Involvement of coenzymes in enzyme catalysed reaction

Enzyme accelerate biochemical reactions by stabilizing the transition state. By utilizing the full repertoire of intermolecular forces they bring substrates together in an optimal orientation so that breaking and making bonds are possible. The catalytic activity of many enzymes depend on the presence of small molecule termed as cofactors. An enzyme without its cofactor is refered to as apoenzyme., the complete catalytically active enzyme is called a holoenzyme.

$$\text{Apoenzyme (inactive)} + \text{cofactor} \rightleftharpoons \text{holoenzyme (active)}$$

Cofactors can be subdivided into two group
i) metal ions e.g Zn^{2+} ii) small organic molecules
e.g. NAD^+ and they are called coenzymes.

So, in general some cofactors, for instance NAD^+, are transiently associated with a given enzyme molecule and other cofactors known as prosthetic group are essentially permanently associated with their protein, often by covalent bonds. For ex. heme prosthetic group of Haemoglobin protein

is tightly bound to its proteins by hydrophobic and hydrogen bonding interactions together with a covalent bond between heme Fe^{2+} ion and (His F8) amino acid residue of globin chains.

Coenzymes are chemically changed by the enzymatic reactions in which they participate. Thus in order to complete the catalytic cycle, the coenzymes must be returned to its original state. For prosthetic groups, this can occur only in a separate phase of enzymatic reaction sequence. For transiently bound coenzyme e.g. NAD^+. The regeneration reaction may be catalyzed by a diffent enzyme.

Some Enzymes requiring or containing inorganic metal ions as cofactor

Cofactor	Enzyme
Fe^{3+} or Fe^{2+}	Cytochrome oxidase Catalase Peroxidase
Cu^{2+}	Cytochrome oxidase
Zn^{2+}	DNA polymerase Carbonic anhydrase Alcohol dehydrogenase
Mg^{2+}	Hexokinase, glucose 6 phosphatase
Mn^{2+}	Arginase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase

The Common Coenzymes

Coenzymes	Reaction Mediated
Biotin	Carboxylation
Cobalamin (B_{12}) coenzymes	Alkylation
Coenzyme A	Acyl transfer
Flavin coenzymes	Oxidation-reduction
Lipoic acid	Acyl transfer
Nicotinamide coenzymes	Oxidation-reduction
Pyridoxal phosphate	Amino group transfer
Tetrahydrofolate	One carbon group transfer
Thiamin pyrophosphate	Aldehyde transfer

Many organisms are unable to synthesise certain portions of essential cofactors and that must be supplied through diet. Infact, many coenzyme were discovered as growth factors for microorganism or substances that cure nutritional deficiency disease in human and animals., e.g. nicotinamide or nicotinic acid relieves the dietary deficiency disease pellegra in human. Since these co-enzymes are essential for body, so they are termed as vitamins. The vitamins in human diet- that are coenzymes precursor are all water soluble vitamins.

Vitamins that are Coenzyme Precursor

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	∝
Cobalamin (B12)	Cobalamin (B12) Coenzyme	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellegra
Pantothenate	Coenzymes A	∝
Pyridoxin (B_6)	Pyridoxal phosphate	∝
Riboflavin (B2)	Flavin Coenzymes	∝
Thiamin (B1)	Thiamin pyrophosphate	Beriberi

∝ - deficiency in human is rare or unobserved.

Biotin : In 1935, Dutch biochemist Frits Kogl, isolated a growth factor called biotin from egg yolks. In human biotin is involved in many important metabolite pathway, e.g gluconeogenesis, fatty acid synthesis, amino acid catabolism.

