

**EFFECTS OF OSMOLARITY, IONS AND COMPATIBLE OSMOLYTES
ON CELL-FREE PROTEIN SYNTHESIS**

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Abbreviations used: ATA2, amino acid transporter A2; BGT-1, betaine/GABA transporter 1;
SMIT, sodium/myo-inositol transporter ; TauT, taurine transporter.

SUMMARY

To mimic what might happen in cells exposed to hypertonicity, the effects of increased osmolarity and ionic strength on cell-free protein synthesis have been examined. Translation of globin mRNA by rabbit reticulocyte lysate decreased by 30-60% when osmolality was increased from 0.35 to 0.53 osmol/kg H₂O by the addition of NaCl, KCl, CH₃COONa or CH₃COOK. In contrast, equivalent additions of the compatible osmolytes betaine or myo-inositol caused a 40-50% increase in the rate of translation, whilst amino acids (50–135 mM) that are transported via System A had no significant effect. Addition of 75 mM KCl caused a dramatic fall in the amount of the 43S pre-initiation complex, whereas it was totally preserved when osmolarity was similarly increased by the addition of 150 mM betaine. The formation of a non-enzymic initiation complex between rabbit [³H]Phe-tRNA, poly(U) and the 80S ribosomes was unaffected by the addition of 75 mM NaCl or KCl, but translation of the complex decreased by 70%. Density gradient centrifugation of reticulocyte extracts translating endogenous mRNA revealed that addition of 150 mM betaine had no effect, whereas addition of 75 mM KCl caused a marked decrease in the polysome peak, concomitant with an increase in the proportion of 80S ribosomes and ribosomal subunits, even when elongation was inhibited with fragment A of diphtheria toxin. These results are consistent with the notion that both initiation and elongation are inhibited by unusually high concentrations of inorganic ions, but not by the compatible osmolytes betaine or *myo*-inositol.

Key words:

Betaine, myo-inositol, translation, hyperosmolarity, ionic strength

INTRODUCTION

Animal cells exposed to hypertonic conditions undergo an immediate shrinkage that causes a marked increase in cellular concentrations of Na^+ and K^+ . This is usually followed by a regulatory volume increase, mediated by the uptake of Na^+ and K^+ via $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport and the accompanying influx of water. The rise in cellular Na^+ concentration presumably increases NaK-ATPase activity, because the net result is a rise in the cell content and concentration of K^+ . Many mammalian cells in culture then adapt to the hyperosmolarity by accumulating “compatible osmolytes”, such as methylamines, polyols, and some amino acids or amino acid derivatives [1,2]. This adaptation involves changes of specific gene expression that result in an increased synthesis of transporters for neutral amino acids (ATA2) [3], betaine (BGT-1), myo-inositol (SMIT), taurine (TauT), or of aldose reductase to catalyse the synthesis of sorbitol [2]. These changes are usually explained in terms of the need to replace the inorganic ions initially concentrated in the cells with compatible osmolytes that, unlike the inorganic ions, do not perturb macromolecular structures [4]. The main evidence supporting this latter contention comes from a number of demonstrations of protective effects of compatible osmolytes on enzyme structure and function. These include the counteraction of the inhibition of malate dehydrogenase by high concentrations of NaCl, the renaturation of acid-denatured lactate dehydrogenase, changes in the accessibility of interior thiol groups in glutamate dehydrogenase, and in the quaternary structure of phosphofructokinase [4].

Although it seems reasonable to extrapolate from the latter findings to the situation inside osmotically shrunken cells, it would be preferable to obtain some more direct evidence. The effects of inorganic ions and compatible osmolytes on cell protein synthesis are particularly important, because impairment of cell protein synthesis occurs immediately after cells shrink in hypertonic media. For example, Petronini et al. [5,6] showed that after 30 min exposure of either chick embryo fibroblasts or porcine endothelial cells to hypertonic (0.5 osmol/kg H_2O) medium, cell protein synthesis was inhibited by more than 40%. When hypertonicity was maintained, however, induced activity of amino acid transport system A was detectable after 2-3 h and restoration of the rate of cell protein synthesis occurred as the cells accumulated amino acids and lost K^+ . Induction of the other transport systems and marked accumulation of the other compatible osmolytes followed later, after 6 – 24 h.

