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HISTONE-H1 INHIBITS TRANSLATION BY RETICULOCYTE LYSATES WITH RELATIVE mRNA SELECTIVITY

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Histone-H1 purified from rat skeletal muscle is a relatively potent inhibitor of peptide chain initiation in a cell free system, the rabbit reticulocyte lysate (50% inhibition at ~0.4 μM). H1 does not inhibit formation of the ternary complex nor its attachment to 40S ribosomes; the data are compatible with H1 binding to mRNA. The inhibition shows mRNA selectivity: translation of β-globin mRNA is more affected than that of α-globin mRNA and hepatic albumin mRNA more than total hepatic mRNA. Whether or not histone-H1 plays a role in translational regulation in intact cells is conjectural, it may serve as a useful model for protein-mRNA interactions.

Control of eucaryotic gene expression at the stage of mRNA translation initiation is increasingly recognized as an important regulatory mechanism (1-4). The first step of initiation, formation of the ternary complex between eIF-2[1], GTP and met-tRNA$_f$ is regulated by phosphorylation-dephosphorylation of eIF-2 (5). After interacting with several initiation factors the 40S ribosome becomes the 43S ribosomal complex, which binds to the ternary complex to form the 43S preinitiation complex (5). Other limiting initiation factors ("message discriminatory factors") e.g. cap binding protein II or eIF-4A, must bind to mRNA before it can interact with the preinitiation complex (6). According to the model proposed by Lodish, when translation is limited at or before mRNA binding to the 43S preinitiation complex (e.g. by eIF-2 availability), the ratio of initiation of mRNAs with low vs. high affinity for ribosomes decreases (7). Similarly, increasing the availability of message discriminatory factors increases the initiation of low affinity vs. high affinity mRNAs (6). Based on these mechanisms

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Abbreviations used: H1, histone H1; H3, histone H3; H2B, histone H2B; eIF, eucarotic initiation factor; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; MSA, mouse serum albumin.

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alone, it is difficult to explain how cells rapidly accelerate the translation of one or a few selected messages during development or in response to environmental stimuli, e.g. heat shock or amino acid deprivation (2). The existence of proteins that act as specific translational repressors and enhancers by binding to particular regions of the 5' untranslated regions of mRNA have been postulated (2).

Insulinopenia causes rapid, translational inhibition in skeletal muscle (8,9). This work evolved from attempts to identify an insulin-regulated translational inhibitor (10,11); a protein, purified from rat skeletal muscle that inhibited peptide chain initiation in reticulocyte lysates, was identified as histone-H1 (H1) (11). This report concerns the mechanism of H1 inhibition and its apparent mRNA selectivity.

MATERIALS AND METHODS

H1 was purified from rat hind limb muscles as described for peak-1 inhibitor in ref. 11, and after the protein was identified, by the Johns procedure (12) adapted to skeletal muscle (11). All preparations of H1 were checked for purity by SDS-PAGE (13), as described in ref 11. In some experiments H1 was purified from muscles of insulin-deficient rats, 2-7 days after the induction of diabetes with streptozotocin (11). Results obtained were identical with both H1 preparations and were not influenced by the metabolic status of the rat.

Rabbit reticulocyte lysates were prepared as described (14); hemin 1.5 μM was included during preparation to prevent activation of heme controlled repressor. Except where indicated in results, mRNA translation was assayed as the incorporation of [^{3}H]leucine or [^{35}S]methionine into TCA precipitable proteins (10,11). Lysates were incubated at 30°C in the presence of 12-25 μM hemin (optimized for different preparations), 40 μg/ml creatine phosphokinase, 10mM creatine phosphate, 100 mM KCl, 1 mM magnesium acetate, 20 mM Tris-Cl (pH 7.3), 0.5 mM dithiothreitol, 0.2 mM GTP, 0.05 mM leucine and 0.05 μCi/μl [^{3}H]leucine (NEN) and a complete mixture of amino acids 0.1 mM each. When [^{35}S]methionine was used as a tracer (1 μCi/μl lysate, NEN) leucine was 0.1 mM, and unlabelled methionine was omitted. Other modifications are described in the figure legends. mRNA dependent lysates were prepared by treatment with micrococcal nuclease (15); mRNAs translated in nuclease treated lysates were globin mRNA (Bethesda Research Laboratories) and mouse liver mRNAs. The latter were prepared by the method of Chirgwin et al (16) and poly(A)+RNA purified by oligodT-cellulose chromatography (17). Other methods are described in the figure legends.