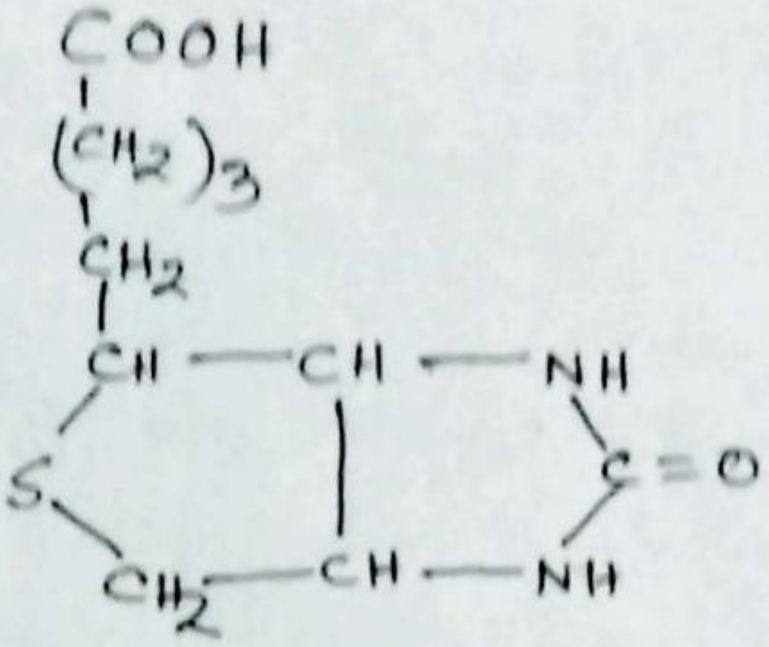
Biotin function as a coenzyme form that helps in transfer of CO_2 to various macromolecules. The enzymes which has got biotin as coenzyme are as following:

Enzyme	Function
i) Pyruvate Carboxylase	Liver gluconeogenesis
ii) Acetyl CoA carboxylase	Lipid synthesis from acetate
iii) Methyl malonyl CoA carboxyl transferase	Propionic acid synthesis
iv) Propionyl CoA carboxylase	Conversion of amino acid and propionate to glucose in liver.