Some correlations between changes of cell cation concentrations and rates of protein synthesis have been noted. For example, Carrasco and Smith [7] showed that inhibition of the initiation step of the translation of globin mRNA during viral infection of the host cell was

accompanied by increased concentration of cellular Na^+ . Similarly, impairment of the rate of protein synthesis in chick-embryo fibroblasts occurred when there was any significant change, an increase or decrease, of intracellular Na^+ concentration [8]. Also, the inhibition of protein synthesis caused by hypertonic culture of SV-3T3 cells or chick embryo fibroblasts was largely prevented by the addition of 10-25 mM betaine [9], which is in keeping with the general ideas about compatible osmolytes. We have now extended these findings by examining the effects of various concentrations of inorganic ions and compatible osmolytes on the cell-free translation of mRNA, endogenous and synthetic. The aim was to mimic with a simplified translation system in vitro what might happen in whole cells exposed to hypertonicity.

EXPERIMENTAL

Materials

$[\text{}^3\text{H}]$ leucine (66 Ci/mmol), [methyl- ^3H]methionine 83 Ci/mmol, $[\text{}^3\text{H}]\text{NAD}^+$ (37 Ci/mmol) and $[\text{}^3\text{H}]$ phenylalanine (120 Ci/mmol) were obtained from Amersham Pharmacia Biotech (Bucks, U.K.). Micrococcal nuclease, creatine phosphokinase, spermidine and all the other chemicals were purchased from Sigma Chemical Co. (St.Louis, MO, U.S.A.).

Methods

Translation of endogenous mRNA by rabbit reticulocyte lysate

Rabbit reticulocyte lysate was prepared as described by [10]. Translation of endogenous mRNA in vitro by the unfractionated rabbit reticulocyte lysate was performed in reaction mixtures (125 μl) containing 30 mM Hepes-KOH, pH 7.5, 80 mM KCl, 1.8 mM magnesium acetate, 50 μM of each amino acid except leucine, 1 mM ATP, 0.25 mM GTP, 5 mM creatine phosphate, 0.18 mg/ml creatine phosphokinase, 0.5 mM dithiothreitol, 0.4 mM spermidine, 0.24 μM (2 μCi) $[\text{}^3\text{H}]$ leucine and 50 μl of lysate [11]. Where indicated, the osmolarity of the reaction mixture was increased by addition of KCl, NaCl, CH_3COOK , CH_3COONa , betaine, myo-inositol or amino acids. The complete mixture was incubated for 5 min at 28°C and then a 62.5 μl sample was taken and added to 1 ml of 0.1 M KOH, the solution was decolourised with two drops of 35% (w/v) H_2O_2 and 1 ml of 20% (w/v) trichloroacetic acid added. The precipitate was collected on a Whatman GF/C filter and its radioactivity measured by scintillation counting. The osmolality of the residual reaction mixture was measured with a vapour pressure osmometer (Wescor), the standard mixture being 0.352 osmol/kg H_2O . Under these standard conditions the $[\text{}^3\text{H}]$ leucine incorporated was 57599 dpm \pm 7225 (mean \pm S.D., n = 15).

Determination of the 43S pre-initiation complex in unfractionated reticulocyte lysate

To measure the 43S pre-initiation complex, translation of endogenous mRNA by unfractionated rabbit reticulocyte lysate was carried out as described above except that the solution contained 0.6 μM [^3H]methionine as the labelled amino acid. After incubation for 2 min at 28°C, the polysome profiles of the reaction mixtures were determined by centrifugation in a sucrose density gradient (see below) and 1 ml fractions were collected. The RNA, including aminoacyl-tRNA, present in each fraction was precipitated by addition of 2 volumes of 2% cetyltrimethylammonium bromide and 2 volumes of 0.5 M sodium acetate (pH 5.2) containing 0.5 mg/ml carrier calf liver RNA [12]. The precipitate was collected on Whatman GF/C filters, washed extensively with water and its radioactivity measured by scintillation counting.

Micrococcal nuclease treatment of rabbit reticulocyte lysate

Rabbit reticulocyte lysate was digested with micrococcal nuclease, to destroy endogenous mRNA and tRNA, as described by Jackson and Hunt [13].

Fractionation of rabbit tRNA

Total tRNA was isolated from rabbit liver with the use of classic phenol extraction procedures and differential precipitation [14]. The tRNA was fractionated on benzoylated DEAE-cellulose and the pure tRNA^{Phe} obtained was charged with [^3H]phenylalanine (41250 dpm/pmol) according to Hultin and Näslund [15]. A tRNA pool minus tRNA^{Phe} was reconstituted by mixing the separated tRNAs obtained from benzoylated DEAE-cellulose according to the relative amount of each tRNA. The amount of lysate assayed for protein synthesis contained approximately 4 μg of tRNA, corresponding to 0.2 μg (8 pmol) of tRNA^{Phe} [16,17].