RESULTS

Hemin deficiency results in eIF-2 phosphorylation in lysates, which prevents the exchange of eIF-2 bound GDP for GTP, resulting in initiation block at the level of ternary complex formation (5). Sucrose gradient analysis of ribosomal subunits (Fig. 1) showed that, as expected, incorporation of [^{35}S]methionine into 43S preinitiation complexes and 80S monomers was inhibited in hemin deficient lysates. H1 added to hemin supplemented lysates also inhibited methionine incorporation into 80S monomers (which, as in hemin deficient lysates, were markedly increased, representing "run off"

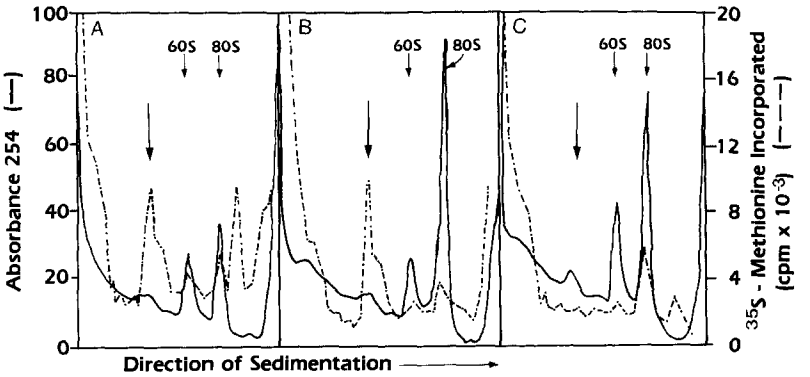


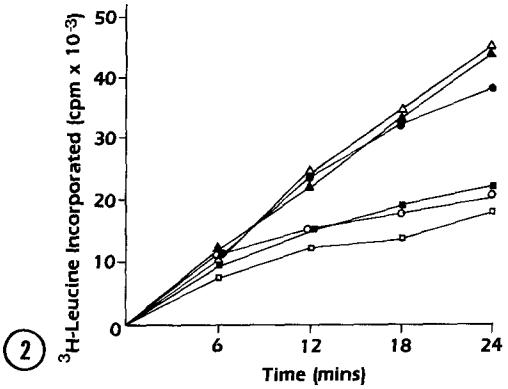
Fig. 1. Ribosomal subunit profile of lysates incubated in the absence of hemin or in the presence of H1. Lysates (250 µl) supplemented with 15 µM hemin and 1 µCi/µl [^{35}S]methionine were incubated for 10 min at 30°C with reagent control (A), or 5 µg H1 (B) or without hemin or H1 addition before incubation (C). Ribosomes and ribosomal subunits were precipitated with magnesium acetate as described (29). The redissolved pellets were layered on 0.44-1.5M sucrose gradients and sedimented at 27000 rpm, 16 hrs at 4°C in a Beckman SW27 rotor (29). Absorbance at 254 nm was recorded as described (10). Fractions (0.5 ml, 0.5 min) were collected on ice, supplemented with 1 mg/ml methionine and 3 mg/ml bovine serum albumin as carrier, precipitated with ice-cold 10% TCA for 1 hr, centrifuged, washed twice with cold 5% TCA, solubilized in 0.1N NaOH and counted as described (10). Normalized absorbance at 254nm (—) and corresponding gradient-associated [^{35}]methionine (---)) are shown. The 60S ribosomal subunit and 80S monomeric complex are indicated. Unlabelled arrows denote the 43S preinitiation complex. A representative profile from 1 of 5 experiments is shown.

monomers characteristic of initiation block), but the labelling of 43S ribosomal complexes was unimpaired (Fig. 1). Hence, H1 did not inhibit ternary complex formation, nor its association with the 40S ribosomal subunit.

Further evidence against eIF-2 involvement in H1 induced initiation block is shown in Fig. 2. While excess GTP relieved translation inhibition in hemin deficient lysates, H1 induced inhibition was unaffected. 2-aminopurine (18), purified eIF-2, eIF-4A (6) and fractions A-C of partially purified ribosomal salt wash containing different groups of initiation factors (19) (gifts of Dr. W.C. Merrick, Case Western University), were ineffective in relieving the H1 induced inhibition of translation (data not shown).

