

# Biosynthesis of Essential Amino Acids

## CHEM-643 Intermediary Metabolism

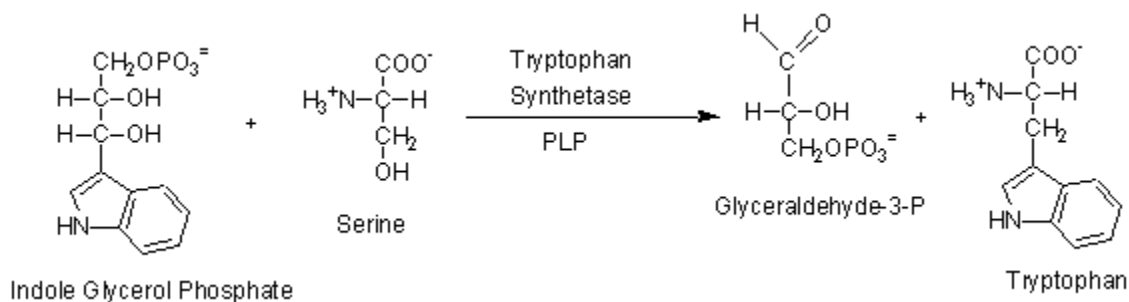
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1. It has been shown in many instances that enzymes catalyzing chemically similar reactions (e.g. malate and lactate dehydrogenases) have amazingly similar tertiary structures which suggests they evolved from a common ancestral protein. Likewise, chemical similarity in sequences of reactions in different metabolic pathways suggest a common evolutionary origin. The sequence of reactions for glutamate synthesis from oxaloacetate and those of leucine synthesis from 2-ketoisovalerate are two such similar pathways.

Write out both pathways so that similar reactions can be compared. By keeping track of the carbons derived from acetyl CoA, show that at least one pair of reactions is not stereochemically homologous.

One set of enzymes having dual substrate specificity accommodates the synthesis of both valine and isoleucine. Assuming that there was a primordial set of enzymes with dual substrate specificity that synthesized both glutamate and leucine, give at least two good reasons for the evolution of two separate sets of enzymes. These must be reasons that do not exist for the valine-isoleucine pathway.

2. The first committed step of isoleucine synthesis is catalyzed by threonine deaminase. This is also the first reaction of threonine catabolism. Bacteria such as *E.coli*. have two isoenzymes of threonine deaminase, one "biosynthetic" and the other "degradative". Due to genetic regulation it is unlikely that significant amounts of both will be present simultaneously. The biosynthetic enzyme is inhibited by isoleucine and the degradative enzyme is activated by AMP [J. Biol. Chem. 248, 512-520(1973)]. Examine the two pathways and provide a rationale for the metabolic regulation indicated above.
3. Tryptophan synthetase catalyses the pyridoxal phosphate-dependent reaction shown below. Enzyme bound indole is an intermediate in this reaction and the enzyme has two non-identical subunits. Propose a reasonable mechanism for the tryptophan synthetase reaction.



4. The intermediates of a biosynthetic pathway must be known before the respective enzymes can be purified and the reaction studied. An extremely useful tool for elucidating metabolic pathways has been mutant bacteria which produce a defective or nonfunctional enzyme. This "genetic block" prevents the normal synthesis of an essential metabolite and thus the mutant must have an exogenous supply of the essential compound in order to grow. Such nutritionally deficient bacteria are auxotrophic.

A series of 8 auxotrophic mutants were isolated for a hypothetical compound X. Extracts of each were made and added to the culture medium of the other seven auxotrophs to see if the requirement for X was eliminated. From the data given below deduce the minimal number of intermediates in the biosynthesis of X and the location in the pathway of the enzyme affected by each mutation. (+ indicates growth, - no growth.)

Auxo troph	Mutant Extract							
	1	2	3	4	5	6	7	8
1	-	+	+	-	+	+	-	-
2	-	-	+	-	-	+	-	-
3	-	-	-	-	-	-	-	-
4	+	+	+	-	+	+	+	-
5	-	+	+	-	-	+	-	-
6	-	-	-	-	-	-	-	-
7	-	+	+	-	+	+	-	-
8	+	+	+	-	+	+	+	-