Methymercury Hydroxide Enhancement of Translation and Transcription of Ovalbumin and Conalbumin mRNA’s*

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Farhang Payvar† § and Robert T. Schimke¶
From the Department of †Pharmacology and ¶Biological Sciences, Stanford University, Stanford California 94305

Translation of total mRNA in heterologous protein-synthesizing systems is often employed as an indirect means of assessing relative mRNA concentrations. However, it is well known that the efficiency of translation of specific mRNAs differs. One such example is the poor translational efficiency of conalbumin mRNA relative to ovalbumin mRNA. In this report we have studied the translation of conalbumin and ovalbumin mRNAs in crude mRNA preparations and with highly purified mRNA preparations. We find that treatment of RNA with methymercury hydroxide prior to translation improves the translational efficiency of both mRNAs and preferentially improves translational efficiency of conalbumin mRNA to the point where it more correctly reflects the relative concentration of these two mRNAs in crude mRNA preparations.

Conalbumin mRNA is also a poor template for the synthesis of full length cDNA synthesis by avian myeloblastosis virus reverse transcriptase, and treatment of this mRNA with methymercury hydroxide increases the size of DNA sequences synthesized. We conclude that treatment with methymercury hydroxide makes it easier to obtain full length cDNA for nucleic acid hybridizations or for cloning mRNA species.

A number of laboratories, including our own, have been studying steroid hormone regulation of egg white protein synthesis. One of the problems encountered in these studies has been the poor translational efficiency of conalbumin mRNA (1, 2) which has resulted in difficulties in quantitating conalbumin mRNA by translational assays and in making accurate estimates of conalbumin mRNA in the course of its purification (3). In addition, conalbumin mRNA is a poor template for complementary DNA synthesis by AM virus reverse transcriptase (3), thereby making it difficult to obtain full length cDNA for nucleic acid hybridizations or for cloning of mRNA sequences.

Because of preliminary observations indicating that conalbumin mRNA aggregates more readily than does ovalbumin mRNA (4, 5), we have explored the possible use of the RNA denaturing agent, methymercury hydroxide (CH3HgOH), to obviate the above problems. Methymercury hydroxide binds to nucleosides by ionic interactions and is readily removed from RNA preparations (6–8). We report herein that treatment of RNA preparations with CH3HgOH increases the translational efficiency of both ovalbumin mRNA and conalbumin mRNA. In crude RNA extracts, the relatively translational efficiency of conalbumin mRNA is increased so that the ratios of incorporation of labeled amino acid into these two proteins reflect their in vivo rates of synthesis in hen oviduct. In addition, we find that CH3HgOH treatment results in larger sized cDNA sequences synthesized from highly purified conalbumin mRNA. Our results suggest that CH3HgOH treatment denatures interactions of the mRNAs both with itself and with contaminating molecules that inhibit translation. Inasmuch as the effects differ for ovalbumin and conalbumin mRNA translation and transcription, we conclude that secondary structural differences between the two mRNAs exist.

Thus, treatment of RNA species with CH3HgOH employed for mRNA quantitation by translational assays may be a useful means for obtaining a more accurate measure of intracellular mRNA content when no information is available concerning the relative efficiencies of translation of individual mRNA species.

**EXPERIMENTAL PROCEDURES**

Materials—Ovalbumin, grade V, conalbumin, type II, and guanidinium HCl were obtained from Sigma, and cycloheximide was from Calbiochem. Thiocyanate was from Tricom/Fluka. Rabbit antiovalbumin was a gift of Jean Feagin, Department of Biological Sciences, Stanford University. Other reagents have been previously described.'

Isolation and Purification of mRNA—Total cellular and polysomal RNA were prepared by Method III, guanidinium thiocyanate-guanidinium HCl procedure as detailed elsewhere. These procedures were adapted from the previously described procedures of Cox (9), Glisin (10), Chargwirn et al. (11) and result in increased specific activity and recovery of conalbumin mRNA as compared to other methods for the preparation of RNA (12, 13). Briefly, oviduct magnum was rapidly homogenized in guanidinium thiocyanate by a polytron homogenizer. DNA and denatured protein were separated from RNA by centrifugation of the homogenate on cesium chloride cushions. The RNA pellets were dissolved in guanidinium HCl and precipitated at −20°C by addition of % volume of ethanol and adjustment of pH to 5.0. The RNA was reprecipitated two additional times by dissolving the RNA pellets in H2O and by addition of 2 volumes of ethanol in the presence of 0.2 M sodium acetate at pH 5.5.

