CONTROL OF HEME SYNTHESIS BY FEEDBACK INHIBITION

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Received November 18, 1964

The capacity of immature erythroid cells to synthesize heme in vitro (London et al, 1950, Shemin et al, 1950, Morell et al, 1958) provides an opportunity for the study of the processes involved in the regulation of this biosynthetic mechanism in animal cells. Burnham and Lascelles (1962) have shown that preparations of deltaaminolevulinic acid (ALA) synthetase from Rhodopseudomonas spheroides are inhibited by hemin. This enzyme catalyzes the condensation of glycine and succinyl-coenzyme A to form deltaaminolevulinic acid (Shemin, 1954). The enzyme ALA dehydrase which catalyzes the condensation of ALA to form porphobilinogen (PBG) was less inhibited. With intact cells of this bacterium, however, inhibition of porphyrin synthesis by hemin was observed when glycine, but not ALA, was the precursor. These findings pointed to ALA synthetase as the site for negative feedback control of porphyrin synthesis by hemin in this bacterium.

In the studies reported here the effects of hemin on the incorporation of glycine-2-C^{14} and ALA-4-C^{14} into heme in immature rabbit erythrocytes (reticulocytes) have been examined. The results suggest that a similar control mechanism, involving feedback inhibition in the conversion of glycine to ALA, is

operative in these animal cells.

Methods: Reticulocyte Preparations. New Zealand rabbits were bled repeatedly (about 30 ml daily for at least 3 days) and reticulocytes were obtained in the top 1-3 ml of packed cells following centrifugation. More prolonged series of bleeding produced reticulocytoses of 15-20%. One ml of such cell preparations plus one ml plasma were added to 8 ml of a saline solution containing the following: 0.76% NaCl, 0.039% KCl, 0.15% $MgCl_2.6H_2O$, 100 mg% glucose, 0.02 M Tris-HCl buffer, pH 7.8 or K_2HPO_4 - KH_2PO_4 buffer, pH 7.4. When a lysate was required, equal volumes of cells and cold distilled water were mixed for 1 minute, the tonicity was adjusted with 10-fold concentrated saline solution and plasma and the saline solution were added as for whole cell suspensions.

Conditions of incubation. The incubation mixture in 12 ml conical tubes included: (1) 1 ml aliquots of cell suspension or lysate; (2) 0.12 ml of hemin solution[1]; (3) penicillin and streptomycin 0.8 to 1.0 mg per ml; (4) glycine-2-C^{14}, 0.25 umole of 8 x 10^5 cpm/umole or ALA-4-C^{14}, 0.5 umole of 4 x 10^5 cpm/umole; (5) saline solution to give total volume of 1.2 to 1.6 ml. In control samples, 0.12 ml of the KOH-buffer-HCl solution without hemin was added. The tubes were incubated in a 37° water bath in room air or in 95% O_2-5% CO_2. At the end of incubation, they were chilled in ice,

1. Hemin solution: (a) 1.2 mg hemin was dissolved in 0.1 ml 1N KOH and diluted with 1.0 ml freshly boiled distilled H_2O and 0.1 ml 1M tris or phosphate buffer; 0.08 ml 1N HCl was added with mixing and the volume was made up to 2 ml with H_2O; (b) for higher concentrations, 8 mg hemin was dissolved in twice the above volumes of KOH, buffer and HCl and the volume was again made up to 2 ml with H_2O. The final concentration of hemin when 0.12 ml of solution (a) was present in an incubation mixture of 1.2 ml was 1 x 10^{-4} M.

and 10 ml of cold red cell lysate (1 vol packed cells and 1 vol H_2O) was added to provide unlabeled heme as carrier. Hemin in solution, equal in amount to that which had been added to the experimental samples, was added to the control samples.

Hemin was crystallized from the hemolysate after treatment with glacial acetic acid (Fischer, 1941). It was recrystallized from pyridine-chloroform, and its radioactivity was measured at infinite thickness. Nearly all experiments were carried out in duplicate with agreement within ± 5 per cent.

Table I

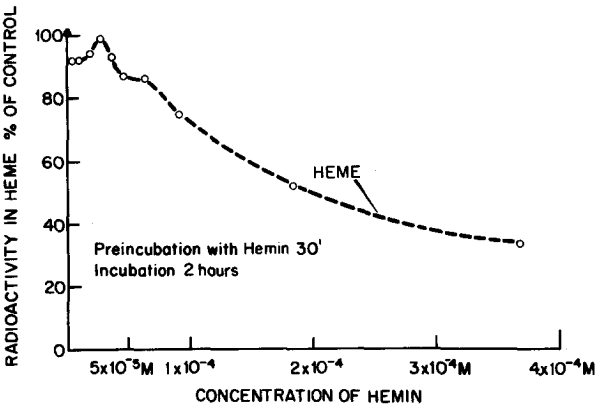
Effect of Hemin on Utilization of Glycine-2-C^{14} and ALA-4-C^{14} for Heme Synthesis in Intact and Lysed Rabbit Reticulocytes

	Incorporation of Glycine-2-C^{14} into Heme	Incorporation of ALA-4-C^{14} into Heme
	% Change from Control	% Change from Control
Number of experiments	42	21
Mean change	-48.6	-12.2
S.E.	± 3.00	± 4.84
	$p < .001$	$.05 > p > .02$

Results: Table I presents the effects of added hemin on the utilization of glycine-2-C^{14} and ALA-4-C^{14} for heme synthesis by intact and lysed rabbit reticulocytes. The data are recorded as the percentage change from the control values obtained in preparations to which no hemin was added. At this concentration (1×10^{-4}M)

the effect of hemin on the utilization of glycine-2-C^{14} was inhibitory in all but one of the 42 experiments. The mean effect was an inhibition of nearly 50 per cent and is highly significant. The mean effect of hemin on the utilization of ALA-4-C^{14} was an inhibition of only 12 per cent and was of only marginal statistical significance. The effects of added hemin on utilization of ALA were variable and in several instances enhanced synthesis of heme was observed.

