Effects of Cations and Cosolvents on Eukaryotic Ribosomal Subunit Conformation†

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ABSTRACT: The effects of various cations and cosolvents on the conformation of wheat germ ribosomes and ribosomal subunits have been investigated by using the techniques of circular dichroism and differential hydrogen exchange. A class of hydrogens on both the 40S and 60S subunits exchange out more rapidly as the Mg^{2+} concentration is raised, indicating that Mg^{2+} causes a change in subunit conformation. Ca^{2+} and the polyamines produce a similar increase in the rate of hydrogen exchange. These results suggest that increases in cation concentrations permit a tightening of ribosome structure and a greater degree of internalization of the rRNA. The cosolvent glycerol causes an alteration in the CD spectrum of 80S ribosomes in both the nucleic acid and protein portions of the spectrum. Glycerol also causes a decrease in the rate of exchange of a number of hydrogens on both the 40S and 60S subunits. These results are interpreted to mean that glycerol favors a more native, less denatured structure in the ribosome.

The ribosome is presently viewed as a dynamic structure that is capable of assuming a wide variety of conformations which are stabilized by interactions among its RNA and protein components. These interactions are, in turn, greatly affected by the ionic environment (Grunberg-Manago et al., 1981) and by the presence of cosolvents (Hui Bon Hoa et al., 1980). Cations, in particular, have been shown to play a central role in maintaining the stability and activity of the ribosome (Grunberg-Manago et al., 1981; Cox et al., 1976; Kimes & Morris, 1973). They are critical for all aspects of protein biosynthesis and affect essentially every step of this process from the association of the subunits to the individual events in amino acid incorporation. For example, Mg^{2+} and the polyamines have been shown to promote the association of ribosomal subunits while the monovalent cations clearly promote subunit dissociation (Zitomer & Flaks, 1972; Hui Bon Hoa et al., 1977; Sperrazza et al., 1980, 1981). Recent studies (Sperrazza & Spremulli, 1983) using wheat germ ribosomes have also shown that a critical number of Mg^{2+} ions must be present on the ribosomes if they are to maintain their activity in protein biosynthesis.

Conformational changes in ribosomes brought about by small variations in the ioinic environment are subtle and not easily detected. Methods used to monitor gross structural changes (ultraviolet absorption spectrum and circular dichroism spectrum, for example) have failed to detect large structural differences in ribosomes in various ionic environments (Miall & Walker, 1968; Kabasheva et al., 1971; Cox & Hirst, 1976). Hence, more sensitive methods of monitoring conformation need to be used in studies of cation and cosolvent effects on ribosome structure. Hydrogen exchange techniques have been applied successfully to monitor conformational changes of peptides (Nabedryk-Viala et al., 1975), proteins (Nabedryk-Viala et al., 1976), and nucleic acids (Teitelbaum & Englander, 1975a,b; Mandal et al., 1979). Early work with rat liver ribosomes (Page et al., 1967) demonstrated the feasibility of applying this technique to the study of ribosomes. More recently, Bonnert et al. (1980) have used hydrogen exchange techniques to examine Mg^{2+} effects on the conformation of the 50S subunit of the *Escherichia coli* ribosome. They observed a conformational change in the large subunit (indicated by an accelerated loss of about 30 hydrogens) when the Mg^{2+} ion concentration was reduced from 10 to 2 mM.

In the present study, we have used both CD and hydrogen exchange to demonstrate that changes in cation and cosolvent concentrations have a significant effect on the conformation of wheat germ ribosomes and ribosomal subunits.

Materials and Methods

Materials. Wheat germ was kindly supplied by J. M. deRosier, International Multifoods Corp. Sephadex G-50-80, spermine hydrochloride, and spermidine hydrochloride were purchased from Sigma. ^3H_2O (specific activity = 1 Ci/mL) was purchased from ICN. Porous polyethylene disks (4-mm diameter, 70-μm pore size, 1.6 mm thick) were obtained by special order from Bel Art Products. Scintiverse was from Fisher. Buffer A contained 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)[1]-KOH, pH 7.6, 10% glycerol, and 2 mM dithiothreitol with KCl and $MgCl_2$ concentrations as indicated.

