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The Regulation of Heme Synthesis: Studies on
Erythrocyte Aminolevulinic Acid Synthetase

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(Slide 1) The synthesis of aminolevulinic acid (ALA) is the first in a series of enzymatic reactions leading to the formation of tetrapyrroles and heme. The reactions shown on this slide as well as the final two steps in heme synthesis are localized within mitochondria. This fact has implicated the synthesis of ALA as the site of a feedback control mechanism whereby heme synthesis may be regulated and integrated with the formation of other parts of the hemoglobin molecule. The following studies were performed: (1) to characterize the kinetics of the synthesis of ALA for enzyme obtained from chicken erythrocytes and (2) to identify and ascertain the importance of potential physiological inhibitors in controlling the rate of heme synthesis.

A crude preparation of mitochondria capable of carrying out the reactions shown on this slide was obtained from reticulocytes of anemic chickens. The preparation was free of hemoglobin and enzymes utilizing ALA so that its formation could be followed. Incubations for one hour were performed at pH optimal for ALA synthetase varying the concentration of substrate to permit construction of standard double-reciprocal Lineweaver-Burke plots. In the present studies Succinyl CoA was generated either from α -ketoglutarate or from succinate in the presence of optimal concentrations of cofactors for the reactions, as noted on the slide, and relying upon α -KG oxidase and succinyl CoA synthetase known to be present in mitochondria of the preparation.

(Slide 2) The right-hand portion of this slide demonstrates the standard double-reciprocal plots with α -ketoglutarate concentration on the abscissa and ALA formed, expressed as μ moles per milliliter per hour, on the ordinate for four concen-

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trations of glycine represented by each of the lines. A secondary plot of the reciprocals of the maximal velocities for each of the lines, as obtained where the lines cross the ordinate, and the reciprocal of the respective glycine concentration, gives a Michaelis constant for glycine of 13mM. This constant represents the concentration of glycine yielding half-maximal velocity for ALA formation. (Slide 3)

When these same data are plotted first for the reciprocal of the glycine concentration on the abscissa and reciprocal of ALA formed on the ordinate, a similar family of curves is obtained, each line now representing a different concentration of α KG. A secondary plot of the reciprocal of the maximal velocity for each line against the reciprocal of the α KG concentration yields a Michaelis constant for α KG of 0.21mM.

(Slide 4) When the formation of ALA is followed utilizing glycine and succinate as substrates, another family of curves is obtained. These curves, which I shall not show, are similar to the ones shown on the previous two slides except that succinate would replace α KG. In these reactions the Michaelis constant for glycine is the same for both α KG and succinate as substrates. However, the Michaelis constant for succinate, obtained from a secondary plot, is 1.7mM compared to the 0.21mM for α KG. The eightfold higher Michaelis constant for succinate compared with α KG indicates that succinyl CoA is generated more effectively from α KG than from succinate. This difference can be accounted for in part by the equilibrium between succinyl CoA and succinate which favors the breakdown of succinyl CoA by a factor of 4 times its synthesis.

The reactions synthesizing ALA from glycine and α ketoglutarate or succinate are described by Michaelis-Menton kinetics for a two substrate reaction with sequential addition of substrates but without specifying their order of addition. The synthesis of ALA is dependent upon the presence of divalent ions, either magnesium (Mg^{++}) or

iron (Fe^{++}). It is of interest that increasing iron concentrations above 0.01 mg/ml are attended by a progressive decrease in ALA synthesized.

(Slide 5) The next series of slides are representative of some experiments designed to evaluate the effectiveness of hemoglobin or parts of the hemoglobin molecule in inhibiting or influencing the enzymatic synthesis of ALA. The slides are constructed showing two panels. The standard double-reciprocal plot is shown above, with the reciprocal of ALA formed on the ordinate, the reciprocal of αKG as substrate on the abscissa, and a fixed optimal concentration of glycine. Each line now represents a different concentration of inhibitor, in this case hemoglobin. The progressive shift of the lines upward with increasing hemoglobin concentration and their intersection to the left of the ordinate is indicative of mixed competitive inhibition by hemoglobin. Shown on the lower panel is an inhibitor plot on which the same points are plotted, but in this case the reciprocal of ALA formed against the concentration of inhibitor at different concentrations of αKG . The intersection of these lines gives an inhibitor constant which represents the concentration of inhibitor at which the reaction is half inhibited. This slide indicates that at a concentration of human hemoglobin of about 8 Gm/100 ml, ALA formation is inhibited by 50 per cent. When succinate was used instead of αKG , a similar group of curves were obtained and a similar inhibitor constant. Chicken hemoglobin as inhibitor gave a comparable group of curves for both αKG and succinate, however the inhibitor constant was 1.0 Gm/100 ml indicating it is a six to eightfold better inhibitor than human hemoglobin.

The inhibition by hemoglobin is independent of the oxidation state of iron in heme. Human methemoglobin, with iron in the ferric valence state, gave a similar group of curves as the ones shown here and an inhibitor constant of 8 to 10 Gm/100ml.

(Slide 6) Human globin prepared by removing the heme group gave a similar pattern

of inhibition and inhibitor constant as hemoglobin, which indicates that the globin moiety is primarily responsible for inhibition. Bovine serum albumin in concentrations similar to those shown here for globin produced no inhibition of ALA synthesis indicating that the inhibition observed for globin is not due to non-specific protein inhibition. The next slide (Slide 7) demonstrates that β -globin chains prepared from human hemoglobin also inhibit enzyme activity with an inhibitor constant of 0.9mg/ml, about a hundredfold smaller concentration than for hemoglobin. Human α -globin chains also inhibited enzymatic activity in a manner identical to the plots shown here with an inhibitor constant of 0.7 mg/ml.

In the data I have just presented the pattern of inhibition and inhibitor constants were similar for both α KG and succinate as substrates, indicating that inhibition is probably on ALA synthetase. Inhibition by heme is somewhat different. (Slide 8) This slide demonstrates the effect of heme on ALA synthesis from α KG in the panel above and from succinate in the panel below. At low concentrations of heme, synthesis of ALA from α KG was not inhibited and was perhaps even accelerated, whereas all concentrations were inhibitory for synthesis from succinate. The inhibitor constant for heme was fourfold ^{to fivefold} greater with α KG as substrate than with succinate.

(Slide 9) In summary the present studies have described the kinetics, iron dependency, and inhibition of ALA synthesis in chicken erythrocyte mitochondria. A summary of the various constants are recorded here. Inhibition by hemoglobin, globin, and polypeptide chains appears to be upon ALA synthetase with isolated chains 100 fold more effective inhibitors than the intact globin or hemoglobin molecule. Heme appears to inhibit ALA synthesis from ^{the} succinate pathway fourfold more effectively than from the α KG pathway. From the magnitude of the inhibitor constants alone, expressed in micromolar concentration units, it would appear that isolated globin

peptide chains are the most effective inhibitors, particularly if succinyl CoA is generated more effectively in the reticulocyte from α KG rather than from succinate. Since the incubations were performed with inhibitors bathing the mitochondria in a manner similar to that occurring in the intact erythrocyte, it would appear that the hemoglobin molecule and its parts are capable of regulating the rate of ALA synthesis, and thereby of heme synthesis. Final interpretation of these data will depend upon the demonstration that globin chains and the hemoglobin molecules are accessible to these series of enzymatic reactions synthesizing ALA in the intact reticulocyte.

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