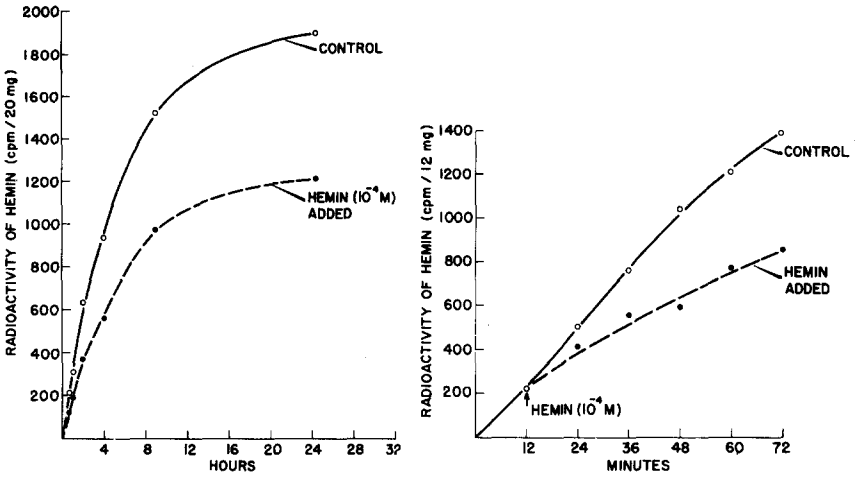
Figure 1 shows the effect of concentration of added hemin on the synthesis of heme from glycine in intact rabbit reticulocytes.



1. The effect of various concentrations of added hemin on the utilization of glycine-2-C^{14} for heme synthesis.

Inhibition is appreciable at 1 x 10^{-4} M and is progressively increased at higher concentrations.

The effects of hemin on the synthesis of heme in a long-term incubation and in a short-term incubation are presented in Figure 2. The inhibitory effect occurs rapidly and is demonstrable throughout a 24-hour period of incubation.



2. Inhibitory effect of hemin on utilization of glycine-2-C^{14} for heme synthesis.

The synthesis of heme with and without added hemin was studied in isotonic phosphate buffer in the range of pH 6.35 to 7.90 and in Tris-NaCl buffer, pH 7.25 to 8.70. Maximal synthesis was observed at pH 7.3-7.4 in the absence of added hemin. The inhibitory effect of hemin was observed over the full range of pH which was studied.

Discussion: The results of these studies indicate significant inhibition by hemin of the utilization of glycine for heme synthesis in rabbit reticulocytes; the effect on the utilization of ALA was variable and less significant. The principal site of inhibition by hemin appears therefore to be found among the reactions which precede the formation of ALA:

$$\text{Succinyl-CoA + Glycine} \xrightarrow[\text{Pyridoxal Phosphate}]{\text{ALA Synthetase}} \underset{\text{adipic acid (AKA)}}{\alpha\text{-amino } \beta\text{-Keto}} \xrightarrow{-CO_2} \text{ALA}$$

These reactions include the activation of succinate, the activation of glycine, probably by a pyridoxal phosphate system; the condensation of activated glycine and succinate, catalyzed by ALA synthetase, to form AKA, and the decarboxylation of AKA to form ALA. It is possible that hemin may inhibit the activation of succinate or glycine, but no information is available on these possibilities at present. The decarboxylation of AKA occurs very readily; it seems unlikely, but it is possible, that hemin may influence the conversion of AKA to ALA. In the light of the studies of Burnham and Lascelles in Rhodopseudomonas spheroides, it seems most likely that a site of feedback control of heme synthesis in the rabbit reticulocyte is at the level of ALA synthetase.

The concentration of hemin within the reticulocyte at which the inhibitory effect is observed is unknown, since the extent to which the hemin can penetrate the cell and the extent of its binding within the cell are not known. It is relevant to note that free protoporphyrin, which is present in reticulocytes in a concentration of the order of 10^{-4}M, can inhibit porphyrin synthesis at this concentration in Rhodopseudomonas spheroides.

Negative feedback control of heme synthesis by heme affords a mechanism for the regulation of porphyrin synthesis. Coupled with the stimulatory effect of hemin on the synthesis of globin (Bruns and London), this control mechanism may participate in the coordination of the synthesis of heme and of globin.

This work was supported by USPHS grant HE-02803 and a grant of the Office of Naval Research Nonr 4094.

REFERENCES

Bruns, G.P. and London, I.M. (accompanying paper)
Burnham, B.F. and Lascelles, J., Biochem. J. 87, 462 (1963)
Fischer, H., Org. Syntheses 21, 53 (1941)
London, I.M., Shemin, D. and Rittenberg, D., J. Biol. Chem. 183, 749 (1950)
Morell, H., Savoie, J.C. and London, I.M., J. Biol. Chem. 233, 923 (1958)
Shemin, D., The Harvey Lectures, Series 50 (1954-1955) (Academic Press Inc., New York, 1956) p. 258
Shemin, D., London, I.M. and Rittenberg, D., J. Biol. Chem. 183, 757 (1950)