Poly(U) translation by rabbit reticulocyte lysate treated with micrococcal nuclease

Translation of poly(U) in vitro by rabbit reticulocyte lysate treated with micrococcal nuclease was performed as described above for the translation of endogenous mRNA, with the following modifications. A non-enzymic initiation complex between [^3H]Phe-tRNA (8 pmol), poly(U) (145 μg) and the 80S ribosomes present in the lysate was obtained during a preliminary incubation in 7 mM $(\text{CH}_3\text{COO})_2\text{Mg}$ [18-20]. After 10 min at 28°C, 3.8 μg of rabbit tRNAs (without tRNA^{Phe}) and the other components of the translation mixture (except [^3H]leucine) were added. The Mg^{2+} concentration was then reduced to 2.5 mM by diluting the mixture and after a further 5 min incubation at 28°C the extent of phenylalanine polymerisation and the osmolality of each sample were measured as described above. Under normal osmotic conditions (0.330 osmol/kg

H₂O) the [³H]phenylalanine incorporated was 68550 ± 5976 (mean ± S.D., n = 8). The formation of the non-enzymic initiation complex was assessed in parallel tubes by collecting the ribosome-bound radioactivity on Millipore filters [21]. The synthesis of oligopeptides during the formation of the initiation complex is likely, since the pre-incubation mixture contained the whole lysate, in which elongation factors and small amounts of ATP and GTP were present. Protein synthesis measured with samples of the pre-incubation mixture, however, gave values similar to the background radioactivity values.

Determination of polysome profiles

Translation of endogenous mRNA by unfractionated rabbit reticulocyte lysate was carried out as described above, except that the solution contained 50 μM NAD⁺ and non-radioactive leucine. Where indicated, the mixtures were treated with 0.2 μM fragment A of diphtheria toxin (see below), or made hyperosmolar by the addition of 75 mM KCl, or both. The polysome profiles of the reaction mixtures were determined by centrifugation in a sucrose density gradient (40 ml, 15-30%, w/v) at 70000 g for 15 h in a Beckman SW 28 rotor. Rabbit reticulocyte ribosomal sub-units [22] and *Artemia salina* 80S ribosomes [23] were used as standards.

ADP-ribosylation of elongation factor 2 (EF2)

Fragment A was prepared as described by Chung and Collier [24] from diphtheria toxin purified by the method of Pope and Stevens [25]. ADP-ribosylation of elongation factor 2 (EF2) was measured with 0.2 μM diphtheria toxin fragment A and 5 μM [³H]NAD⁺ in the reaction mixtures described above for the translation of endogenous mRNA by unfractionated reticulocyte lysate, modified to contain non-radioactive leucine. After 5 min incubation at 28°C the acid-insoluble radioactivity of 20 μl samples was collected on Whatman GF/C filters and counted.

RESULTS AND DISCUSSION

Effects of solutes on cell-free protein synthesis

Fig. 1 shows the effect of increased osmolarity and ionic strength on the translation of globin mRNA by unfractionated rabbit reticulocyte lysate. Increased osmolarity alone clearly did not inhibit translation, because high concentrations of either betaine or myo-inositol actually increased protein synthesis. When both osmolarity and ionic strength were increased by addition of NaCl or KCl, however, the rate of translation fell, NaCl inhibiting more than equimolar additions of

KCl. CH_3COONa mimicked the effect of NaCl, whereas only the highest concentration of CH_3COOK inhibited.