Conalbumin mRNA was prepared by immunoprecipitation of its polyclonal antibody-Sepharose chromatography, and sucrose gradient centrifugation as described. Ovalbumin mRNA was prepared as detailed (9) except for an additional four sucrose gradient centrifugations.

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mRNAs as well as ribosomal RNA. By these criteria ovalbumin and conalbumin mRNAs each appeared to be better than 90% pure with respect to other mRNAs as well as ribosomal RNA.\(^1\)

**CH\(_3\)HgOH Treatment of RNA—CH\(_3\)HgOH stock solutions (IM, alfalfa) in water were diluted to 2.0 to 40 mM CH\(_3\)HgOH with water in a fumehood and added to RNA samples to make the final concentration of CH\(_3\)HgOH 10 mM in total cellular and polysomal RNA and 2.5 mM in purified mRNA (A) and poly(A) RNA. The latter were made at site that were not treated with CH\(_3\)HgOH were diluted with an equivalent volume of water. Unless otherwise stated, the concentration of CH\(_3\)HgOH in protein synthesis and reverse transcription systems did not exceed 0.25 and 2 mM, respectively. The CH\(_3\)HgOH treatment was performed immediately prior to addition of RNA to protein synthesis or cDNA synthesis systems and was between 3 and 5 min.

**Reticulocyte Lysate Assay—**Rabbit reticulocyte lysates were prepared as described previously (14). mRNA-dependent lysates were made by modification of published procedures (15) as detailed (3, 16). The conditions for cell-free synthesis were as before (14), with the following additional comments and modifications. 1) 112 mM potassium acetate replaced 75 mM potassium chloride. This substitution has been described by several other investigators on total RNA. This substitution increases mRNA-specific activity, while the specific activity of conalbumin was increased by 40 to 60%. Similar ionic adjustments have been shown to result in improvement of translation of other mRNAs through improvement in initiation (17). 2) The standard assay mixture contained 60 pg of mRNA-dependent lysate, 2 pl of 1.0 M Hepes buffer, pH 7.4, 3.7 \(\mu\)L of [\(^{14}C\)leucine (5 Ci/mmol), and 23 pl of water and RNA. 3) The cell-free protein synthesis was for 2 h at 25°C. 4) The reaction was stopped by cooling to 4°C and addition of 50 pl of a mixture containing 1.4% Triton X-100 and 1.4% sodium deoxycholate, 40 mM leucine or methionine, and 15 \(\mu\)g of bovine serum albumin. In experiments dealing with determination of transit times, this mixture also contained 5 \(\mu\)M cycloheximide. 5) Incorporation into total protein and ovalbumin and conalbumin was linear up to 30 to 60 min, had pH optimal of 7.4, and a broad temperature optimal between 22°C and 27°C. 6) The mRNA-dependent lysate was stored under liquid nitrogen in small aliquots for no longer than 7 days before use. Lysates kept under nitrogen were thawed only once.

