THE BIOLOGICAL UTILIZATION OF GLYCINE FOR THE SYNTHESIS OF THE PROTOPORPHYRIN OF HEMOGLOBIN

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Structural considerations and experimental findings (1–3) suggest that at least two organic precursors are involved in the biological synthesis of the protoporphyrin of hemoglobin. An obvious nitrogenous precursor may be an amino acid but this need not be one having a cyclic structure. In fact, from theoretical considerations there are many objections to the postulation of biological incorporation of some preformed ring into the porphyrin structure.

Evidence has been obtained in this laboratory that the nitrogenous precursor of the protoporphyrin of hemoglobin of the human erythrocyte is glycine (1, 2). As a further study of this subject in man would be expensive, we have used the rat for an investigation of the utilization of isotopic glycine, glutamic acid, proline, leucine, and ammonia for the synthesis of heme. Proline and glutamic acid were selected since it has often been suggested, because of the similarity of their structures to that of the pyrroles, that proline and the anhydride of glutamic acid, pyrrolidonecarboxylic acid, may be precursors of the protoporphyrins (4). Leucine was chosen as a representative \( \alpha \)-amino acid whose intact carbon chain is unlikely to be used for pyrrole synthesis. Ammonia was chosen in order to test the non-specific utilization of nitrogen liberated by deamination of amino acids.

In order to compare the utilization of these compounds for porphyrin synthesis, they were labeled with isotopic nitrogen and fed individually to rats on a protein-free diet. Glycine and ammonia, as ammonium citrate, were fed to rats at a level of 1.33 mM per 100 gm. of body weight per day over a period of 3 days. In the case of the racemic amino acids twice the amount was administered over a period of 3 days, 2.66 mM per 100 gm. of body weight per day. The animals were given a protein-free diet in order to avoid dilutions of varying magnitudes of the test substance which would be caused by amino acids of dietary proteins. The hemin was isolated 2 weeks after the feeding of the isotopic test substances, for it had previously been found in a human experiment that at this time the N\(^{15}\) concentration of the porphyrin was near to its maximum value (2). The isotope concentrations in the compounds fed and in the hemin isolated are given in Columns 2 and 3 of Table I.
Since the N\textsuperscript{15} concentrations of the test compounds were different, the isotopic values obtained in the hemin preparations were calculated on the basis that the compound fed contained 100 per cent N\textsuperscript{15}. This is done to facilitate the comparison of the utilization of the different compounds for porphyrin formation (see Column 4, Table I). These results clearly demonstrate that glycine is by far the most effective source of nitrogen for the synthesis of heme. The values given for dl-leucine and dl-proline are not actually on a comparable basis to those for glycine or ammonia. In the case of these dl-amino acids an equal amount of the labeled d isomer was given along with the l isomer. It is known that a large part of the nitrogen

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Compound fed} & \textbf{N\textsuperscript{15} concentration (1)} & \textbf{Hemin N\textsuperscript{15} concentration} & \textbf{Hemin N\textsuperscript{15} concentration*} \\
& \textit{atom per cent excess} & \textit{atom per cent excess} & \textit{atom per cent excess} \\
\hline
Glycine & 11.6 & 0.108 & 0.93 \\
\text{Ammonium citrate} & 19.0 & 0.169 & 0.89 \\
\text{dl-Glutamic acid} & 13.0 & 0.012 & 0.09 \\
\text{dl-Proline} & 18.6 & 0.032 & 0.17 \\
\text{dl-Leucine} & 11.6 & 0.031 & 0.27\textsuperscript{t} (0.18) \\
\text{dl-Proline} & 11.6 & 0.028 & 0.24\textsuperscript{t} (0.15) \\
\text{dl-Leucine} & 32.7 & 0.051 & 0.16\textsuperscript{t} (0.07) \\
\hline
\end{tabular}
\caption{Comparison of N\textsuperscript{15} Concentration in Hemin after Feeding Isotopic Compounds}
\end{table}

* Calculated on the basis that the compound fed contained 100 per cent N\textsuperscript{15}, 
N\textsuperscript{15} concentration in hemin (Column 3) \times 100 = Column 4 
N\textsuperscript{15} concentration of compound fed (Column 2) 
\textsuperscript{t} To correct for the d isomers 0.09 per cent should be subtracted from these values. Corrected values would be 0.18, 0.15, and 0.07 per cent respectively. See the text for explanation.

of these unnatural isomers is liberated, probably in the form of ammonia. In these cases we were thus feeding not only the labeled l-amino acid but also, in potential form, an equivalent amount of labeled ammonia. To bring the values of these compounds to a comparable basis with ammonia and glycine one should subtract 0.09 atom per cent excess N\textsuperscript{15}, the isotope concentration found in the hemin after the feeding of ammonia, from the values given in Column 4. The corrected isotope concentration in the hemin is therefore only 0.18 and 0.15 per cent for the two proline feedings and 0.07 per cent for the leucine feeding. These considerations do not apply to the experiment in which dl-glutamic acid was administered, for it is known that d-glutamic acid is not metabolized, but largely excreted (5).