When rabbit globin mRNA was preincubated with H1 before addition to nuclease treated lysates, the inhibitory activity of H1 was markedly enhanced as compared to the inhibition seen when H1 and mRNA were added separately (Fig. 3). This interaction of H1 with globin mRNA is reversible, since the translatability of the mRNA upon extraction from H1 inhibited lysates is unimpaired (11).

The translation products of reticulocyte lysates are predominantly the α- and β-chains of globin. Reticulocytes contain more α- than β-globin mRNA,



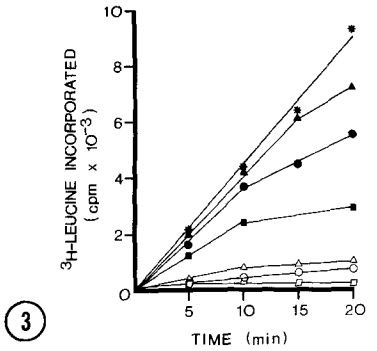


Fig. 2. Excess GTP "rescues" hemin deficient but not H1 inhibited lysates. A representative time course (1 of 4 experiments) of [^{35}H]leucine incorporation into TCA precipitable proteins (10) is shown. Lysates (50 µl) were incubated at 30°C with optimal hemin (20 µM)(△); low hemin (1.5 µM) (○); 20 µM hemin + excess GTP (1mM) (▲); low hemin + excess GTP (●); 20 µM hemin + H1 (0.5 µg) (□); or 20 µM hemin + H1 + excess GTP (■); and sampled at the times indicated in the abscissa. When excess GTP was added, Mg++ was increased to 2 mM. Additions were made in 3-5 µl lysate buffer, all samples were diluted equally.

Fig. 3. Preincubation of globin mRNA with H1 enhances H1 induced translation inhibition in nuclease treated lysates. [^{3}H]leucine (25 µCi) was added to 500 µl micrococcal-nuclease-treated lysate (15) which was distributed into 40 µl aliquots on ice. Globin mRNA (0.5 µg/100 µl lysate) and H1 were incubated at 20°C separately (●, ▲, ■) or together (○, △, □) in lysate buffer for 6 min before addition to the lysates which were then incubated at 30°C and sampled (4 µl) in duplicate at the times indicated. The incorporation of [^{3}H]leucine into TCA precipitable protein is shown in the absence of H1 (*) and in the presence of 1.5 (■, □), or 0.75 (▲, △) or 0.3 (●, ○) µg H1/100 µl lysate. The data represent means of triplicate samples obtained in 1 of 5 similar experiments.

while β-globin mRNA binds more efficiently to ribosomes and is initiated more frequently (1,7). The preferential initiation of β-globin mRNA may reflect a more relaxed conformation of its 5' untranslated and initiator codon region as compared to α (20). Under the conditions used here, the ratio of α/β globin mRNA translation was 0.71 ± 0.04 (n = 12 experiments, Fig. 4) in hemin supplemented lysates translating endogenous mRNA (21). In accordance with the Lodish model (7) hemin deficiency decreased the α/β globin synthesis ratio. Interestingly, the effect of H1 was the opposite of that seen with hemin deficiency. At low concentrations of H1 (~0.2 µM) β- but not α-globin chain translation was inhibited (Fig. 4). With increasing concentrations of H1 the translation of both α- and β-globin mRNA decreased progressively, with concommitant increases in the α/β synthesis ratios.

The apparent mRNA selectivity of H1 inhibition was not restricted to the globin message. When hepatic poly(A)+RNA was translated in nuclease treated lysates, the translation of mouse serum albumin mRNA was ~2-fold more inhibited by H1 than that of total mouse liver RNA (Fig. 5).

The effect of H1 on translation was compared to that of other histones and small basic peptides, e.g. polyamines (Sigma Chemical Co.) in experi-