Some of the inhibitory effects of these inorganic ions are in keeping with the findings of early studies that established optimal experimental conditions for translation in cell-free systems. For example, translation specifically requires the K^+ concentration to be within a relatively narrow range, but tolerates higher concentrations of CH_3COOK than of KCl. The actions of salts on the conformation and function of macromolecules are usually related to the destabilising or stabilising properties of their anions and cations according to the Hofmeister series [26,27]. The Hofmeister series for cations indicates that both Na^+ and K^+ destabilise macromolecules, Na^+ being more perturbing than K^+ . The series for anions indicates that Cl^- also has a destabilising effect, whereas CH_3COO^- has stabilising properties. Since the actions of the different ions are additive, however, the overall effects of the salts are not simply predicable. The results in Fig. 1 are as expected from the Hofmeister series for the cations (NaCl versus KCl, and CH_3COONa versus CH_3COOK), but the effects of the anions are not so clear cut. The different effects of KCl and CH_3COOK can be explained in terms of the stabilising CH_3COO^- counteracting the inhibition caused by the mildly destabilising K^+ , whereas Cl^- increases destabilisation. On the other hand, the perturbing effect of Na^+ appears to be unmodified by the nature of the accompanying anion (NaCl versus CH_3COONa). However, the specific requirements for K^+ and Mg^{2+} make it difficult to distinguish between specific and general salt effects. For example, added Na^+ would be expected to inhibit by competing with K^+ . It is clear, however, that the increased concentrations of the common physiologically important ions, Na^+ , K^+ and Cl^- inhibited cell-free translation, whereas equivalent concentrations of the typical compatible osmolytes betaine and myo-inositol did not. With NaCl or KCl at osmolality values close to 0.5 osmol/kg H_2O , the extent of inhibition in the cell-free system was almost 60%, well within the 40 - 75% range found when intact cells were exposed to the same osmolality [6,28].

To mimic cell shrinkage a little more closely, we checked the effect of simultaneously increasing the concentrations of everything in the reaction mixture by reducing its final volume to 63% of that normally used. This inhibited translation by about 18%, compared with the approximately 25% inhibition predicted by the curve in Fig. 1 from the increased KCl concentration (80 to 127 mM).

In general, these results are in keeping with the notion that impairment of translation in whole cells exposed to hypertonicity is the consequence of the increase in the concentrations of inorganic ions, particularly Na^+ , K^+ and Cl^- . Such increases are unavoidable during the initial rapid shrinkage of the cells, and are only modified in detail, not dissipated, during regulatory volume

increase brought about by $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transport and Na^+/H^+ exchange. The latter processes are also likely to change the relative concentrations of the ions, which might also be inhibitory.

Fig. 2 shows the result of performing cell-free protein synthesis in the presence of different combinations of KCl and betaine, such that the total osmolality remained constant at about 0.5 osmol/kg H_2O . Clearly, the gradual iso-osmotic replacement of the inhibiting KCl with betaine restored protein synthesis to the control rate. We also checked the effects of high concentrations of amino acids on cell-free protein synthesis because, as explained in the introduction, many intact cells respond to hypertonic incubation by increasing their uptake of amino acids via transport system A. With the exception of valine, which was inhibitory when present at 135 mM, the neutral amino acids tested caused no significant inhibition of the rate of translation (Table 1). In contrast, an equimolar mixture of lysine and glutamic acid strongly inhibited protein synthesis. Since system A transports small neutral amino acids, not the larger more hydrophobic ones or those bearing a net charge, such as lysine and glutamate [29], these findings support the concept that induction of system A provides cells with amino acids as compatible osmolytes. The finding that only certain amino acids behave as compatible solutes is in keeping with early observations of enzyme activity being unaffected by amino acids such as glycine, alanine or proline, but markedly impaired by lysine or arginine (see [4]).

Effect of osmolarity and ionic strength on the initiation and elongation steps of translation

In the experiments illustrated in Fig. 1, a marked inhibition of protein synthesis by NaCl and KCl was observed with the use of unfractionated rabbit reticulocyte lysate, a system consisting mainly of polysomes engaged in the elongation step of protein synthesis. Several reports, however, indicate that the initiation step of translation is inhibited in whole cells exposed to hypertonic conditions [7, 30-32]. Lu et al. [31], for example, showed that osmotic stress induced the phosphorylation of eukaryotic initiation factor 2α (eIF2 α) in erythroid cells, thus blocking the assembly of the 43S pre-initiation complex. The formation of the 43S pre-initiation complex in unfractionated rabbit reticulocyte lysate can be easily detected by labelling the 40S subunit with the initiator [^3H]Met-tRNA_f [12], so we examined the sensitivity of this process to increased osmolarity. Fig 3 shows that addition of 75 mM KCl caused a dramatic fall in the amount of the 43S pre-initiation complex, whereas it was totally preserved when osmolarity was similarly increased by the addition of 150 mM betaine. On the other hand, the persistence of the first peak of radioactivity, corresponding to the [^3H]Met-tRNAs, indicates that the aminoacylation step was not affected by the added KCl.