The isotope concentrations found in the porphyrin after the feeding of ammonia, glutamic acid, proline, and leucine are therefore not significantly
different from each other and about one-thirteenth to one-fifth of that found after the feeding of glycine. The isotope concentration found in the hemin after the feeding of these compounds represents values one would expect to find after the feeding of a non-specific source of isotopic nitrogen which would only enrich the $N^{15}$ concentration of the body nitrogen from which the precursor of heme is synthesized. The slightly higher values found after the feeding of proline and glutamic acid may be the result of a more direct utilization of the $l$ isomers of these two acids for the formation of glycine than of leucine or ammonia. An indication of this difference has been obtained (6).

\[
\begin{align*}
R & \quad \text{CH}_3 \\
\text{C} \quad \text{C} & \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{C} \quad \text{H} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{N} \\
\text{N} & \quad \text{H} \\
\end{align*}
\]

Enol of $\beta$-ketoaldehyde + glycine

\[
\begin{align*}
R & \quad \text{CH}_3 \\
\text{C} \quad \text{C} & \quad \text{H} \\
\text{H} & \quad \text{C} \quad \text{H} \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{N} \\
\end{align*}
\]

The results indicate that the nitrogen of glycine is directly employed for the synthesis of the protoporphyrin of hemoglobin, while the nitrogen of the other compounds is used indirectly, presumably by way of glycine. While the above findings prove that the nitrogen of glycine is utilized for the synthesis of a porphyrin, there seems little doubt that this conversion involves the $\alpha$-carbon atom of glycine and the carboxyl carbon as well.

As glycine contains but 2 carbon atoms, other compounds probably participate with it to form the pyrrole ring. It has previously been shown that the feeding of sodium deuterioacetate to rats resulted in the formation of deuteriohemin (3). This finding showed only that some of the carbon atoms of the side chains of heme are derived from acetate, for none of the carbon atoms of the pyrrole rings is bonded to hydrogen. The feeding of compounds labeled with deuterium will therefore furnish only indirect evidence for the participation of these compounds in pyrrole ring formation.

Fischer and Fink (7) have recently shown that formylacetone and glycine readily condense to yield a product which gave the positive Ehrlich
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color reaction for pyrroles. Since the product was not isolated, its structure is unknown. While insufficient data are yet available to permit definite formulation of the reactions by which porphyrins are biologically synthesized, it is possible that the synthesis involves the condensation of glycine with a β-ketoaldehyde formed in part at least from acetic acid. The reaction may occur as shown in the accompanying diagram. According to this formulation the α-carbon atom of the pyrrole rings and the carbon atoms of the methine bridges are derived from glycine.

EXPERIMENTAL

Isotopic Nitrogen Compounds—Glycine, dl-glutamic acid, dl-leucine, and ammonium citrate containing N\textsuperscript{15} were synthesized by the methods previously described (8). The dl-proline was synthesized from isotopic potassium phthalimide and trimethylene bromide by the method of Sorensen and Andersen (9).

Feeding Experiment—A group of rats (220 to 280 gm.) was given the following diet: starch 83 per cent, yeast 5 per cent, salt mixture 4 per cent (10), cottonseed oil (Wesson oil) 6 per cent, and cod liver oil 2 per cent. After the rats were kept on this diet for 2 days, each test substance was incorporated into the diet of at least two animals for 3 days. The rats were then kept on the basal diet for the next 2 weeks. 1.33 mM of glycine and ammonia and 2.66 μM of dl-glutamic acid, dl-proline, and dl-leucine per 100 gm. of body weight were given over a period of 3 days.

Isolation of Hemin—The rats were killed by exsanguination. The oxidized blood was centrifuged and the red cells, after being washed twice with physiological saline, were hemolyzed with an equal volume of water. The hemoglobin solution was added dropwise over a period of 20 minutes to 3 volumes of acetic acid containing about 0.5 cc. of a saturated sodium chloride solution at 95–100°. After the addition of the hemoglobin solution, the mixture was kept on a steam bath for 1 hour. The hemin crystals were centrifuged, washed twice with a 50 per cent acetic acid solution, twice with water, twice with alcohol, and finally with ether (11).

SUMMARY

1. Glycine has been shown to be a nitrogenous precursor of the protoporphyin of hemoglobin in the rat.

2. The finding of N\textsuperscript{15} in heme after the feeding of isotopic proline, glutamic acid, leucine, and ammonia is due to the N\textsuperscript{15} enrichment, by the nitrogen of these compounds, of the body nitrogen from which the precursor of heme is synthesized rather than to a direct utilization of these compounds.

3. The biological formation of the porphyrin structure is briefly discussed.
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