Preparation of Ribosomes and Ribosomal Subunits. Wheat germ ribosomes were prepared as described previously (Spremulli et al., 1977) with the modifications described by Russell & Spremulli (1979). Ribosomal subunits were prepared as described (Spremulli et al., 1977) except that 10% glycerol was added to all buffer solutions. Each preparation of ribosomal subunits had less than 2% cross-contamination. Both ribosomes and subunits were capable of undergoing reversible association and dissociation as judged by analysis on sucrose gradients (Russell & Spremulli, 1978), and both functioned catalytically in the poly(U-directed polymerization of phenylalanine.

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[1] Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Microdetermination of Phosphorus. Phosphate was released from the ribosome by using the method of Ames & Dubin (1960), and the amount of phosphorus was then determined through the reduction of a phosphomolybdate complex by ascorbic acid (Chen et al., 1956). These data were used to determine the molar ellipticity for CD spectra.

Circular Dichroism Measurements. Circular dichroism spectra were recorded on a Cary 60 spectropolarimeter with a CD attachment. The instrument was calibrated with *d*-10-camphorsulfonic acid. The spectra were recorded at 27 °C in a thermostated cell (0.5-mm path length) connected to a circulating waterbath. All spectra were repeated at least 6 times. The signal to noise ratio was at least 15:1 at the maxima. The results, shown as the average of all spectra minus the base line, are reported as molar ellipticity, $[\theta]$, per mole of phosphate. The value for $[\theta]$ was proporational to concentration, indicating that there were no artifacts due to light scattering.

Hydrogen–Tritium Exchange Procedure. Small columns were prepared by the method of Penefsky (1977). Briefly, Sephadex G-50 (fine) equilibrated in the appropriate buffer was placed in a Becton-Dickinson 1-mL TB syringe fitted with a porous polyethylene disk, and the resin was then reduced to a gel cake by centrifugation for 1 min at 150*g* in a Model 103 Dynac II table-top centrifuge using a fixed-angle rotor (0113). This procedure was scaled up to accommodate larger volumes as needed.

Ribosomal subunits (21 mg/mL, 0.4–0.6 mL) in buffer A containing 5 mM $MgCl_2$ and 50 mM KCl were labeled with ^3H by incubation on ice for 1 h in the presence of $^1/_{19}$th volume of ^3H_2O. A series of control experiments was carried out to determine the most appropriate labeling time. Fewer Mg^{2+}-sensitive hydrogens were labeled at less than 1 h while longer incubations produced too much labeling of the slowly exchanging hydrogens. It was, therefore, concluded that the 1-h exchange-in time period was sufficiently long to label the Mg^{2+}-sensitive hydrogens and yet label only a few of the slowly exchanging hydrogens. To remove excess ^3H, the sample was diluted to 1 mL with buffer A containing 5 mM $MgCl_2$ and 50 mM KCl and applied to a 10-mL Sephadex G-50 column prepared as described above and equilibrated in buffer A containing 0.5 mM $MgCl_2$ and 50 mM KCl unless otherwise indicated. The column was subjected to a 2-min, 150*g* centrifugation step at 4 °C in which the eluant containing the labeled ribosomes was collected in a siliconized 15 × 45 mm shell vial. A clock was started, and the eluant was then divided into the desired number of aliquots. Exchange out was allowed to occur for 30 min to permit the loss of rapidly exchanging hydrogens. Following this initial incubation, the conditions were altered in the appropriate aliquots as described in the figure legends. The exchange-out rate was followed by removing three samples of 100 μL each at various times and placing each sample on a 1-mL G-50 column prepared as described above and equilibrated with the appropriate buffer. The syringe was suspended on the tube shield and subjected to a 1 min, 150*g* centrifugation step at 4 °C and the eluant containing the ribosomes was collected as above. The ribosome concentration in the eluate was determined by duplicate A_{260} readings, and the amount of ^3H present was measured by counting several 50-μL aliquots of the eluant in 10 mL of Scintiverse. The number of hydrogens remaining per ribosome was calculated by the method of Englander & Englander (1972).

Results

Effect of Mg^{2+} Concentration on Subunit Conformation.