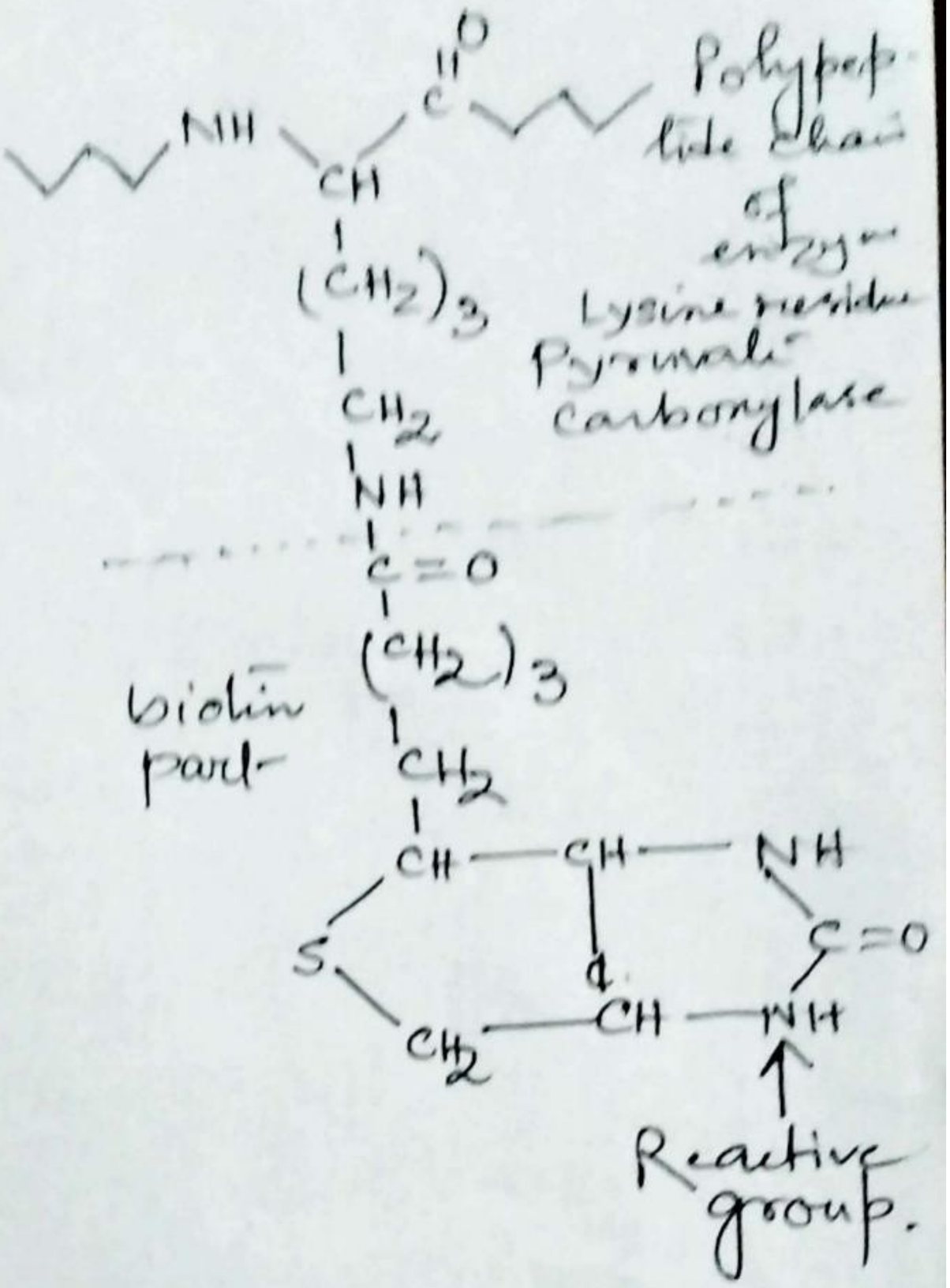
In biotin-dependent enzymes, the biotin molecule is covalently attached to the enzyme protein through an amide linkage with the ϵ-amino group of a specific lysine residue at the enzyme active site. The name <u>biocytin</u> is given to this <u>biotinyl-lysine</u> residue. Biotin is a transient carrier of a carboxy ($-COO^-$) group in a number of enzymatic carboxylation reactions requiring ATP. The carboxy group is transiently attached to a nitrogen atom of the double ring system of biotin. Pyruvate carboxylase is an enzyme which carboxylates pyruvate to yield oxaloacetate

$$CH_3-\overset{O}{\overset{\|}{C}}-COO^- + ATP + HCO_3^- \underset{\text{Carboxylase}}{\overset{\text{Pyruvate}}{\rightleftharpoons}} \text{Oxaloacetate} + ADP + P_i$$

