TRANSFER RNA CONTENT AND DISSOCIABILITY OF MOUSE LIVER MONOSOMES PRODUCED BY HISTIDINOL

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SUMMARY

A single injection of the amino acid analog L-histidinol dihydrochloride reduced [^{3}H]-leucine incorporation into protein in vivo and partially disaggregated mouse liver polysomes into monosomes within 30 minutes. Isolated monosomes dissociated into 40S and 60S subunits during centrifugation in linear sucrose gradients containing 0.3 M KCl and had a 4S to 5S molar RNA ratio of 0.7, indicating 0.7 tRNA and/or aminoacyl tRNA molecule per ribosome. No peptidyl tRNA was present. These results suggest that a single injection of histidinol rapidly produces monosomes which resemble runoff ribosomes.

Histidinol is an amino acid analog, which reversibly inhibits protein synthesis in vitro (1). Balkow and Rabinovitz demonstrated that administration of histidinol to reticulocyte lysates results in a stabilization of polysomes (2), presumably because the decreased translation of histidine codons limits the rate of elongation. Vaughan and Hansen (3) demonstrated that histidinol competitively inhibits the charging of $tRNA^{His}$ and causes polysome disaggregation in HeLa cells. In these cells they described a decreased elongation rate but in addition a stronger effect on initiation of translation so that the net effect was to decrease the average size and abundance of polysomes. Thus, histidinol has been demonstrated to inhibit protein synthesis in two in vitro systems by a mechanism involving uncharged $tRNA^{His}$.

We investigated the effect of a single intraperitoneal injection of histidinol on mouse liver and found that histidinol inhibits protein synthesis and partially disaggregates polysomes. In order to further elucidate the mechanism by which histidinol disaggregates mouse liver polysomes, the dissociability and transfer RNA content of the monosomes produced by this analog were

studied. We have previously shown that these parameters can determine whether monosomes are complexed, runoff, or falloff ribosomes (4-7).

METHODS

Inhibition of Protein Synthesis. Male Swiss Webster albino mice weighing 25 to 30 grams were given a single intraperitoneal (i.p.) injection of 1.0 gm L-histidinol dihydrochloride (Sigma Chemical Co.) per kg body weight, dissolved in 0.15 ml of distilled water. Control mice were given a single i.p. injection of 0.15 ml of a 4% NaCl solution, which is approximately the same osmolality, 1200 mOsM, and volume as the histidinol solution.

Thirty minutes later experimental and control mice were injected i.p. with 25 μCi per 100 gm body weight of [^{3}H]-leucine (New England Nuclear Corp., specific activity, 45 curies/mmole). Twenty minutes later the animals were sacrificed and trichloracetic acid (TCA) precipitable protein was prepared from one gram samples of liver as previously described (8), except that the hot TCA-insoluble residue was twice solubilized in 0.5 N NaOH and reprecipitated with 10% TCA. The final TCA-insoluble residue was solubilized in 5.0 ml of 0.5 N NaOH and 0.2 ml of this solution was suspended in 15 ml Aquasol (New England Nuclear Corp.). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. Counting efficiency was approximately 14% as determined by the channels ratio method (9). Protein content was determined by the Lowry method (10).

Disaggregation of Polysomes. Mice were given a single i.p. injection of histidinol, in doses ranging from 0.01 to 1.00 gm per kg body weight, dissolved in 0.15 ml of distilled water. Control mice were given a single i.p. injection of 0.15 ml of a 4% NaCl solution. Thirty minutes later the animals were sacrificed, livers were removed, and ribosome pellets were prepared as previously described (5). The pellets were resuspended in TKM buffer containing 10 mM Tris-HCl (pH 7.1), 25 mM KCl, and 5 mM $MgSO_4$ and were centrifuged for 90 minutes at 114,000 x g in 0.5 to 1.2 M linear sucrose gradients. The gradients

were then scanned at 260 nm in a Gilford spectrophotometer equipped with a flow cell.