**Immunoprecipitation Assay—**Immunoprecipitation assays were performed by the modification of previous procedures (14, 15, 10) as described above, but without the 10-min centrifugation step previously noted (2, 19). This modification reduces the specific loss of conalbumin synthesized in lysate that sometimes occurs. All other reagents used in immunoprecipitation were centrifuged at 4°C for 10 min to remove any particulate aggregate that may settle to sediment with specific immunoprecipitates. 2.5 to 90 pl of reaction mixtures were incubated in the presence of 0.4% Triton X-100, 0.4% sodium deoxycholate, 6 \(\mu\)g of ovalbumin, or 17 \(\mu\)g of conalbumin in 10 mM phosphate buffer, pH 7.2, containing 100 mM sodium chloride and either 0.7 mg of rabbit antiovalbumin or 1.3 mg of goat anticonalbumin in a final volume of 300 pl. The immunoprecipitation reaction was carried out for 2 h at 25°C and subsequently layered on 90 pl of a 1.0 M sucrose cushion, overlayed by 25 \(\mu\)L of 0.5 M sucrose in 400 pl of polypropylene tubes. Sucrose solutions contained 0.4% Triton X-100, 25 mM Hepes, pH 7.4, 0.2 M sodium chloride, and 5 mM EDTA. Immunoprecipitates were collected by centrifugation at 13,200 \(\times g\) for 5 min at 4°C. The immunoprecipitates either were eluted (with 1 M NaDodSO\(_4\)) or kept in NaDodSO\(_4\)-gel buffer. For sedimentation counting, polypropylene tubes were frozen in Dry Ice-ethanol bath, and the bottom of tubes containing the immunoprecipitates was cut. For scintillation counting, polypropylene tubes were frozen in Dry Ice-ethanol bath, and the bottom of tubes containing the immunoprecipitates was cut. For scintillation counting, polypropylene tubes were frozen in Dry Ice-ethanol bath, and the bottom of tubes containing the immunoprecipitates was cut. For scintillation counting, polypropylene tubes were frozen in Dry Ice-ethanol bath, and the bottom of tubes containing the immunoprecipitates was cut. The immunoprecipitates were dissolved in 0.1 M NaOH by 15 min of constant shaking at room temperature. The samples were neutralized by addition of HCl and counted in a Trition-xylene-based scintillation mixture. Co-sedimentation of samples with reticulocyte lysate and conalbumin synthesized in vitro was determined by incubation of samples with rotation of microwells, by autoradiography of polyacrylamide gels, by fluorographic procedures (21) using preflashed films (22).
Conformational Control of mRNA Translation and Transcription

Fig. 1. Optimal concentration of CH₃HgOH for the translation of ovalbumin and conalbumin mRNA. Total cellular RNA from hen oviduct was prepared and treated to obtain the indicated concentrations of CH₃HgOH. The treated RNA was added to mRNA-dependent reticulocyte lysates in the presence of [³⁵S]methionine. Following 2 h of synthesis, aliquots were removed and used either for the determination of trichloroacetic acid-precipitable radioactivity (a), or for immunoprecipitation with antiovalbumin (b), or anticonalbumin antibodies (c). Specific activities (counts per minute/µg of added RNA) for each point were calculated by the subtraction of endogenous incorporation of lysates containing no added RNA from those receiving RNA. The endogenous incorporation was approximately 5000 cpm and was not affected by the presence of CH₃HgOH at concentrations indicated under “Experimental Procedures.” Trichloroacetic acid precipitation was used for the determination of total incorporation as described (14).

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total product of mRNA-dependent lysate programmed with hen oviduct RNA in the presence or absence of CH₃HgOH. Reticulocytes lysates were programmed with either no RNA or 3.6 µg of total cellular RNA from hen oviduct. RNA samples were either treated to “0” mM or “10” mM CH₃HgOH prior to their addition to reticulocyte lysates. Following synthesis equal aliquots were removed and electrophoresed on 4 to 10% gels, as described under “Experimental Procedures.” Track 1, endogenous incorporation, 4,600 cpm; Track 2, RNA treated with “0” mM CH₃HgOH, 7,300 cpm; and Track 3, CH₃HgOH-treated RNA, 15,000 cpm. Films were exposed for 12 days at -80°C. CON, OV, and globin refer to migration of authentic conalbumin, ovalbumin, and globin markers. The two species of ovalbumin (OVA I and OVA II) that are resolved under these conditions have not been previously observed (23). OVA I and OVA II are differentially induced by various steroid hormones and show different kinetics of induction following steroid hormone stimulation of hormone-withdrawn chicks.₄

Multiples from the same lysate mixture. Ovalbumin synthesized in vitro migrates slightly less readily than authentic ovalbumin, consistent with previous findings (23, 24). This behavior of in vitro synthesized ovalbumin may result from the inability of in vitro protein-synthesizing systems to promote glycosylation in the absence of membrane fractions (24, 25).