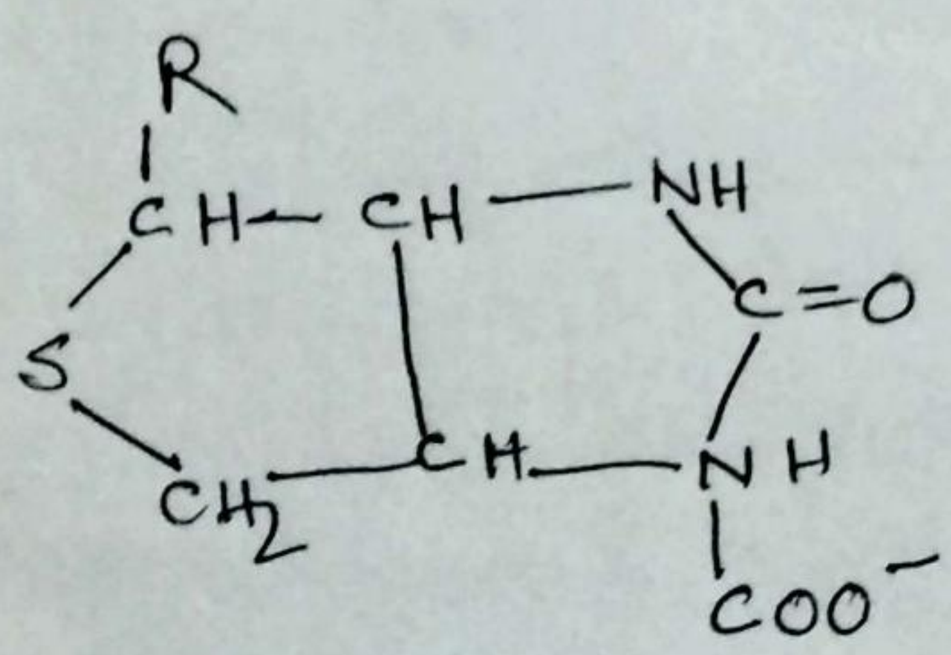
pyruvate



Biotin molecule



During the transfer of $-COO^-$ to pyruvate to yield oxaloacetate, $-COO^-$ group is attached transiently to the Reactive group of biotinyl lysine. (biocytin)



Vitamin B12 or Cobalamin coenzyme (B12)

Vitamin B12 is unique among all the vitamins in that it contains not only a complex organic molecule but also an essential trace element, cobalt. Vitamin B12 is isolated as cyanocobalamin because it contains a cyano group attached to the cobalt. The complex corrin ring system of vitamin B12, to which cobalt is coordinated, is chemically related to the porphyrin ring system of heme and heme proteins. In the coenzyme form of vitamin B12, the cyano group is replaced by 5' deoxyadenosyl group and the coenzyme form is known as 5' deoxyadenosyl cobalamine.

coenzyme function:

Enzymes requiring coenzyme B12 have an ability to carry out the shift of a hydrogen atom from one carbon atom to an adjacent one, or exchange of an alkyl, carboxyl, hydroxyl or amino group.

Three classes of enzymes require B12 for their function.

a) Isomerase, b) Methyl transferase, c) Dehalogenase

Isomerase rearranges hydrogen atom to another adjacent carbon

For. ex. Methylmalonyl Coenzyme A mutase (MUT)

Methylmalonyl CoA $\xrightleftharpoons{MUT}$ Succinyl CoA

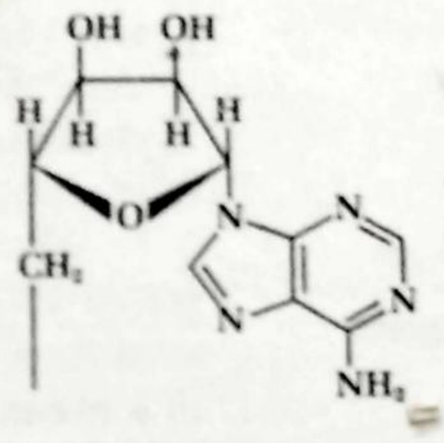
MUT require cobalamin coenzyme for its activity

Methylcobalamin is another coenzyme form of vitamin B12, which participate in enzymatic reactions involving the transfer of methyl group. for ex. Methionine synthase

5 methyltetrahydrofolate + Homocysteine $\xrightleftharpoons[\text{synthase}]{\text{Methion}}$ THF + Methione

