deficiency. Again e^- flow occurs from a small delocalized ring system to a larger one with lower lying electronic levels.**

   The flow would be assisted by the –ve charge from the extra e^- on nearby Tyr-59. although in the absence of π electron cloud overlap, charge transfer from 59 → 67 would be inhibited.

3. **A conformation change occurs, leading from the obsd. oxidized structure to reduced one, bringing Tyr-59 and Tyr-67 into parallelism and 'π e^- cloud overlap' . This conformation change may be triggered or assisted by the electrostatic attraction of e^- rich Tyr-59 and e^- deficient Tyr-67.**

4. **An e^- is then transferred from Try-59 to Tyr-67 , leaving both gps electrically neutral and the molecule is obsd reduced conformation.**

   The two e^-s transfers of step-1 in this model are facilitated because they both involve e^-s flow from a small Tyr to a larger delocalized e^- aromatic system.

- **The final transfer in step-4 is enhanced because it eliminates an ion pair in a medium of low dielectric constt in the interior of the molecule. The normal activation barriers of charge transfer between aromatic systems are hence at least partially removed.**

- **Cyt a acts essentially as a redox carrier, transferring reducing equivalents from one molecule to another in much the same way as NAD does.**

- **It is therefore, truly a cofactor in the respiratory system. It does not act like an enzyme by activating specific substrates of its own. Cyt aa3 (cyt oxidase, E.C. 1.9.3.1) brings about the rapid oxidation of reduced cyt c by molecular O2, a reaction which is very slow in its absence.**

- **It is the terminal oxidase of the main mitochondrial respiratory chain.**

e^- e^-

Reductase complex → Tyr-74 ⟶ Trp-59