Dissociation of Monosomes. Ribosome pellets were prepared, resuspended in TKM buffer containing 0.3 M KCl, centrifuged for 90 minutes at 114,000 x g in 0.5 to 1.2 M linear sucrose gradients containing 0.3 M KCl TKM buffer, and scanned at 260 nm as described above. We have previously shown that runoff and falloff ribosomes dissociate into 40S and 60S ribosome subunits in 0.3 M KCl, whereas complexed ribosomes do not dissociate (4-7).

Transfer RNA Content. The transfer RNA content of isolated histidinol monosomes was determined as previously described (7). This determination is based upon the theory that since each ribosome contains one 5S rRNA molecule, the number of 4S tRNA molecules per ribosome can be estimated by calculating the 4S to 5S molar RNA ratio, with and without prior treatment with Pronase (5). Incubation with Pronase prior to RNA extraction removes nascent peptides from peptidyl tRNA and permits extraction of all tRNA (from tRNA, aminoacyl tRNA, and peptidyl tRNA). Extraction of RNA with no prior Pronase incubation, permits only the extraction of tRNA originally present as tRNA and aminoacyl tRNA.

Table I. EFFECT OF HISTIDINOL ON THE SYNTHESIS OF PROTEIN IN MOUSE LIVER

	dpm per mg protein	% inhibition of protein synthesis
1. Control mice (2)	2210, 2075	--
2. Histidinol mice (2)	600, 660	70

Two control mice were injected i.p. with 0.15 ml of a 4% NaCl solution or two histidinol mice were injected i.p. with 1 gm of L-histidinol dihydrochloride per kg body weight. Thirty minutes later [^{3}H]-leucine (25 µCi per 100 gm body weight) was injected i.p. into both groups of mice. The animals were sacrificed 20 minutes later and the amount of [^{3}H]-leucine incorporated into liver protein determined.

RESULTS

Inhibition of Protein Synthesis. From Table I it can be seen that a single injection of 1 gm histidinol per kg body weight reduced the incorporation of [^{3}H]-leucine into TCA precipitable protein by 70%.

Disaggregation of Polysomes. Figure 1A demonstrates the polysome profile from a normal (untreated) mouse liver. The dotted line in Figure 1B shows the hepatic polysome profile from a mouse injected with 1.00 gm histidinol per kg body weight. A prominent breakdown of polysomes with an increase in the size

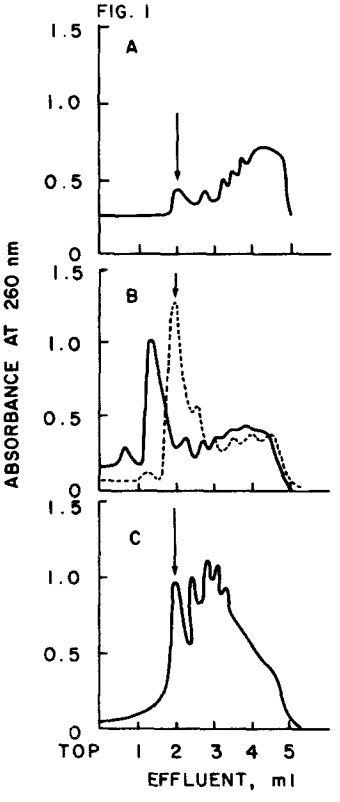

Figure 1. Ribosome-polysome profiles of mouse livers in 0.5 to 1.2 M linear sucrose gradients. The arrow indicates the position of the 80S peak. A. Normal ribosome-polysome profile; B. 30 minutes after a single i.p. injection of 1.00 gm L-histidinol dihydrochloride per kg body weight, ---------- 25 mM KCl treatment, ——— 0.3 M KCl treatment; C. 30 minutes after a single i.p. injection of 0.15 ml of a 4% NaCl solution (control), 25 mM KCl treatment.

of the 80S peak can be seen. A similar disaggregation of polysomes was observed within 30 minutes after a single intraperitoneal injection of doses varying from 0.01 to 1.00 gm histidinol per kg body weight; whereas a dose of 0.001 gm histidinol per kg body weight had no observable effect on the polysome profile. Figure 1C, the profile of a control mouse injected with 0.15 ml of a 4% NaCl solution, shows the presence of trimers, tetramers, and larger polysome aggregates without a prominent 80S peak.