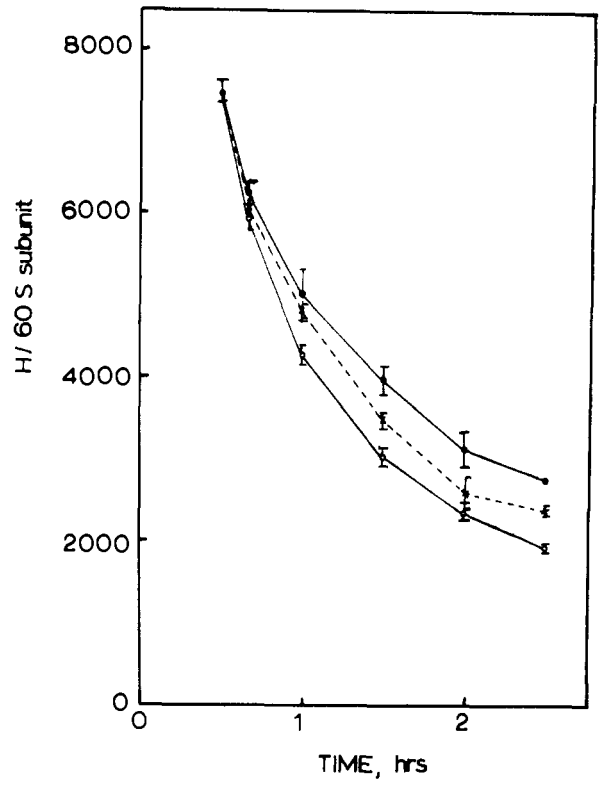

FIGURE 1: Hydrogen exchange-out curves for 60S ribosomal subunits at different Mg^{2+} concentrations. The 60S ribosomal subunits were labeled with ^3H as described under Materials and Methods. Exchange out at (●) 0.5, (×) 3, or (○) 5 mM $MgCl_2$. Where no error bars are indicated, the error is contained within the size of the point.

The effect of small changes in the Mg^{2+} ion concentration on the conformation of wheat germ ribosomal subunits was investigated by the sensitive technique of hydrogen–tritium exchange. In initial experiments, the large subunit was labeled with tritiated water at 5 mM Mg^{2+}. Free tritiated water was removed; the Mg^{2+} concentration was then lowered to 0.5 mM and incubation continued for a short period of time to permit exchange out of the hydrogens with fast rates of exchange. The Mg^{2+} concentrtaion was subsequently raised and exchange out followed for several hours. Figure 1 illustrates exchange-out curves for 60S subunits at 0.5, 3, and 5 mM Mg^{2+}. Within the first 0.5 h tested, about 600 hydrogens were more rapidly lost from the subunits at 5 mM Mg^{2+} than at 0.5 mM Mg^{2+}. The remaining hydrogens were lost at similar rates in the two samples. The curve obtained for ^3H loss at 3 mM Mg^{2+} fell in between the curves obtained at 0.5 and 5 mM Mg^{2+}, indicating a gradual change in the exchange-out rates with a change in the Mg^{2+} concentration. Additional experiments at Mg^{2+} concentrations of 2 and 4 mM confirmed this observation (data not shown). As the Mg^{2+} concentration was raised further, the trend of a faster exchange-out rate continued. About 1000 hydrogens per subunit exchanged out more rapidly at 10 mM Mg^{2+} than at 0.5 mM. These observations suggest that the 60S subunit undergoes a gradual change in its conformation as the Mg^{2+} concentration is raised.

The above results were obtained at several different ribosome concentrations and with many different subunit preparations. The total number of hydrogens labeled varied by about 10%, but the hydrogens always exchanged out faster as the Mg^{2+} concentration was raised. This trend was evident even when the subunits were labeled at low Mg^{2+} concentrations, but the difference between the two curves was more erratic (data not shown) possibly because labeling at this Mg^{2+} concentration does not preferentially label the Mg^{2+}-sensitive