The improvements made by the modifications described here result in relative in vitro translational efficiencies of ovalbumin and conalbumin mRNA that are comparable to those obtained in vivo. The ratio of [³⁵S]methionine or of [³⁴H]leucine incorporated into ovalbumin relative to conalbumin by in vivo labeling of hen oviduct fragments ranges between 10 and 16 (Met) and 4.5 and 8 (Leu) (26). These ratios compare favorably to those obtained in vitro by translation of hen oviduct total cellular RNA in the presence of CH₃HgOH (10 to 17 -Met and 6 to 10 -Leu) and not with those in its absence (30 to 60 -Leu and 25 to 50 -Met) (see Table I and Fig. 1). The differential labeling noted with the two amino acids results from the fact that there are 34 to 50 residues of leucine and 16 and 8 residues of methionine/molecule of ovalbumin and conalbumin, respectively (27, 28).

Enhancement of translational efficiency by CH₃HgOH requires that the RNA samples be treated with CH₃HgOH prior to translation. The addition of CH₃HgOH to lysates prior to the addition of RNA has no effect on the translational efficiency of mRNAs (data not shown). This probably results from the fact that the ionic conditions of reticulocyte lysate do not favor the ionic interaction of CH₃HgOH with mRNA (5–7). Therefore, we conclude that CH₃HgOH enhances the utilization of mRNA by reducing mRNA aggregation and not by affecting the protein synthetic machinery.

₄ F. Payvar and R. T. Schimke, manuscript in preparation.
aggregates under our poly(U)-Sepharose chromatography conditions. The particular step in the procedure that could have resulted in the partial removal of RNA aggregates may have been tunicamidine employed both in washing of the bound RNA and in the final elution. The experiments detailed below suggest, however, that improvement in the translation of conalbumin mRNA can occur without the use of a denaturant and, furthermore, suggest that the poor translation of conalbumin mRNA in crude RNA preparation relative to purified mRNA may be explained at least in part by its interaction with an inhibitory molecule.

RNA and polysomal RNA were prepared by the same methods ("Experimental Procedures") in the presence of guanidinium salts. The specific activity of ovalbumin and conalbumin mRNAs in total cellular RNA was approximately 50% higher than in the polysomal RNA when translated with pretreatment with CH₃HgOH. The result of translation of these RNAs in the absence of CH₃HgOH is, however, both qualitatively and quantitatively different with respect to conalbumin synthesized. Total cellular RNA, polysomal RNA, and purified conalbumin mRNA were translated in the absence and presence of CH₃HgOH, and conalbumin immunoprecipitates were subjected to electrophoresis on sodium dodecyl sulfate polyacrylamide gels (Fig. 3). The results confirm and extend those presented above. In each case pretreatment with CH₃HgOH leads to enhancement of conalbumin mRNA-specific activity. The magnitude of the CH₃HgOH effect is greatest with total RNA (6-fold), smaller with polysomal RNA (4-fold), and smallest in the case of purified mRNA (50% greater). Also, the increase in conalbumin mRNA activity was associated with an increase in the proportion of the radioactivity that migrates at the position of authentic conalbumin. 2) In the absence of CH₃HgOH the specific activity of conalbumin mRNA is higher in polysomal RNA than in total cellular RNA, a result opposite to that obtained when translation was performed in the presence of CH₃HgOH. In addition, the proportion of conalbumin synthesized in the absence of CH₃HgOH (Fig. 3, lane I, A, B, and C) and which migrates in the position of authentic conalbumin appears highest in the case of purified mRNA, less in the case of polysomal RNA, and practically undetectable in the case of total cellular RNA. The radioactivity immunoprecipitated with conalbumin antibody from the translation mixture programmed with total cellular RNA in the absence of CH₃HgOH was typically smaller than authentic conalbumin (76,000 dal-

### Table I

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>Methylmercury hydroxide treatment</th>
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<tr>
<td>min at 68°C</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>0</td>
<td>1200</td>
</tr>
<tr>
<td>2</td>
<td>2300</td>
</tr>
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<td>5</td>
<td>2600</td>
</tr>
<tr>
<td>10</td>
<td>2800</td>
</tr>
<tr>
<td>15</td>
<td>2300</td>
</tr>
</tbody>
</table>