Dissociation of Monosomes. The solid line in Figure 1B demonstrates that the 80S monosomes produced by injection of 1.00 gm histidinol per kg body weight dissociated into 40S and 60S subunits in linear sucrose gradients containing 0.3 M KCl. This indicates that the monosomes produced by histidinol dissociate into subunits as do monosomes which are free of messenger RNA (4-7).

Transfer RNA Content. From Table II, line 1, it can be seen that polysomes contain 0.9 molecule of tRNA and/or aminoacyl tRNA and 0.8 molecule of peptidyl

Table II. TRANSFER RNA CONTENT OF RIBOSOMES

Type of ribosome	4S:5S ratio with Pronase	4S:5S ratio without Pronase
(1) Normal polysomal ribosomes	1.7	0.9
(2) Control ribosomes (4% NaCl solution)	1.7	0.9
(3) Starvation monosomes	0.7	0.7
(4) Histidinol monosomes	0.7	0.7

Ribosome pellets were resuspended in 5 mM Tris-HCl (pH 7.4) and RNA was extracted immediately or the suspension was incubated for 1 hour at 30° C with 3 mg per ml Pronase and 4 mg per ml sodium dodecyl sulfate prior to extraction. RNA was precipitated overnight and analyzed by electrophoresis in 4% polyacrylamide gels, as previously described (5). Gels were scanned at 260 nm and the 4S to 5S molar RNA ratio was calculated by multiplying the value of the 4S peak by 1.5 and by dividing by the value of the 5S peak.

tRNA. Ribosomes prepared from livers of mice who had been injected with 4% NaCl (control ribosomes) do not differ from polysomes in their tRNA content. Table II, line 3, reveals that monosomes produced by starvation of mice for 72 hours contain 0.7 molecule of tRNA and/or aminoacyl tRNA but lack peptidyl tRNA (5,6, 7). Likewise, histidinol monosomes contain 0.7 molecule of tRNA and/or aminoacyl tRNA but no peptidyl tRNA.

DISCUSSION

Our results show that the amino acid analog histidinol reduces incorporation of [^{3}H]-leucine into liver proteins and produces hepatic polysome disaggregation within 30 minutes after injection into mice. This effect is transient; the polysome profile returns to normal within 12 hours following injection of histidinol (unpublished results).

Since the histidinol solutions used were hypertonic, hypertonicity is a possible mechanism for the observed disaggregation. Previous investigators (11, 12) have reported that intraperitoneal administration of hypertonic solutions causes polysome disaggregation and inhibition of protein synthesis in mouse liver. However, these investigators injected 1 ml of a 4% NaCl solution into 16 gram mice. Whereas, at the maximum dose of 1.00 gm histidinol per kg body weight, we administered only 0.15 ml of a 1200 mOsM solution, which is of comparable osmolality to a 4% NaCl solution, to 25 to 30 gram mice. Further evidence ruling out an osmotic effect is afforded by the presence of polysomes in the profiles from control mice.

The observations that the monosomes produced by histidinol dissociate into subunits in 0.3 M KCl solutions and lack peptidyl tRNA indicate that they are runoff ribosomes similar to the monosomes produced by total starvation for 72 hours (5). It was suggested by Vaughan and Hansen (3) that histidinol inhibits initiation of translation in HeLa cells due to uncharged tRNA acting as a co-repressor of initiation. Presumably starvation could increase cellular levels of uncharged tRNA thus inhibiting initiation. In this sense the histidinol effect may be equated to a starvation effect, only it occurs acutely.

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