# DIRECT EFFECTS OF 1,3-DIAMINOPROPANE ON RETICULOCYTE LYSATE PROTEIN SYNTHESIS

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## 1. Introduction

The precise functions of polyamines in mammalian cells have not yet been established, but the strong association between the rate of cell growth and proliferation and the rates of putrescine and spermidine synthesis suggests that they have an important regulatory role [1]. The short half-lives of some enzymes required for polyamine synthesis, in particular the first enzyme of the pathway ornithine decarboxylase, and the consequent very rapid and extensive variation in its activity, reinforce this suggestion.

Specific inhibitors of the production of polyamines would be of great value in further elucidating their functions, and one drug which has been widely used in this respect is the putrescine analogue 1,3-diaminopropane [2–17]. Diaminopropane does not directly affect the activity of isolated ornithine decarboxylase, but greatly reduces the activity of the enzyme in the intact animal or cell [2,8,10,11]. As the enzyme synthesis can also be inhibited, apparently by a feedback repression mechanism, by low concentrations of its natural product putrescine [4,18,19], it seemed likely that diaminopropane was acting by a similar mechanism, although substantially higher concentrations of the analogue were required.

However, it has been inferred from studies with virus-infected baby hamster kidney cells that the effects of diaminopropane may be due at least in part of a direct effect on cellular protein synthesis [20]. The dramatic effects on ornithine decarboxylase may thus be a function of its unusually rapid turnover rate rather than, or in addition to, any specific inhibition of its synthesis by a feedback repression mechanism.

Here we report that diaminopropane, at concentrations within the range normally used, does indeed have a direct inhibitory effect on the rate of protein synthesis by a cell-free system from rabbit reticulocytes. The effect of diaminopropane is not due to any antagonistic effect against naturally occurring polyamines, but is simply a cation effect consequent upon the high levels used. In the absence of normal divalent cations, diaminopropane will itself support protein synthesis in this system. These results lend strong support to the doubts about the specificity of diaminopropane as an inhibitor of polyamine synthesis raised in [20].

## 2. Materials and methods

Spermidine and 1,3-diaminopropane were obtained as the hydrochlorides from Sigma (St Louis MO) and dissolved in $H_2O$.

Procedures for the preparation of rabbit reticulocyte lysate and for determination of [$^{14}$C]leucine incorporation into protein were as in [21]. Each 30 $\mu$l incubation contained the following components: lysate, 15 $\mu$l; KCl, 100 mM; Tris–HCl (pH 7.6) 25 mM; ATP, 1 mM; GTP, 0.2 mM; creatine phosphate, 4 mM; creatine phosphokinase, 100 $\mu$g/ml; leucine, 25 $\mu$M including 1 $\mu$Ci/ml [$^{14}$C]leucine; the other 19 amino acids, each at 75 $\mu$M; haemin, 40 $\mu$M. The amounts of $Mg^{2+}$, spermidine and diaminopropane added are indicated for each experiment. After incubation for 45 min at 30°C, 10 $\mu$l aliquots were taken for determination of [$^{14}$C]leucine incorporation into protein. At least 4 replicate determinations were made for each value, and the error bars indicate the range of values obtained.

## 3. Results and discussion

Preliminary experiments demonstrated that addition of 1,3-diaminopropane to reticulocyte lysate cell-free systems led to substantial inhibition of protein synthesis (fig.1). Significant inhibition was seen with as little as 0.5 mM diaminopropane, while 50% inhibition required only 1.5 mM. As at least 1 mM diaminopropane must be added to cultured cells to inhibit ornithine decarboxylase activity and levels used have been generally 5–13 mM [13–17], while intracellular levels >1 mM are achieved with the injection regimes used for intact animals [6–8], a direct inhibition of protein synthesis seems probable.

As the effects of polyamines are frequently synergistic with those of $Mg^{2+}$, and the rate of protein synthesis is critically dependent on the $Mg^{2+}$ concentration, the effects of independent variation of the concentration of diaminopropane, $Mg^{2+}$ and spermidine, the natural polyamine implicated in protein synthesis in the reticulocyte lysate [22], were determined (fig.2a). Each cation when added alone stimulated protein synthesis, and the concentration curves obtained were of similar shapes. Maximum protein synthesis was seen with 1 mM $Mg^{2+}$, 2 mM diaminopropane or 0.2 mM spermidine. Diaminopropane was only a little less effective than $Mg^{2+}$, and substantially better than the optimum spermidine concentration. The synergism between diaminopropane and $Mg^{2+}$ is illustrated in fig.2b, which shows that in the presence of 2.5 mM diaminopropane the $Mg^{2+}$ concentration curve is displaced downwards by ~1 mM.

