synthesis of several polypeptides, whereas at later times the protein pattern appears to be similar to control. Our observations on the behaviour of such parameters as polyribosome profile, cation content and amino acid transport have shown a strong perturbation of these parameters in the first few hours of hyperosmolar treatment followed by a slow but persistent return to the values of the control. These results are an indication of the alteration of some functions in hypermodulation of protein synthesis. Of course, the possibility that the change in the expression of specific proteins might be referred to the alteration of some functions in hyperosmolarity-treated cells deserves further study.

Pyrophosphate analogues as inhibitors of viral polymerases

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Analogues of pyrophosphoric acid, e.g. phosphonoacetic (I), phosphonoformic (II) and C-substituted methylene bisphosphonic acids (III) and the related tetraviole (IV) can act as product inhibitors of viral polymerases. For example, the replication of herpes viruses can be inhibited by (I) (Boezi, 1979) or (II) (Oberg, 1983), both compounds inhibiting the DNA polymerases induced by these viruses. In addition, (I), (II), (III, X = Y = Cl) and (IV) can inhibit the RNA transcriptase activity of influenza virus A (Cloud & Hutchinson, 1983; Stridh & Datema, 1984).

\[
\begin{align*}
\text{HO}_2\text{P(O)}\text{CH}_2\text{CO}_2\text{H} & \quad \text{HO}_2\text{P(O)}\text{CO}_2\text{H} \\
\text{(I)} & \quad \text{(II)} \\
\text{HO}_2\text{P(O)}\text{CXYP(O)(OH)}_2 & \quad \text{HO}_2\text{P(O)}\text{CXYP(O)(OH)}_2 \\
\text{(III)} & \quad \text{(IV)} \\
\text{HO}_2\text{P(S)}\text{OP(O)(OH)}_2 & \quad \text{HO}_2\text{P(S)}\text{OP(OR)}_2 \\
\text{(V)} & \quad \text{(VI)}
\end{align*}
\]

The inhibition of the RNA transcriptase activity of influenza virus by these compounds appears to be related to their abilities to form complexes with an essential metal ion (probably zinc) in the transcriptase (Cloud & Hutchinson, 1983; Hutchinson, 1985). Thus (III, X = Y = H) forms only weak complexes with zinc ions at physiological pH while (III, X = Y = Cl) forms considerably stronger complexes. This behaviour is paralleled in their inhibitory activities and (III, X = Y = Cl) is a good inhibitor of the RNA transcriptase activity while (III, X = Y = H) is a poor inhibitor.

The metal chelating properties of inorganic pyrophosphate are affected if oxygen atoms are replaced by sulphur. Oxygen is a 'hard' centre of the Pearson Hard and Soft Acid and Base Scale while sulphur is a 'soft' centre (Pearson, 1968). Thus, thiopyrophosphates might be expected to bind to metal ions in a manner different from that observed with pyrophosphate. We have shown by $^{31}$P n.m.r. that bisthiopyrophosphate (VI) appears to bind to Zn$^{2+}$ through sulphur while it binds to Mg$^{2+}$ through oxygen (Hutchinson et al., 1985). Mono- (V) and bis-thiopyrophosphate (VI) form strong complexes with Zn$^{2+}$ ions under physiological conditions. They are as good inhibitors of the RNA transcriptase activity of influenza virus A as compounds (I)-(IV) and are better inhibitors of the transcriptase than inorganic pyrophosphate. Mono- and bis-thiopyrophosphate inhibit the replication of influenza virus A in MDCK cells and do not appear to be cytotoxic to these cells after 3 days at concentrations which cause marked inhibition of virus replication.