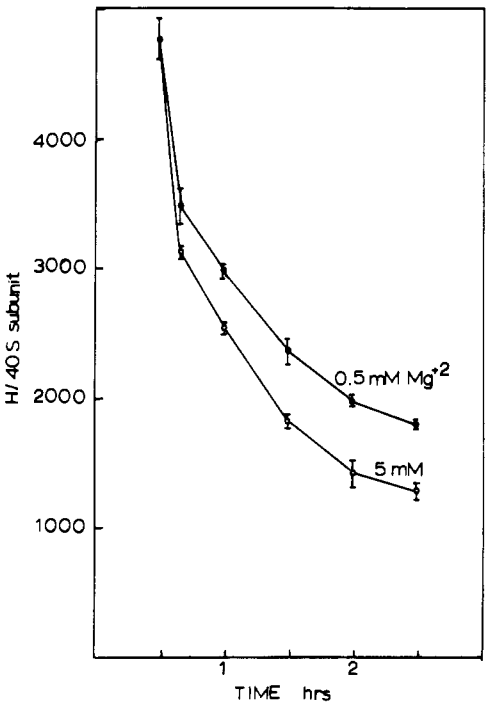

FIGURE 2: Hydrogen exchange-out curves for 40S ribosomal subunits at different Mg^{2+} concentrations. The 40S ribosomal subunits were labeled with ^3H as described. Exchange out was followed at either 0.5 (●) or 5 mM $MgCl_2$ (○).

hydrogen. Graphs of ln number of hydrogens remaining per 60S subunit vs. time did not yield linear plots. It was therefore concluded that these exchange-out curves did not represent first-order exponential decay and that the observed rate must be a mixture of several rates.

In order to show that the effect of Mg^{2+} concentration on the exchange-out rate of ^3H was not due merely to a change in the ionic strength, we obtained hydrogen exchange-out curves under conditions in which the K^+ concentration was raised to 65 mM, giving an increase in ionic strength equivalent to that produced by the increase in Mg^{2+} concentration from 0.5 to 5 mM. The exchange-out curves obtained under these conditions were essentially identical, indicating that the Mg^{2+} effect was not simply the result of an increase in the ionic strength but was due to the presence of the divalent cation itself.

Experiments similar to those described above were carried out with wheat germ 40S subunits at 0.5 and 5 mM Mg^{2+} (Figure 2). Again a class composed of about 500 hydrogens per subunit was observed that exchanged out faster at the higher concentration of Mg^{2+}. Following the loss of these hydrogens in the first 10 min, the curves then paralleled one another.

The above results indicated that changes in the concentration of Mg^{2+} affected the conformation of wheat germ ribosomal subunits. In order to get an idea of the magnitude of these changes, we examined the circular dichroism (CD) spectra of the ribosome and its subunits under a variety of ionic conditions. The CD spectrum observed when 40S subunits were incubated at 5 mM Mg^{2+} and 50 mM K^+ was essentially the same as that obtained when the subunits were incubated at 0.5 mM Mg^{2+} (data not shown). Similar results were obtained with 60S subunits. These results suggest that although changes in the Mg^{2+} concentration caused subtle changes in ribosome structure which can be detected in hydrogen exchange experiments, the overall conformations of the subunits were not grossly altered over the range of Mg^{2+} concentrations tested.

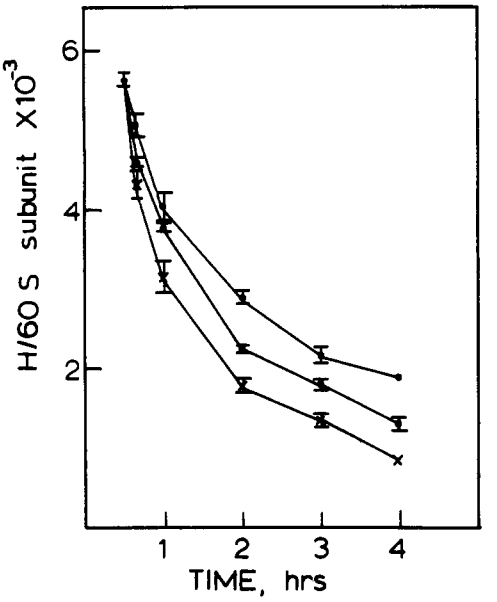

FIGURE 3: Hydrogen exchange-out curves for 60S ribosomal subunits in the presence of divalent cations. The 60S ribosomal subunits were labeled with ^3H as described. Exchange out was followed at either 0.5 mM $MgCl_2$ (●), 5 mM $MgCl_2$ (○), or 0.5 mM $MgCl_2$ and 4.5 mM $CaCl_2$ (×).