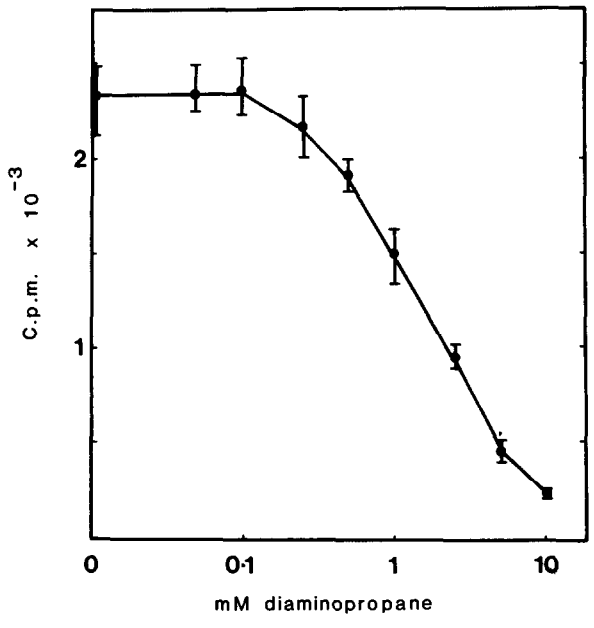



Fig.1. Effect of 1,3-diaminopropane on protein synthesis by reticulocyte lysates supplemented with 2 mM $Mg^{2+}$.

To determine whether any part of the inhibitory effect of diaminopropane on protein synthesis was due to an action antagonistic to that of spermidine, the natural polyamine implicated as playing an essential role in protein synthesis [22], the effect of diaminopropane on lysates containing 1.5 mM $Mg^{2+}$ with or without supplementation with spermidine was compared (fig.3). The lysate itself contains trace amounts of spermidine which are sufficient to support protein synthesis in the presence of $Mg^{2+}$, but if the action of diaminopropane was due to competition with this, then the inhibition would be expected to be proportionately reduced when the lysate was supplemented with a comparatively large amount of spermidine. Fig.3 shows clearly that there was no evidence at all for any such effect.

These results provide a direct confirmation of the suggestion in [20] that diaminopropane at concentrations comparable to those generally used might inhibit protein synthesis. The extent of the inhibition would be expected to vary from tissue to tissue, depending on the effective concentration of $Mg^{2+}$ and free polyamines in the vicinity of the ribosomes, and perhaps also on other factors. While these parameters in intact cells are extremely difficult to determine with sufficient accuracy to be certain, it seems extremely probable in view of the high concentrations of diaminopropane used that at least part of the inhibition of ornithine decarboxylase activity and polyamine accumulation seen in [2–17] will have been due to this non-specific effect, and that other effects of the drug, such as the inhibition of DNA replication hitherto thought to be secondary to the inhibition of polyamine synthesis, may require reevaluation. The activity of ornithine decarboxylase would be reduced much more rapidly than that of other enzymes with a lower turnover rate after inhibition of protein synthesis, although additional specific feedback repression or induction of a specific inhibitor by diaminopropane may also be involved. It may be relevant that diaminopropane has been reported to reduce the activity of *S*-adenosylmethionine decarboxylase also [6,7], which also has quite a rapid turnover rate.

