FORMATION OF A RIBOSOMAL LESION IN RABBIT RETICULOCYTES BY THE LYSINE ANTAGONIST, S-(β-AMINOETHYL) CYSTEINE

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Received December 18, 1961

Most amino acid antimetabolites have been found to enter complete protein molecules rather than to interfere with their synthesis (Vaughan and Steinberg, 1959). However, a valine analogue, α-amino-β-chlorobutyric acid, has been shown to block hemoglobin formation by rabbit reticulocytes and to cause an accumulation of what appears to be precursor protein on the ribosomes (Rabinovitz and McGrath, 1959). These results suggested a possible unique role of valine in hemoglobin completion or release from the ribosomes. We have now found that an antagonist of lysine, S-(β-aminoethyl) cysteine, causes a similar interruption. This report is concerned with a few of the characteristics of this inhibition.

S-(β-Aminoethyl)-L-cysteine hydrochloride was synthesized by a modification of the method of Cavallini et al. (1955). It was a very potent competitive inhibitor for the incorporation of lysine into total reticulocyte protein in vitro, as had been found with rat bone marrow cells (Rabinovitz and Tuve, 1959). Current studies have shown that when a radioactive amino acid is added to cells treated with the inhibitor, a marked increase in labeled protein associated with the ribosomes occurs, together with a decrease in the synthesis of hemoglobin. The effect of various concentrations of S-(β-aminoethyl) cysteine on the incorporation of histidine is shown in Figure 1. The limiting level of inhibition of hemoglobin synthesis observed with increasing concentrations of inhibitor was also found when valine was the amino acid incorporated.

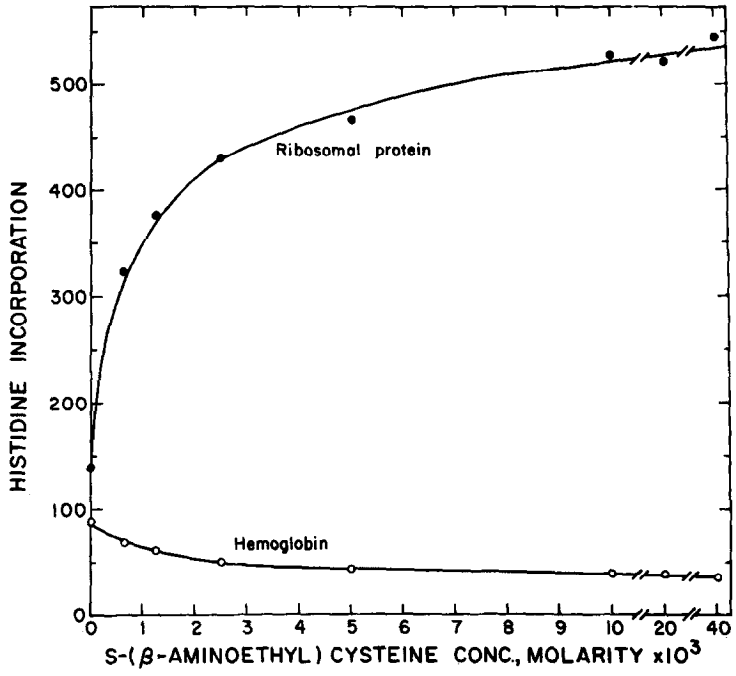

Fig. 1. Effect of Concentration of S-(β-Aminoethyl) cysteine on Incorporation of Histidine into Reticulocyte Ribosomal Protein and Hemoglobin.

One ml. of packed cells was incubated with inhibitor for 20 minutes prior to addition of L-histidine-2(ring)-C^{14} (8.2×10^4 c.p.m. in 50 mμ moles) in 4.0 ml. total volume. The incubation with radioactive amino acid was for 15 minutes. Incubation conditions, procedures for isolation of cell fractions by differential centrifugation and preparation of proteins were as previously described (Rabinovitz and Olson, 1959; Rabinovitz and McGrath, 1959) except that the ribosomes were washed with medium A of Keller and Zamecnik (1956) prior to preparation of protein for counting. Hemoglobin was isolated from the 100,000 x g. supernatant by column chromatography (Rabinovitz and Olson, 1959). Incorporation is expressed as counts per minute per milligram protein.

Lysine prevented the accumulation of labeled protein on the ribosome (Figure 2). A lower ratio of lysine to inhibitor concentration was needed to completely prevent inhibition of hemoglobin synthesis than to prevent the increased labeling of ribosomal protein.

The results may be interpreted in terms of the substitution of S-(β-aminoethyl) cysteine for lysine in protein precursors of hemoglobin. This alteration in composition of the intermediate may interfere with the conformation of the α or β chains of globin, their association, or the release of hemoglobin from the ribosome.

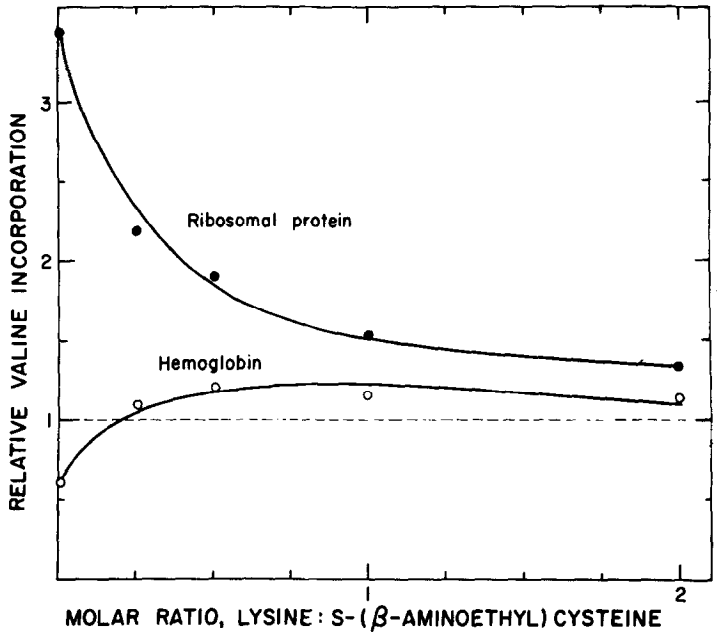

Fig. 2. Prevention by Lysine of both S-(β-Aminoethyl) cysteine Inhibition of Hemoglobin Synthesis and the Associated Accumulation of Newly Formed Protein on Reticulocyte Ribosomes.

Conditions of incubation were as described in Figure 1. The cells were incubated with L-lysine and inhibitor (0.01 M) for 20 minutes before addition of DL-valine-1-C^{14} (1.4×10^6 c.p.m. in 2 μ moles). The specific activity of the uninhibited controls were as follows: ribosomal protein, 280 c.p.m. per mg. protein; hemoglobin, 88 c.p.m. per mg. protein. Relative incorporation refers to the specific activity of the sample with that of the corresponding uninhibited control taken as unity.

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