Effect of Other Cations on Subunit Conformation. Previous studies (Grunberg-Manago et al., 1981; Sperrazza et al., 1981) have shown that a number of different divalent cations and the polyamines are capable of promoting the association of ribosomal subunits. To investigate whether some of these other cations, like Mg^{2+}, cause the rapid loss of a class of hydrogens, several additional cations were tested. As indicated in Figure 3, Ca^{2+} promoted a faster exchange-out rate for certain hydrogens. In this case, about 1100 hydrogens/subunit were lost more rapidly when the divalent ion concentration was shifted from 0.5 mM Mg^{2+} to 0.5 mM Mg^{2+} and 4.5 mM Ca^{2+}. This value is nearly twice that obtained with a similar increase in the Mg^{2+} ion concentration. Since Ca^{2+} is also more effective than Mg^{2+} in promoting subunit association, these results suggest that the effects being observed are not simply the result of total divalent cation concentration but reflect specific effects of the ions on the structure of the 60S subunit.

Polyamines, like divalent cations, are capable of promoting the association of ribosomal subunits and are important for their activity in protein synthesis. When the polyamines were tested for their effect on ribosomal subunit conformation, they were observed to cause an increase in the exchange-out rate of a class of hydrogens similar to that observed with Mg^{2+}. In these experiments, the addition of 2 mM spermidine or 0.3 mM spermine to subunits in the presence of 0.3 mM Mg^{2+} caused an increase in hydrogen exchange out equivalent to that observed when the Mg^{2+} concentration was raised to 5 mM (data not shown).

Effect of Increased Monovalent Cation Concentration on Subunit Conformation. The results presented above indicate that there is a correlation between the rate of hydrogen exchange and the concentration of multivalent cations. Increases in monovalent ion concentrations giving equivalent increases in ionic strength do not produce noticeable changes in the exchange rate. However, monovalent cations at elevated concentrations have been shown to compete with Mg^{2+} for binding to ribosomes and to promote the dissociation of ribosomal subunits. It was therefore of interest to determine whether higher concentrations of K^+ would be able to prevent the increased rate of hydrogen exchange observed when the

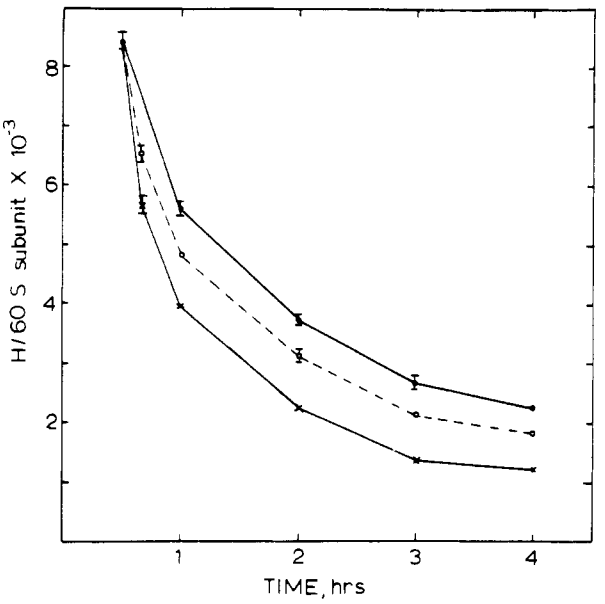

FIGURE 4: Hydrogen exchange-out curves for 60S ribosomal subunits at several Mg^{2+} and K^+ concentrations. The 60S ribosomal subunits were labeled with ^3H, and exchange out was followed as described for samples containing 50 mM KCl and 0.5 mM KCl (●), 50 mM KCl and 3 mM $MgCl_2$ (○), or 500 mM KCl and 3 mM $MgCl_2$ (×).

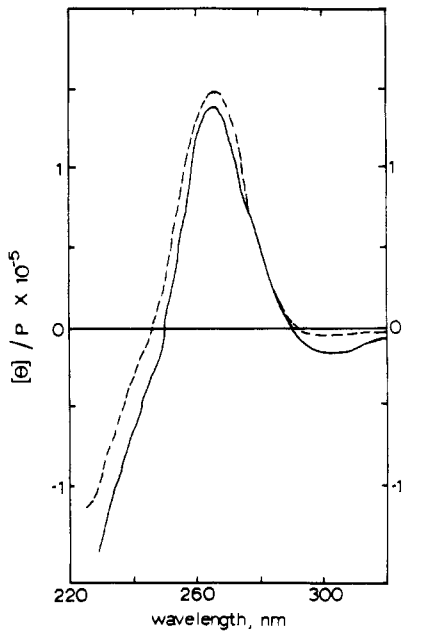

FIGURE 5: Ultraviolet CD spectra of 80S wheat germ ribosomes in the presence (—) or absence (- - -) of glycerol in buffer A containing 50 mM KCl and 5 mM $MgCl_2$. [θ] is reported as degrees centimeter squared per mole.