Lipid-soluble pyrophosphate analogues should be taken up by cells more readily than highly polar compounds, e.g. (I)-(III). The presence of an electron-withdrawing group on the bridge carbon atom of methylene bisphosphonates is necessary if these bisphosphonates are to be good inhibitors of the RNA transcriptase of influenza virus A (Cloud & Hutchinson, 1983). We have developed a method for the synthesis of C-alkylated monochloro-bisphosphonates (VII) in high yield:

\[
\begin{align*}
\text{(R'O)}_2\text{P(O)CIP(O)(OR')}_2 & \quad \text{(V)} \\
\text{(R'O)}_2\text{P(O)CIP(O)(OR')}_2 \\
\text{(VII)} & \quad \text{(VII)}
\end{align*}
\]

The key steps in our synthetic route are the use of thallium(I) ethoxide as base to form the anion from a tetra-alkyl ester of monochloromethylene bisphosphonate followed by reaction with an alkyl iodide and deesterification with trimethylsilyl bromide (Hutchinson & Semple, 1985). In this way (VII) can be prepared in over 80% yield overall. If the sodio- or lithio-salts of the monoanion of the tetra ester of monochloromethylene bisphosphonate are used in the alkylation step or if deesterification is carried out by hydrolysis with concentrated acid, marked reductions in the yield of (VII) occur. In preliminary experiments, we have shown that (VII, R = Me, n-Pr, n-Bu, n-Hex) are effective inhibitors of sulphur.
The major P$_1$P$_4$-bis-(5'-adenosy1)-tetraphosphate-binding protein in *Artemia* is a protein kinase

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Several acid-soluble nucleotides act as specific signals in the regulation of metabolic reactions (Tomkins, 1975). The past decade has seen a considerable growth in interest in the novel purine nucleotide P$_1$P$_4$-bis-(5'-adenosy1)-tetraphosphate (Ap$_4$A). This apparently ubiquitous component of living cells has been shown to alter its intracellular concentration up to 1000-fold during the G$_1$ to S progression of a synchronized cell cycle (Weinmann-Dorsch et al., 1984). It may be involved in a number of metabolic processes in the cell including the initiation of DNA replication (Weinmann-Dorsch et al., 1984) and stimulation of processing of ADP-ribosylated proteins (Surowy & Berger, 1983). It is synthesized by the aminocyl-tRNA synthetases in the presence of Zn$^{2+}$ ions (Goerlich et al., 1982). Evidence to date suggests a role as a messenger molecule in cellular proliferation.

As part of our investigations into the function of Ap$_4$A we have previously examined the levels of this nucleotide during development of the brine shrimp *Artemia*. When ameboid encysted gastrulae of *Artemia* resume development they do so in the total absence of DNA replication and cell division until the point of hatching (16 h) although protein and RNA synthesis proceed unhindered. Upon re-initiation of development, a 125-fold rise in the intracellular concentration of Ap$_4$A was observed, the maximum level being 93 000 on sucrose gradients. Since most protein kinases undergo autophosphorylation in *vitro* when incubated with [$\gamma$-32P]ATP of high specific activity (de Jonge & Rosen, 1977), such a mechanism may be responsible for the incorporation observed here. The precise subunit composition of the native kinase is at present unclear, but our observations may be the result of partial proteolysis of a larger precursor. Such an observation has been made by several investigators (Kuo & Shoji, 1982).

The polypeptides of M$_r$ 35 000 and 40 000 or 42 000 may represent the proteolytic products of the M$_r$ 72 000 polypeptide which, when associated with the third polypeptide of low molecular weight, yields the native kinase of M$_r$ 93 000. This interpretation is supported by the fact that autophosphorylation of crude extracts in the presence of sodium fluoride, an ATPase and protein phosphatase inhibitor, yields only the polypeptide of M$_r$ 72 000 on sodium dodecyl sulphate/polyacrylamide gel. This polypeptide co-sediments with the native Ap$_4$A-binding protein of M$_r$ 93 000 on sucrose gradients.

Cyclic AMP (1 $\mu$M) or cyclic GMP (1 $\mu$M) completely suppress the phosphorylation of the bands of M$_r$ 42 000 and 40 000 while cyclic GMP also reduces phosphorylation of the polypeptides of M$_r$ 72 000 and 35 000. The possible role of this kinase in growth regulation and development in *Artemia* and the significance of Ap$_4$A binding are currently under investigation.