To test whether the elongation, not just the initiation, is affected by increased ionic strength, translation from a non-enzymic initiation complex was examined. A non-enzymic initiation complex between rabbit [³H]Phe-tRNA, poly(U) and the 80S ribosomes present in the micrococcal nuclease-treated lysate was formed first during a preliminary incubation. Then poly(U) translation was measured in the presence and absence of added salts or compatible osmolytes. Fig. 4 shows that increased concentrations of NaCl and KCl caused an inhibition of polyphenylalanine synthesis similar to that observed with globin synthesis (Fig. 1). Checks with the highest concentration of the salts used in Fig. 4 showed that formation of the non-enzymic initiation complex was not affected (data not shown), perhaps because poly(U) is a relatively robust molecule having less stringent folding requirements than endogenous mRNA. Thus it seems clear that the elongation step also is inhibited by increased ionic strength due to added KCl or NaCl. In contrast, neither the betaine nor the myo-inositol significantly affected the rate of elongation (Fig. 4), suggesting that their stimulation of protein synthesis shown in Fig.1 resulted from an effect on initiation alone.

Site of action of destabilising ions

We examined the effect of increased osmolarity and ionic strength on polysome structure by density gradient centrifugation of rabbit reticulocyte extracts translating endogenous mRNA (Fig. 5). The polysome profile of the standard reaction mixture (*A*) was compared with those of mixtures made about 0.5 osmol/kg H₂O by the addition of 150 mM betaine (*B*), or 75 mM KCl (*C*). Addition of betaine did not affect the polysome profile, whereas KCl caused a decrease in the polysome peak concomitant with an increase in the proportion of 80S ribosomes and ribosomal subunits. Thus high concentrations of the salt, but not of betaine, markedly decreased the number of polysomes. Since this effect of KCl could be explained either by inhibition of polysome formation or by disaggregation of existing polysomes, we tested the effect of inhibiting elongation with fragment A of diphtheria toxin. This inhibits the elongation step of translation by ADP-ribosylation of EF2, with no obvious effect on ribosome structure. The activity of fragment A was evaluated directly by measuring the transfer of the [³H]ADP-ribose moiety of NAD to the EF2 present in the translation mixture: it was not affected by the addition of 75 mM KCl (data not shown). This allowed us to check the effect of increased salt concentration on polysomes when nascent peptides could not be completed because EF2 was unavailable. Addition of fragment A of diphtheria toxin to the standard reaction mixture caused a strong inhibition (91%) of protein synthesis, “freezing” the proportion of polysomes carrying nascent peptides (Fig. 5D). When 75 mM KCl was also added, however, the effect was very similar to that seen in the absence of the toxin inhibitor - a loss of polysomes accompanied by an increase in the proportion of 80S ribosomes and ribosomal subunits

(Fig. 5E). This indicates that the high salt concentration does cause disaggregation of existing polysomes, though there are at least two possible interpretations of how this occurs. High ionic strength might simply cause the ribosome subunits to come off the mRNA and then form an increased pool of free 80S ribosomes. Alternatively, the high salt concentration might perturb the polysome structure sufficiently to enable endogenous nucleases to attack the mRNA, splitting off ribosomes still attached to small remnants of the mRNA.

Effect of hypo-osmolarity

As expected from known properties of the reticulocyte lysate system, progressive decrease of the 80 mM KCl normally present in the assay buffer caused the rate of protein synthesis to fall. In a single experiment, it decreased almost linearly with the KCl concentration, to $35 \pm 2\%$ (mean \pm SD, $n=3$) of the control when KCl was omitted from the assay buffer. The addition of betaine to maintain constant osmolarity afforded some protection, giving a value of $51 \pm 4\%$ under the same conditions. Hence, although the specific requirement for K^+ dominates, decreased osmolarity seems to be partly responsible for this effect. We did not pursue this aspect further, however, because there is no evidence for accumulation of compatible osmolytes following cell swelling, only the reverse.

Physiological implications

This simple cell-free system is limited by the obvious differences between it and cell cytosols, with their higher viscosity, macromolecules, cytoskeleton and organelles. Nevertheless, the results described above are consistent with the notion that both the initiation and elongation steps of protein synthesis are inhibited by unusually high concentration of inorganic ions, but not when the osmolarity is similarly increased by the addition of a compatible osmolyte, such as betaine or myo-inositol. We have detected both increased Na^+ concentration (100%) and inhibition of protein synthesis (40%) as soon as 5 minutes after exposure of porcine endothelial cell to hypertonicity (0.5 osmol/ kg H_2O) (unpublished results), suggesting that these are important early responses to cell shrinkage. These cells undergo apoptosis during prolonged culture in hypertonic media depleted of compatible osmolytes, whereas in their presence hypertonicity causes induction of the membrane transporters for these osmolytes, the cells accumulate them and survive [33]. Thus the results reported here provide new experimental evidence that supports the usual interpretation, outlined in the introduction, of the responses many cells show to hypertonic stress. However, as discussed previously [33], it remains unclear why so many cells possess this ability to adapt to a circumstance that they apparently are unlikely to encounter.