The effectiveness of CH₃HgOH in disaggregating RNA samples and the subsequent differential improvement in the translational efficiency also compares favorably with brief heat treatment of RNA at 68°C prior to translation. RNA samples were either heated to 68°C for from 1 to 15 min or pretreated with 10 mM CH₃HgOH and then translated in mRNA-dependent reticulocyte lysate. The results are presented in Table I and demonstrate that heat treatment of RNA samples increases the total incorporation and ovalbumin mRNA-specific activity to an extent comparable with pretreatment with CH₃HgOH. The specific activity of conalbumin mRNA is, however, not substantially affected by heat treatment. Therefore, the net effect of heat treatment appears as one of reduced translational efficiency of conalbumin mRNA relative to ovalbumin mRNA.

From the data thus far presented we conclude that the aggregation of mRNA may limit its translation, and that differential efficiencies of translation of various mRNAs may be related to their variable propensities for aggregation.

**Purified Conalbumin and Ovalbumin mRNA are Translated with Similar Efficiencies**—The results of translation of poly(A) RNA from hen oviduct in the absence or presence of various concentrations of CH₃HgOH are presented in Table II. These results indicate that CH₃HgOH pretreatment results in only a small increase in the specific activities of ovalbumin and conalbumin mRNAs as compared to total cellular RNA, that the maximal effect of CH₃HgOH occurs at 2.5 mM as compared to 10 mM in the case of total cellular RNA, and, finally, that the ratios of ovalbumin and conalbumin mRNA activities are similar in the absence or presence of various concentrations of CH₃HgOH and resemble the in vivo ratios noted previously. Since greater than 70% of the activity of both mRNAs is recovered in the purification procedure, and since the purification procedure leads to isolation of mRNAs equal in size to those in unfractionated RNA and capable of coding for authentic ovalbumin and conalbumin, we conclude that separation of ribosomal contaminants employing poly(U)-Sepharose leads to translation of conalbumin and ovalbumin mRNAs with similar efficiencies.

**Inefficient Translation of Conalbumin mRNA Relative to Ovalbumin mRNA May be Explained by the Interaction of Conalbumin mRNA with an Inhibitory Molecule**—The improved efficiencies of conalbumin mRNA translation that was observed following its separation from ribosomal RNA could potentially result both from its separation from poly(A) mRNA and from the removal of aggregates under our poly(U)-Sepharose chromatography conditions. The particular step in the procedure that could have resulted in the partial removal of RNA aggregates may have been tunicamidine employed both in washing of the bound RNA and in the final elution. The experiments detailed below suggest, however, that improvement in the translation of conalbumin mRNA can occur without the use of a denaturant and, furthermore, suggest that the poor translation of conalbumin mRNA in crude RNA preparation relative to purified mRNA may be explained at least in part by its interaction with an inhibitory molecule.

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### Table II

<table>
<thead>
<tr>
<th>Methylmercury hydroxide</th>
<th>Conalbumin</th>
<th>Ovalbumin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.60</td>
<td>25.0</td>
<td>49.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.00</td>
<td>25.0</td>
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</tr>
<tr>
<td>2.0</td>
<td>2.10</td>
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</tr>
<tr>
<td>2.5</td>
<td>2.20</td>
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<td>3.8</td>
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<td>29.0</td>
<td>58.0</td>
</tr>
<tr>
<td>5.0</td>
<td>1.60</td>
<td>n.d.</td>
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</tr>
<tr>
<td>7.5</td>
<td>1.46</td>
<td>25.0</td>
<td>48.0</td>
</tr>
</tbody>
</table>

* n.d., not determined.
There is a slight decrease in the transit time for total proteins, and presence of CH$_3$HgOH (Fig. 4). While it is evident that time course of synthesis of total oviduct protein in the absence of mRNA resulting from treatment with CH$_3$HgOH may be due to improvement in initiation, elongation, and termination of CH$_3$HgOH appears to act by increasing such movement. A mRNA, however, the smaller fragments represent less than 10% of total radioactivity and may constitute conalbumin mRNA that would otherwise remain inactive or be subject to degradation in the absence of CH$_3$HgOH.

