Hemin: An Inhibitor of Erythroid Cell Ribonuclease

Abstract. *Hemin, in concentrations which stimulate protein synthesis and stabilize polyribosomes in reticulocytes, is a potent inhibitor of ribonuclease activity in the erythroid cell. This may offer a partial explanation for the way in which hemin acts to accelerate globin synthesis and thus synchronize the synthesis of hemin and globin in the maturing erythroid cell.*

Hemin stimulates the synthesis of globin and promotes and stabilizes the formation of polyribosomes in the intact reticulocyte in vitro (*1, 2*) and in lysate systems from reticulocytes (*3*). This action may be of importance in maintaining the close synchrony of heme and globin production in the maturing erythroid cell (*4*). The exact mechanism by which this stimulatory effect occurs is unknown, but it has been suggested that hemin accelerates the rate of initiation of peptide chains (*2, 5*). The similarity between the reticulocyte lysate system (*3, 6*) and an assay system developed in this laboratory for the study of erythroid cell ribonuclease suggested that hemin-induced moderation of protein synthesis might be related to changes in ribonuclease activity. Investigation has confirmed that hemin, in concentrations which stimulate globin synthesis in reticulocytes, is a potent inhibitor of ribonuclease activity in the erythroid cell.

Ribonuclease activity was assayed in hemolyzates of rabbit reticulocytes prepared by lysis with four volumes of 20 milliosmolar phosphate buffer, *p*H 7.4 (*7*). Activity was present in whole cell hemolyzates and in membrane-free hemolyzates. The substrate was rabbit reticulocyte RNA uniformly labeled in vivo with ^{32}P (*7*). The quantity of acid-soluble ^{32}P was determined at the beginning and end of a 4-hour incubation at 37°C. This system differs from reticulocyte lysate systems which incorporate amino acids into globin only by the absence of energy-generating reagents (*3, 6*). Under these conditions, the ribonuclease activity followed Michaelis-Menten kinetics and was dependent upon both the concentration of the enzyme and the concentration of the substrate (*8*). The hemin solution ($4 \times 10^{-3} M$) was prepared as previously described (*2*).

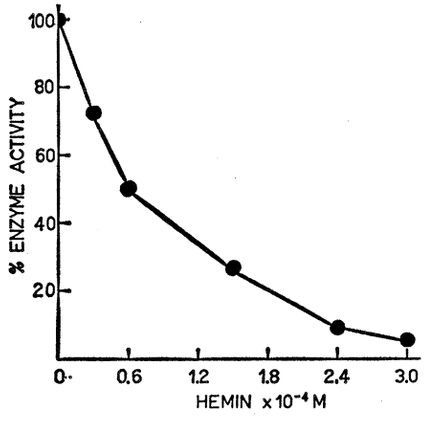

Fig. 1. Effect of hemin on ribonuclease activity in the erythroid cell. The assay system contained 1 ml of a 1 to 4 dilution of reticulocyte hemolyzate and 0.07 mg of ^{32}P-labeled reticulocyte RNA (5.73×10^4 count min^{-1} mg^{-1}). During the 4-hour incubation, 59.6 percent of the substrate was destroyed in the control assay.

The effect of hemin, in concentrations which maximally stimulate heme synthesis in reticulocytes (*2, 3*), on ribonuclease activity in the erythroid cell is shown in Fig. 1. The presence of hemin inhibited ribonuclease activity in a concentration-dependent fashion. Hemin also inhibited the activity of pancreatic ribonuclease, but to a lesser extent. Hemin ($3 \times 10^{-4} M$) reduced the activity of pancreatic ribonuclease (10 μg/ml) against rabbit reticulocyte RNA to 34 percent of control values.

Other substances reported to influence globin synthesis and polyribosome stability were also examined for their ability to alter ribonuclease activity in the erythroid cell (Table 1). Ferrous ions, which stimulate globin synthesis in reticulocytes (*2, 9*), did not inhibit ribonuclease activity in the reticulocyte. Cobaltous ions, which have an effect qualitatively similar to hemin but lesser in degree (*2*), were moderately effective in inhibiting ribonuclease activity. Bipyridine, an iron chelating agent which induces breakdown of polyribosomes (*9*), had no effect on ribonuclease activity. The presence of bipyridine in the incubation mixture did not prevent the heme-mediated inhibition of ribonuclease activity. Lead, which appears to inhibit globin synthesis and induce breakdown of polyribosomes by two separate mechanisms (*9*), was a mild inhibitor of ribonuclease activity at 10^{-3} molar concentration.

In attempting to explain the action of hemin in stimulating globin synthesis in reticulocytes, investigators have stressed acceleration at the translational level, but have made only passing mention of the possibility that an inhibitor of ribosomal aggregation might be affected (*2*). The present data indicate that hemin, and, to a lesser degree, cobaltous ions, inhibit ribonuclease activity against a natural substrate. These findings offer a plausible explanation for the effect of hemin in prolonging the period of protein synthesis in the reticulocyte lysate system and in cell-free systems of avian erythrocyte nuclei without changing the rate of protein synthesis (*3, 10*). The effect on ribonuclease activity may not, of course, be the sole mechanism by which these substances act to stimulate protein synthesis in reticulocytes, but alteration of ribonuclease activity by free hemin may be responsible, at least in part, for synchronizing production of hemin and globin in the maturing erythroid cell. The experimental findings raise the more general question of the mode of action of other substances, such as RNA, reported to stimulate protein synthesis in reticulocyte systems (*11*).

Table 1. Inhibition of erythroid cell ribonuclease activity. Conditions of incubation were as described for Fig. 1.

Addition	Concentration (*M*)	Ribonuclease activity (%)
None		100
Hemin	0.3×10^{-4}	72
	3.0×10^{-4}	7
$CoCl_2$	10^{-4}	91
	10^{-3}	61
$Fe(NH_4)_2(SO_4)_2$	10^{-4}	96
	10^{-3}	98
$Pb(CH_3COO)_2$	10^{-4}	99
	10^{-3}	77
Bipyridine	3×10^{-4}	101

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