In conclusion, it seems advisable that extreme

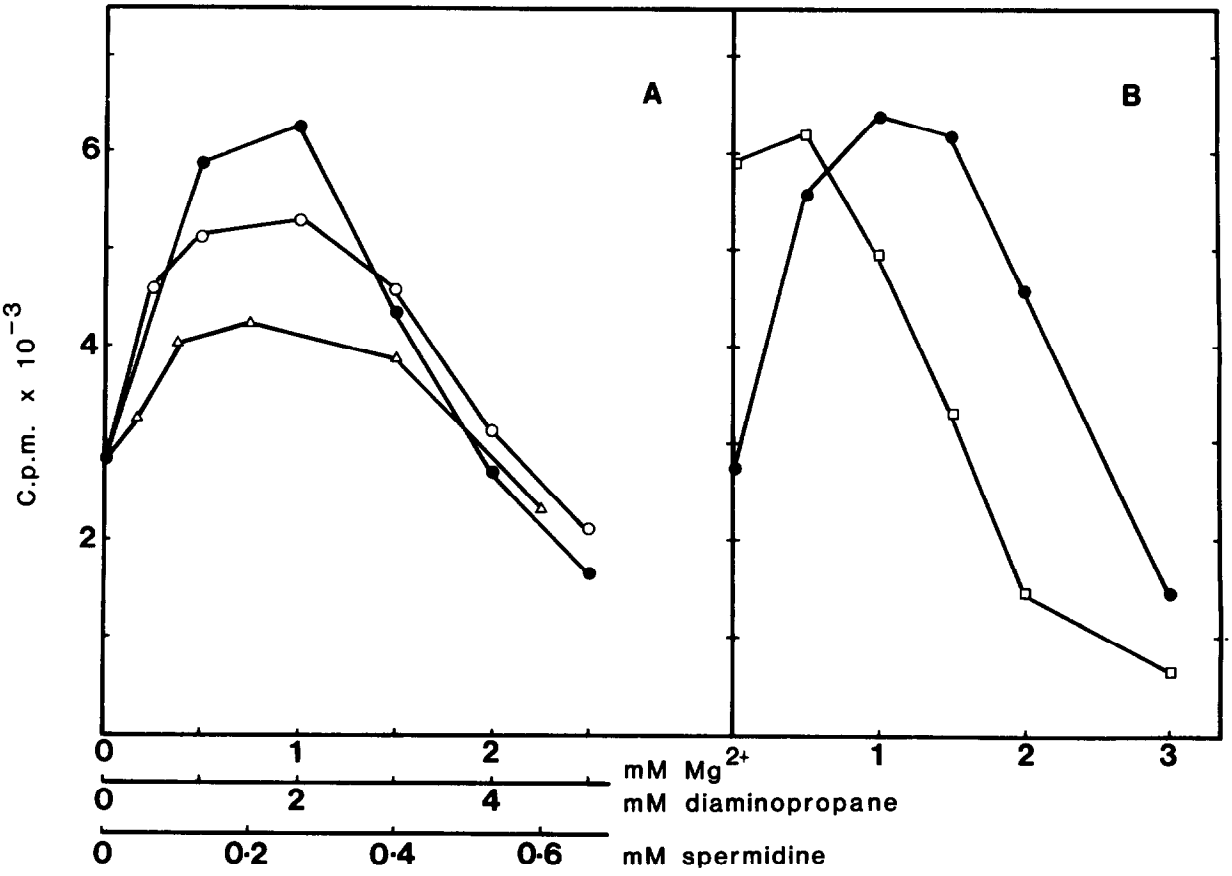
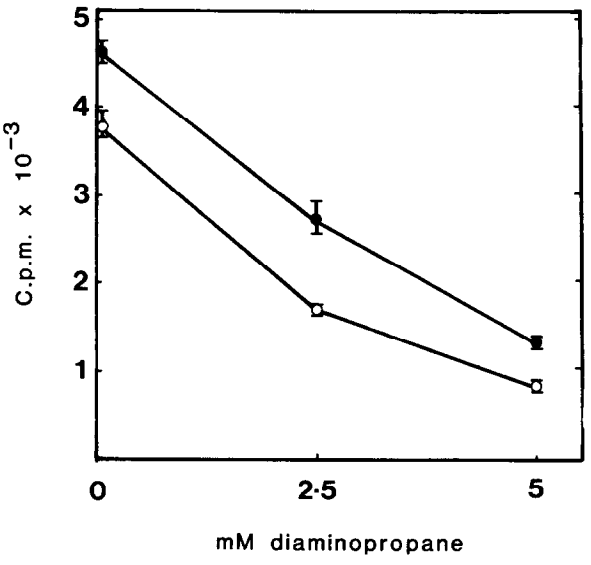



Fig.2. Protein synthesis by reticulocyte lysate incubated with the concentrations shown of $Mg^{2+}$ (•), spermidine (△), 1,3-diaminopropane (○) or $Mg^{2+}$ in the presence of 2.5 mM 1,3-diaminopropane (□).




caution should be used in the interpretation of changes occurring in cells exposed to high concentrations of 1,3-diaminopropane (or, other polyamine analogues active only at high concentrations) as necessarily being consequences of inhibition of polyamine synthesis, unless very careful controls are carried out. Selective inhibition of ornithine decarboxylase may be more satisfactorily achieved using alternative antagonists such as $\beta$, $\gamma$-unsaturated ornithine analogues [23] or $\alpha$-difluoromethylornithine [24–26].

Fig.3. Effect of 1,3-diaminopropane on protein synthesis by reticulocyte lysates supplemented with 1.5 mM $Mg^{2+}$ (•) or 1.5 mM $Mg^{2+}$ plus 0.2 mM spermidine (○).

## Acknowledgement

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