Mg^{2+} concentration was raised or whether they would in turn cause a new series of subunit conformations. To examine this question, experiments were performed in which 60S subunits were labeled with ^3H at 0.5 mM Mg^{2+} and 50 mM K^+ and exchange out was followed at a variety of Mg^{2+} and K^+ concentrations. As indicated in Figure 4, the addition of high concentrations of K^+ accelerated the rate of H exchange significantly. Further experiments indicated that the effect of increased monovalent ion concentration on the conformation of the 60S subunit was a gradual rather than an abrupt one (data not shown).

The results presented above suggest that the 60S subunit is capable of assuming a variety of conformations. It is difficult to determine how the conformational change caused by K^+ relates to that produced by the multivalent ions. The data do indicate that there is not necessarily any direct correlation between the more rapid exchange-out rate observed at elevated Mg^{2+} concentrations and the ability of the subunits to associate under these conditions.

Effect of Cosolvents on Monomer and Subunit Conformation. Using *E. coli* ribosomes, Hui Bon Hoa et al. (1980) found that cosolvents enhanced the following two aspects of ribosomal activity: the association of subunits to the 70S monomer and the binding of fMet-tRNA to 30S ribosomal subunits. However, binding constants of divalent cations to RNA and nucleotides were not significantly affected by the presence of cosolvent, indicating that cosolvents do not affect the interaction of divalent cations with phosphate groups on the rRNA. The concentrations of Mg^{2+} and Ca^{2+} required for 50% association of the ribosomal subunits were both lowered to the same extent, suggesting that cosolvents were able to substitute to some degree for cations in promoting association of the subunits or that the cosolvents affected the hydration of the subunits in a manner that promoted association.

In the studies reported here, the cosolvent glycerol has been present in all the buffers used for the preparation of wheat germ ribosomes since ribosomes prepared in the preesence of glycerol are more active in polymerization. We were, therefore, interested in determining whether glycerol was able to produce a detectable effect on the conformation of the 80S monosome or the individual subunits. Differences in the conformation of the 80S monomer in the absence and presence of glycerol were first probed by using circular dichroism measurements. As shown in Figure 5, in the absence of glycerol there was a small increase in the maxima at 265 nm and some decrease in ellipticity below 250 nm. The difference in the 265-nm peak is indicative of differences in ribosomal RNA structure due to changes in base pairing or stacking. The differences below 250 nm result from changes in protein conformation. These changes in protein conformation could in turn result in the alteration in RNA structure observed.

The effects of glycerol on the rates of hydrogen exchange in the 80S ribosome were also studied. Ribosomes were labeled with ^3H in buffer A containing 50 mM KCl and 5 mM $MgCl_2$. The sample was then divided into two aliquots; free ^3H_2O was removed from one aliquot, and both ^3H_2O and glycerol were removed from the other sample by centrifugation on Sephadex G-50 columns, and the exchange-out rate of both samples was followed. The hydrogens on the 80S monomer exchanged out more slowly in the presence of glycerol. This difference was small but reproducible (data not shown). The ribosomes remained fully active in polymerization for at least 5 h under the conditions of this experiment.

Similar studies were carried out on the isolated subunits. The CD spectra of 60S subunits showed on significant differences in the absence or presence of glycerol. However, at either 0.5 or 5 mM Mg^{2+}, about 300 hydrogens/subunit exchanged out more slowly in the presence of glycerol (data not shown). The activity of 60S subunits maintained in the absence of glycerol was tested in poly(U)-directed phenylalanine polymerization when supplied with an excess of 40S subunits. The 60S subunits lost approximately 25% of their activity after 3 h in the absence of glycerol.

The CD spectra of 40S subunits showed no differences in the absence or presence of glycerol. However, at either 5 or 0.5 mM Mg^{2+}, about 300 hydrogens/subunit exchanged out more slowly in the presence of glycerol than in its absence (data not shown). In a control experiment, the activity of these subunits in poly(U)-directed polymerization of phenylalanine was assayed with an excess of 60S subunits. After 5 h, the

40S subunits had lost no activity in the absence of glycerol.