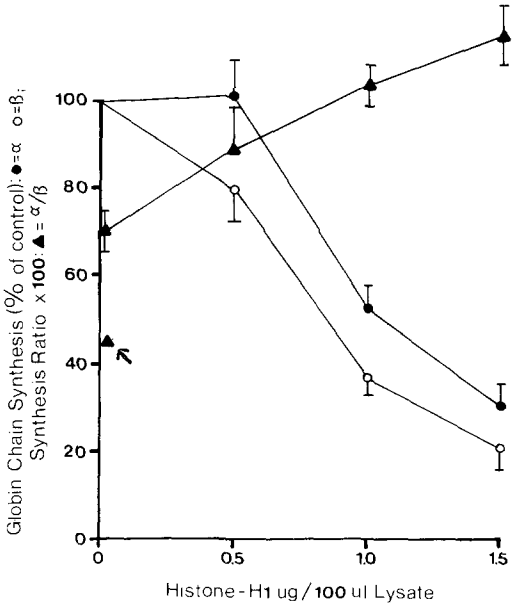


Fig. 4. Effect of H1 on α- and β-globin chain synthesis by reticulocyte lysates. Lysates (30-50 µl) were supplemented with hemin and [^{3}H]leucine as described in Methods, except the concentration of unlabelled leucine was decreased to 10 µM and [^{3}H]leucine increased to 0.5 µCi/µl. Incorporation of [^{3}H]leucine into protein was linear with time, with slopes identical to those observed with unmodified lysate buffer. Lysates were translating endogenous mRNA at 30°C in the presence of reagent control or increasing concentrations of H1. Samples were withdrawn at 6 min intervals to monitor total protein synthesis. After 18 min the lysates were placed on ice, and 5 µl samples diluted 1:10 with acid urea sample buffer (30); 5 µl aliquots were electrophoresed on 12% polyacrylamide slab gels containing 6M urea, 2% Triton-X100 in 5% acetic acid, 17 hrs at 8.5 mAmps (30). Gels were stained and destained as described (30), coated with EN^{3}hance (NEN), dried and exposed to film (Kodak X-omat AR5) for 3-7 days at -80°C. Radioactivity incorporated into the α- and β-globin chains was quantitated by excising the gel slices, solubilizing overnight in 1 ml 30% H_2O_2 and counting in a liquid scintillation counter in Scintiverse E (Fischer). Duplicate samples were electrophoresed on 2 or 3 separate gels, hence each determination is the mean of 4-6 measurements. α-(●) and β-globin chain (○) synthesis is normalized to the corresponding reagent controls in each experiment, ▲ represents the α/β globin chain synthesis ratios. The arrow indicates a hemin deficient lysate. Means ± SEM from 6-12 separate experiments are shown, 6 different H1 preparations were tested in 3 different rabbit reticulocyte lysate preparations.

ments designed as in Fig. 4 (data not shown). On a molar basis the inhibitory activity was H1>H3>H2b, with a relative activity of ~ 3:2:1. Below 0.5 mM polyamines caused no inhibition. Putrescine was not inhibitory up to 1.5 mM, the highest dose tested. Spermine (0.5 mM) and spermidine (1 mM) inhibited translation 70% and 30% respectively. In contrast to H1, at inhibitory concentrations, polyamines decreased the α/β globin synthesis ratios.