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Table 1. Effect of high concentrations of amino acids on the translation of globin mRNA by rabbit reticulocyte lysate.

Translation was measured in terms of the rate of incorporation of [³H]-labelled leucine into protein, as described under Experimental, and expressed as a percentage of the value obtained with the standard reaction mixture (0.352 osmol/kg H₂O). . Mean values (\pm S.E.M.) from four experiments are given. The standard medium was made hyperosmolar by addition of amino acids, as indicated, giving final osmolalities (mean value \pm S.D.) of 0.383 ± 0.004 osmol/kg H₂O with 50 mM and 0.454 ± 0.019 osmol/kg H₂O, with 135 mM. (*An equimolar mixture of glutamic acid and lysine.)

Amino acid	Cell-free protein synthesis (%)	
	50 mM	135 mM
Alanine	107 \pm 3	103 \pm 1
Serine	138 \pm 3	107 \pm 4
Glutamine	117 \pm 3	96 \pm 2
Threonine	104 \pm 10	99 \pm 1
Glycine	136 \pm 2	100 \pm 5
Proline	117 \pm 14	111 \pm 10
Valine	81 \pm 4	44 \pm 3
Glutamic acid + lysine *	43 \pm 1	4 \pm 1

Figure 1. Effects of increased osmolarity and ionic strength on the translation of globin mRNA by rabbit reticulocyte lysate.

Translation was measured in terms of the rate of incorporation of [³H]-labelled leucine into protein, as described under Experimental, and expressed as a percentage of the value obtained with the standard reaction mixture (0.352 osmol/kg H₂O). The latter was made hyperosmolar by addition of 10-75 mM KCl (filled triangles), NaCl (filled circles), CH₃COOK (open triangles) or CH₃COONa (open circles), or of 20-150 mM betaine (filled squares) or myo-inositol (open squares). Final osmolalities were measured as described under Experimental. Mean values (± S.E.M.) from 3 measurements are given.

Figure 2. Effect on translation of hyperosmolarity produced with different ratios of KCl and betaine.

The experiments were performed as describe in the legend to Fig.1, except that hyperosmolarity was produced by the combined addition of KCl (0-75 mM) and betaine (150-0 mM), maintaining osmolality at about 0.5 osmol/kg H₂O. The rates of translation are expressed as percentages of the value obtained with the standard medium (0.352 osmol/kg H₂O). Mean values (± S.E.M.) from 3 measurements are given.

Figure 3. Formation of 43S pre-initiation complex

Labelling of the 40S subunit with [³H]methionine was measured in unfractionated rabbit reticulocyte lysate as described under Experimental. Control reaction mixture (open circles), reaction mixtures made hypertonic by the addition of 75 mM KCl (filled triangles), or 150 mM betaine (filled squares).

Figure 4. Effect of increased ionic strength on the translation of poly(U) in vitro

Rabbit reticulocyte lysate was treated with micrococcal nuclease and then used to translate poly(U), as described under Experimental. The media were made hyperosmolar by addition of 10-75 mM KCl (filled triangles) or NaCl (filled circles), or of 20-150 mM betaine (filled squares) or myo-inositol (open squares). The rates of translation are expressed as percentages of the value obtained with the standard medium (0.352 osmol/kg H₂O). Mean values (± S.E.M.) from 3 measurements are given.

Figure 5. Effects of increased osmolality and ionic strength on polysomes

Rabbit reticulocyte extracts translating endogenous mRNA were analysed by density gradient centrifugation as described under Experimental. (A), standard mixture (0.352 osmol/kgH₂O); (B), medium made about 0.5 osmol/kgH₂O by addition of 150 mM betaine; (C), medium made about 0.5 osmol/kgH₂O by addition of 75 mM KCl; (D), standard mixture treated with diphtheria toxin fragment A; (E), as in (D), but also made about 0.5 osmol/kgH₂O by addition of 75 mM KCl.