Discussion

The lack oif significant differences in the CD spectra of wheat germ ribosomal subunits at 0.5 and 5 mM Mg^{2+} suggests that no gross conformational differences occur as a result of the binding of additional Mg^{2+}. However, the hydrogen exchange data indicate that subtle conformational changes occur as the Mg^{2+} concentration is raised. Hydrogens on both 40S and 60S subunits exchange out more rapidly at elevated Mg^{2+} concentrations (Figures 1 and 2). The current picture of hydrogen exchange is a breathing model (Englander, 1975) which suggests a transient denaturing of the structure under study. This denaturing is envisioned not as a pulsation but as a dynamic process in which part of the structure statistically will be unfolded at any given time. A more open structure will have more frequent and larger breathing sites and thus more rapidly exchanging hydrogens. A tighter structure will contain more hydrogens which are hydrogen bonded or shielded from the solvent by being buried within the molecule, and these hydrogens would exchange more slowly. The increase in the hydrogen exchange rate upon the addition of Mg^{2+} to ribosomal subunits suggests that their conformation must be more open in some regions as a result of Mg^{2+} binding.

Due to the complexity of ribosome structure, it is difficult to determine whether the Mg^{2+}-sensitive hydrogens come primarily from the rRNA or the ribosomal proteins. However, the exchange rates examined strongly suggest that the hydrogens under study are those of the amide bond in the protein backbone (Woodward & Hilton, 1977). One can imagine that the binding of additional Mg^{2+} to the ribosomal subunits might reduce the electrostatic repulsion between portions of the rRNA, allowing for a tightening of its structure and perhaps more internalization of the RNA. This conformational change might result in more exposure of the proteins on the surface of the subunit, increasing the exposure of the peptide backbones and the rate of exchange of the amide hydrogens.

Most of the conditions that were observed in this study to produce an increase in hydrogen exchange also promote subunit association. However, large increases in the K^+ concentration which promote subunit dissociation also resulted in an enhanced rate of hydrogen exchange. These hydrogens might reflect K^+-sensitive hydrogens which are representative of a native, more open structure. The increased rate of hydrogen exchange thus does not correlate with the ability of the subunits to associate or with charge neutralization of the phosphates of the RNA backbone (Sperrazza & Spremulli, 1983). At the K^+ concentration used here, charge neutralization by Mg^{2+} is complete by 3 mM Mg^{2+}, yet the increase in exchange out is seen at Mg^{2+} concentrations up to 10 mM. From the hydrogen exchange data presented, it is clear that increased concentrations of divalent ions or polyamines cause a range of subtle conformational changes in the ribosomal subunits, some of which may permit subunit association.

Hydrogen exchange studies on *E. coli* 50S subunits (Bonnet et al., 1980) have shown an effect opposite to that observed with the eukaryotic ribosomes used in this study; i.e., there was a decrease in the hydrogen exchange rate as the Mg^{2+} concentration was raised. The marked difference in the effect of increasing Mg^{2+} concentration on the hydrogen exchange rate with *E. coli* and wheat germ ribosomes suggests that changes in divalent cation concentration might have quite different conformational consequences for prokaryotic and eukaryotic ribosomes.

The conformation of the wheat germ ribosomal subunits is influenced not only by the concentration of the cations present but also by cosolvents such as glycerol. Gekko & Timasheff (1981a,b) observed that proteins are preferentially hydrated in the presence of glycerol which would compress nonpolar residues to the interior of the molecule and favor the more folded state of the protein. The results described in this paper indicate that hydrogens exchanged out more slowly in the presence of glycerol when either the 80S monomer or the 40S or 60S ribosomal subunits were studied. The decrease in the rate of hydrogen exchange indicates a decrease in solvent accessibility due to increased hydrogen bonding, steric constraints, or changes in microenvironmental effects of local apolar or charged groups (Gekko & Timasheff, 1981a,b). Glycerol, therefore, favors a less denatured state of the ribosome.

The results presented here indicate that the eukaryotic ribosome is a dynamic structure that is capable of assuming a very wide range of related conformations all influenced by the nature and concentrations of the cations present. The influence of cations on the structure of the ribosome and on the various reactions of protein biosynthesis emphasizes the necessity of viewing the ribosome not only in terms of its RNA and protein components but also in the context of the various cations associated with the structure.