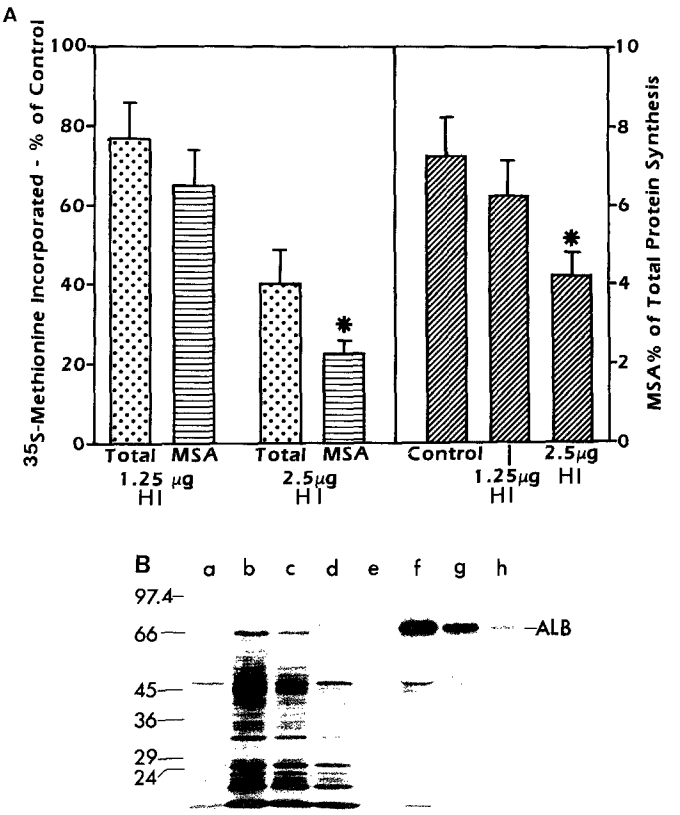


Fig. 5. Differential effect of Hl on translation of total mouse liver and mouse serum albumin (MSA) mRNAs. Nuclease treated lysates (100 µl) were incubated at 30°C for 40 min with 2.5 µg mouse liver poly(A)+RNA and 0.1 mCi [^{35}S]methionine, without or with 1.25 or 2.5 µg Hl; then duplicate 40 µl aliquots were added to 1 ml ice-cold stopping buffer (10,31), and centrifuged at 50,000 rpm (Beckman Type 65 rotor) for 2 hrs at 4°C. (31). Two 450 µl aliquots of the supernatant were immunoprecipitated with antibody to MSA (gift of Dr. B. Ledford, MUSC), one precipitate was analyzed by SDS-PAGE and the other used for measurement of [^{35}S]methionine incorporation into MSA (10). Aliquots (50 µl) of the total translation products were analyzed in parallel. Immunoprecipitation was under conditions of antibody excess (31,32) in the presence of 4 µg carrier MSA for 1 h at 37°C, then at 4°C overnight. Immunoprecipitates were pelleted at 2000 x g 10 min, and washed 5 times with 0.9% NaCl.

A. The left panel shows [^{35}S]methionine incorporation into total protein (⊡) and immunoprecipitable MSA (▤) during incubation with 1.25 or 2.5 µg Hl. Values are normalized to the incorporation by controls in the same experiment. Control lysates incorporated ~200,000 cpm into total protein and 14000 cpm into MSA in 40 min. The right panel shows MSA synthesis expressed as % of total protein synthesis in the absence or presence of 1.25 or 2.5 µg Hl. Means ± SEM of 8 experiments are shown. *$p < 0.05$ vs. control by paired analysis.

B. Representative autoradiogram of translation products resolved by SDS-PAGE (13). Samples were boiled in Laemmli's sample buffer and subjected to electrophoresis on 7.5%-10%-12.5% polyacrylamide slab gels (ratio 21:21:12 ml). Gels were processed for fluorography as described (31). Total translation products are in lanes a-d and immunoprecipitated MSA in lanes e-h; lysates incubated in the absence of added RNA (lanes a & c); or in the presence of mouse liver poly(A)+RNA and 0 (lanes b & f), 1.25 (lanes c & g) or 2.5 (lanes d & h) µg Hl.

DISCUSSION

H1 is synthesized in the cytosol but is rapidly transported to the nucleus where it is predominantly found (22). Estimates of extranuclear H1 vary between 0.2% (23) and 10% of total cellular H1 (24); the association of extranuclear H1 with polysomes and ribosomes has been suggested (24). While the synthesis of H1, as that of the core histones, is closely coordinated with DNA synthesis in dividing cells (25,26), it does proceed, albeit at a slow rate in non-dividing cells (23,25-27) and the synthesis of some H1 subspecies is relatively unaffected when cell division is inhibited (27). Since we did not fractionate H1, a more inhibitory subspecies may be included in our preparation. Inhibition of protein synthesis results in stabilization of histone mRNAs and their accumulation in the cytosol (25,26,28). The negative feedback regulation of histone synthesis has been suggested to represent specific binding of histones to their own mRNAs signalling them for degradation (28). Whether or not H1 has any role in translational regulation in the intact cell is unknown.

Since H1 binds to DNA in chromatin (22), its binding to RNA is not surprising. Our data suggest that H1 interacts preferentially with mRNA vs. other RNA species, e.g. initiation complex formation was not affected. The apparent mRNA selectivity of H1 at low concentrations (Fig. 4-5) suggests that, at least with respect to globin chain mRNA selection, H1 preferentially inhibited the translation of high affinity mRNA. Possible hypotheses include preferential interaction of H1 with mRNA 5' untranslated regions of defined structure. The structural differences between α- and β-globin mRNA 5' untranslated regions (20) may render β-globin mRNA more accessible to H1 binding, resulting in preferential initiation inhibition. Alternatively, H1 may prevent the binding of message discriminatory factors and abolish the competitive advantage of high affinity mRNAs or H1 may inhibit initiation at a stage after the 40S ribosome binds to mRNA.

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