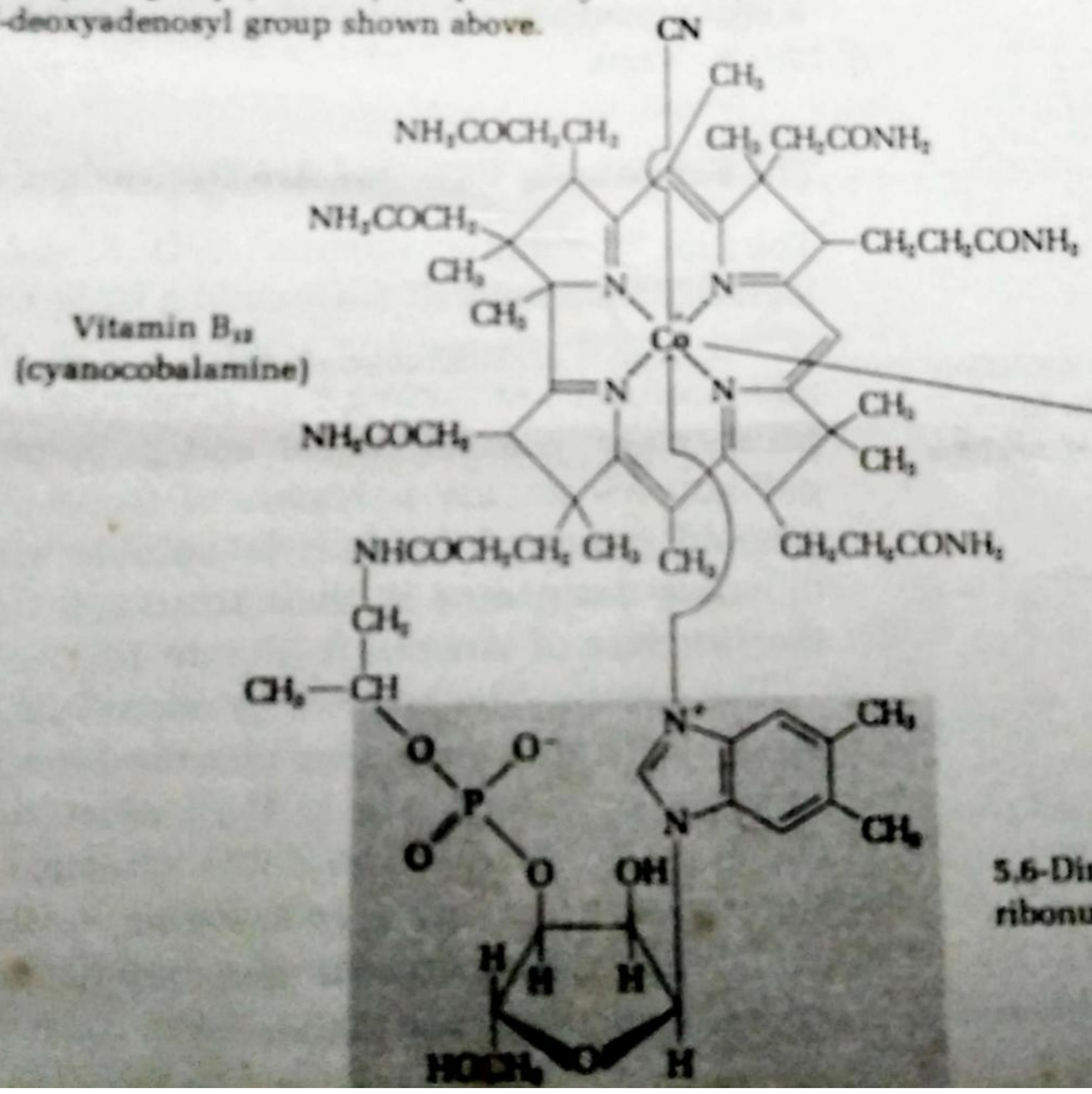
Methionine synthase require methylcobalamin as coenzyme. THF plays an important role in DNA synthesis. Thus megaloblastic anemia or pernicious anemia may result from cobalamin deficiency and it can be reversed if sufficient dietary folate is absorbed from diet.

Figure 10-15
Vitamin B_{12} and its coenzyme form.



5′-Deoxyadenosyl group of coenzyme B_{12}

In the coenzyme form of vitamin B_{12}, which is called adenosyl cobalamine (also coenzyme B_{12}), the cyano group (in color) is replaced by the 5′-deoxyadenosyl group shown above.



Vitamin B_{12} (cyanocobalamine)

The corrin ring system

Reactive site of coenzyme B_{12}

5,6-Dimethylbenzimidazole ribonucleotide