Registry No. Spermine, 71-44-3; spermidine, 124-20-9; calcium, 7440-70-2; magnesium, 7439-95-4; hydrogen, 1333-74-0; potassium, 7440-09-7; glycerol, 56-81-5.

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Transformed Mouse Glucocorticoid Receptor: Generation and Interconversion of the 3.8S, Monomeric and 5.2S, Oligomeric Species†

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ABSTRACT: Recent studies have implicated subunit dissociation as a possible mechanism of glucocorticoid receptor transformation [Vedeckis, W. V. (1983) *Biochemistry 22*, 1983-1989; Raaka, B. M., & Samuels, H. H. (1983) *J. Biol. Chem. 258*, 417-425]. While it is becoming increasingly evident that the untransformed (non-nuclear-binding and non-DNA-binding) glucocorticoid receptor from mouse AtT-20 cells is a 9.1S oligomeric species (M_r 290 000-360 000), two transformed species have been described for this receptor. One of these has a sedimentation coefficient of 5.2 S (on molybdate-containing gradients), while the smallest nonproteolyzed, monomeric subunit is 3.8 S. The present study was undertaken to determine which is the most common form generated both in vitro and in vivo and the structural relationship between these two forms. A wide variety of in vitro transformation protocols all yielded the 5.2S form when analyzed on molybdate-containing sucrose gradients by using a vertical tube rotor. Kinetic studies showed that the appearance of the 5.2S form coincided precisely with the appearance of transformed receptor, as defined by DEAE-cellulose elution. Furthermore, when the 3.8S and 5.2S peaks were collected from sucrose gradients directly, they were transformed receptors as defined by both DEAE-cellulose and DNA-cellulose chromatography, while the 9.1S sucrose gradient peak was untransformed when the same criteria were used. The 3.8S monomer, when isolated from high-salt sucrose gradients and then desalted, reverted to the 5.2S form (molybdate-containing gradients) or a 6.6S form (low-salt, molybdate-free gradients). Additionally, when the receptor was transformed in vivo by incubating mouse AtT-20 cells with radioactive hormone at 37 °C, the transformed receptor obtained was the 5.2S species. Treatment of the in vivo generated transformed receptor with ribonuclease A caused a decrease in sedimentation coefficient of the 5.2S form to the 3.8S species, suggesting that RNA may be a component of the intermediate, 5.2S transformed receptor complex. The appearance of this 5.2S, intermediate-transformed receptor species under a wide variety of experimental conditions suggests that it is a discrete, and perhaps physiologically relevant, entity.

After steroid hormone ligands are bound, steroid receptor proteins undergo a process called transformation,[1] which results in a conversion of the protein from a non-DNA-binding and non-nuclear-binding form to a species that binds to genomic constituents. Although it has been studied for many years, the molecular mechanism of steroid receptor transformation has remained obscure. Recent studies on transformation of the mouse and rat glucocorticoid receptors (GC-R)[2] have suggested that this process may involve the dissociation of an oligomeric, 9-10S, untransformed complex into transformed receptor subunits (Vedeckis, 1981, 1983a,b; Holbrook et al., 1983; Raaka & Samuels, 1983; Eastman-Reks et al., 1984).

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[1] We have previously used the more traditional term, activation, to describe this process. However, as is shown here and elsewhere (Holbrook et al., 1983; Raaka & Samuels, 1983; Vedeckis, 1983b; Eastman-Reks et al., 1984), a physicochemical alteration in structure ("change in form") does occur in this process. Therefore, as suggested by Wheeler et al. (1981), we will use the term transformation to indicate the change in receptor form accompanying its conversion to a species which binds to nuclear constituents. Activation will be reserved for the conversion of the receptor from a form which is incapable of binding ligand to one which can bind hormone.

[2] Abbreviations: GC-R, glucocorticoid receptor; Tris, tris(hydroxymethyl)aminomethane; Dex, dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione); Na_2EDTA, disodium ethylenediaminetetraacetate; DEAE, diethylaminoethyl; SDS, sodium dodecyl sulfate; RBF, receptor binding factor; TA, triamcinolone acetonide (9α-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-acetal with acetone).

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