The improved translation of conalbumin and ovalbumin mRNA resulting from treatment with CH$_3$HgOH may be due to improvement in initiation, elongation, and termination of conalbumin mRNA or prevention of degradation. An analysis of transit times for conalbumin and ovalbumin mRNA (29, 30) revealed that CH$_3$HgOH acted to shorten the transit time on both mRNAs. For conalbumin the reduction was from 14 to 11 min and for ovalbumin from 7 to 5 min (data not shown). Therefore, it appears that in the absence of CH$_3$HgOH movement of ribosomes on both mRNAs is hindered, and CH$_3$HgOH appears to act by increasing such movement. A more dramatic feature of these analyses is illustrated by the time course of synthesis of total oviduct protein in the absence and presence of CH$_3$HgOH (Fig. 4). While it is evident that there is a slight decrease in the transit time for total proteins, the major effect is an increase in the rate of incorporation. This indicates that the major effect is on the utilization of mRNAs that would otherwise remain inactive or be subject to degradation in the absence of CH$_3$HgOH.

We propose that the lower translational efficiency of conalbumin mRNA may result from its interaction with a secondary class of molecules, which exist in highest concentrations in the total cellular RNA preparations and which appear to interact with the mRNA so as to create a translationally inactive pool of conalbumin mRNA and further interact to limit elongation or termination. The improvement in the translational efficiency of conalbumin mRNA can be achieved by either the reduction in the concentration of these proposed inhibitory molecules through preparation of polysomal RNA, the purification of the mRNA, or by the reduction of its interaction with the use of CH$_3$HgOH.

Preliminary experiments indicate that molecules can be isolated from the total RNA preparations which reduce the efficiency of translation of purified conalbumin mRNA and that CH$_3$HgOH is able to abolish such reduction in efficiency. The particular nature of these molecules, the mode of their interaction with conalbumin mRNA, and the possibility of a regulatory role in vivo remain to be investigated.

Methylmercury Hydroxide Increased the Efficiency and the Size of the cDNA Synthesized from Conalbumin mRNA—We have previously investigated various parameters that could potentially affect the proportion of full length cDNA from partially purified mRNAs, ovalbumin, conalbumin, ovomucoid, and lysozyme. All mRNAs with the exception of conalbumin mRNA gave rise to large portions of full length cDNA under the conditions examined (3).

The effect of treatment of total RNA samples as well as purified ovalbumin and conalbumin mRNA with CH$_3$HgOH prior to addition to the cDNA synthesis reaction is presented in Table III. CH$_3$HgOH increases the efficiency of cDNA synthesis from total RNA (3-fold) and from ovalbumin and conalbumin mRNA (1.8-fold). The optimal concentration of CH$_3$HgOH required was 5 to 10 mM total RNA and 3.5 mM with the purified mRNAs. The optimal concentrations of CH$_3$HgOH required and the fold stimulation of cDNA synthesis observed were similar to those previously noted for optimal translation of RNA samples.

The effect of CH$_3$HgOH on the size of cDNA products is...
Effect of CH₃HgOH on synthesis of cDNA from hen total cellular RNA and purified ovalbumin and conalbumin mRNA

Hen total cellular RNA, ovalbumin mRNA, and conalbumin mRNA were prepared as described. The RNA samples were treated with the indicated concentrations of CH₃HgOH prior to their addition to reaction mixture containing [³²P]dGTP. Following the synthesis at 42°C for 30 min, the reaction was stopped and trichloroacetic acid-precipitable radioactivity was determined.

<table>
<thead>
<tr>
<th>Methymercury hydroxide</th>
<th>Total cellular RNA (cpm/µg x 10⁶)</th>
<th>Conalbumin mRNA (cpm/µg x 10⁶)</th>
<th>Ovalbumin mRNA (cpm/µg x 10⁶)</th>
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<tr>
<td>m M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
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</tr>
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<td>27.0</td>
<td>n.d.a</td>
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</tr>
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<td>22.0</td>
<td>n.d.a</td>
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</tr>
<tr>
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<td>17.0</td>
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Not determined.

DISCUSSION

Our efforts to quantitate intracellular conalbumin mRNA activity, to purify conalbumin mRNA, and to synthesize full length conalbumin cDNA for hybridization studies and insertion into bacterial plasmids have been difficult. These difficulties have stemmed from the fact that conalbumin mRNA is translated inefficiently in vitro protein-synthesizing systems, has high propensity for aggregation, is lost and degraded more readily than ovalbumin mRNA, and is a poor template for cDNA synthesis (1-5).

We have found that most of these properties of conalbumin mRNA result from its greater ability to interact with itself and with other molecules that is the case for ovalbumin mRNA. This is evidenced by the fact that the reduction of these interactions by the use of the nucleic acid denaturant CH₃HgOH results in a greater improvement in the efficiency of translation of conalbumin than ovalbumin mRNA. This improved efficiency allows for a more accurate estimation of the intracellular concentration of these mRNAs. In addition, CH₃HgOH also increases the efficiency of cDNA synthesis from both mRNAs as well as an increase in proportion of larger sized cDNA from conalbumin mRNA. Finally, since CH₃HgOH treatment of RNA samples affects the translation and cDNA synthesis differently in the case of conalbumin and ovalbumin mRNA, it appears that secondary structural differences between the two mRNAs exist. In the absence of data for expected translational efficiency of a given mRNA, the treatment of RNA with CH₃HgOH may provide a more accurate measure of relative mRNA concentrations when assayed in translational assays.
The finding that the effects of CH$_3$HgOH reported here necessitates its addition to RNA samples prior to synthesis eliminates the possibility that CH$_3$HgOH exerts its effect through modification of the systems for the synthesis of DNA and protein. It is unlikely that the enhanced efficiencies observed are the result of a permanent chemical modification of the mRNAs. Under the conditions employed for translation and cDNA synthesis dissociation of CH$_3$HgOH from nucleic acids is a highly favored process (6-8).

The propensity exhibited by RNA species for aggregation occurs with various methods of RNA preparation. We have found variable degrees of RNA aggregation with numerous methods for RNA extraction involving: phenol-chloroform (12), sucrose gradients NaDodSO$_4$ (13) guanidinium hydrochloride (9), guanidinium thiocyanate, and cesium chloride (10, 11).

That conformational considerations may be of general importance in the utilization of mRNA is suggested from the work of others. Leinwand and Ruddle (31) have found a 2-fold increase in the translational efficiency of myeloma mRNA, tobacco mosaic virus RNA, and endogenous reticulocyte mRNA in reticulocyte lysates in the presence of actinomycin D. These increases, however, were not found to differentially affect various mRNAs and did not occur above and beyond that produced by a heat treatment similar to that reported here. In addition, Lodish and Robertson (32) have found that formaldehyde treatment of bacteriophage f2 RNA enhances the efficiency of initiation as judged by binding of formylmethionine tRNA to Escherichia coli ribosomes. For reviews of other related literature see Refs. 33 to 35.

In avian ovoviduct both concentration and utilization of egg white mRNAs have been shown to be regulated by both estrogen and progesterone (36-40).

At the present time details of the processes involved in the control of accumulation and utilization of the mRNA or for the secretion of those proteins are not known. An intriguing possibility, however, exists that steroid hormone-induced conformational changes in mRNA structure may be important in the complex cascade of events that lead to increase in accumulation and utilization of mRNA. Our findings suggest that the conformational state of conalbumin and ovalbumin mRNA is important in determining their utilization in heterologous protein-synthesizing systems. The ionic interactions of conalbumin and ovalbumin mRNA with other molecules in the total RNA samples appear to differentially limit their utilization using this system. Since steroid hormones have been shown to be intimately involved in promotion of polyoma formation and mRNA utilization (41, 42), one possibility is that steroid hormones may act in some fashion and, in part, to promote mRNA utilization through alteration in interactions and changes in mRNA secondary structure.

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