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DINUCLEOSIDE OLIGOPHOSPHATES - SIGNAL MOLECULES?

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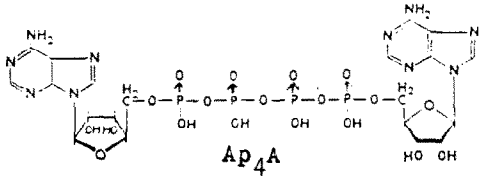
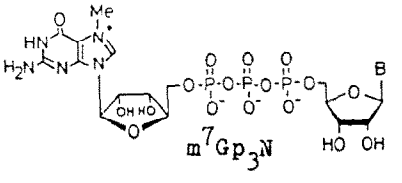
Dinucleoside oligophosphates (DNOPs) of the general form $N(5')p_n(5')N'$, or simply Np_nN', where N and N' are nucleosides, and n = 2 - 6, are ubiquitous components of living cells, both in the free form and as "cap" structures. The most widely investigated and best-known are Ap_4A, diadenosine 5',5'''-P^1,P^4-tetraphosphate or (IUPAC) adenosine(5')tetraphospho(5') adenosine, and the 5'-terminal cap m^7Gp_3N of eukaryotic and viral mRNA, as follows:





The first such natural compound isolated was Gp_4G (followed by Gp_3G, Gp_2G, Gp_3A, and more recently Ap_4A and Ap_3A) in the brine shrimp Artemia salina; in dormant cysts Gp_4G comprises 45% of acid-soluble nucleotides and 2% of the dry weight. It is synthesized by a GTP:GTP guanylyltransferase and is the primary source of all purine-containing compounds for development [1].

Subsequently, following detection of Ap_4A as a product of the back-reaction of aminoacyl-tRNA synthetase, its presence was demonstrated in a variety of prokaryotes and eukaryotes at levels of 10^{-8} - 10^{-6} M, cf. to an ATP level of 10^{-3} M. In cell lines in culture, and normal mouse liver and hepatoma, its level varies over a 100- to 1000-fold range with proliferative activity of the cells. It has been reported to stimulate DNA synthesis in whole cells, to accelerate DNA polymerase α in vitro, to serve as a primer for replication, and to exhibit a high affinity for a subunit of DNA polymerase α, leading to the proposal that it is a "signal molecule" like cAMP or pppGpp [2]. If so, recent detailed reports indicate that this is not the case in all cellular systems. It should be recalled that the signal molecule pppGpp mediates the stringent response in prokaryotes, but to a doubtful extent, if at all, in eukaryotes [3].

Added impetus to the foregoing was furnished by the observation that levels of a variety of DNOPs in E. coli and S. typhimurium increase enormously following heat-shock or oxidative stress [4], a finding duplicated, albeit to a lesser extent, in eukaryotic systems, and leading to the, perhaps premature [5], proposal that these molecules are "alarmones".

Some significant cellular role(s) for DNOPs is further supported by existence of several enzyme systems which can account for their synthesis, as well as a variety of hydrolases, and at least one phosphorylase, which degrade such molecules in the free form and as cap structures. Nucleotide pyrophosphatase from higher plants [6] cleaves all of these, including the cap of intact mRNA, in a defined manner; and, together with snake venom phosphodiesterase I, is a useful tool for identification of a given DNOP.

Somewhat suprisingly, little attension has been devoted to possible existence of DNOPs with linkages other than (5')-(5'). In one instance, this was given considerations [4]. In another it was noted that, although dT(3')p_2(3')dT is bound almost as effectively as its (5')-(5') congener, by nucleotide phyrophosphatase, it is a much poorer substrate [6]. It has also been found that RNA 3' terminal phosphate cyclase from HeLa cells, an enzyme possibly involving in splicing, converts the terminal 3'-phosphate to the 2',3'-cyclic phosphate via an N(3')p_2(5')A intermediate [7].

Numerous DNOPs are potent inhibitors in various enzyme systems, of in vivo significance in some instances. Particularly striking is their utility as probes of the active site(s) of enzymes in which nucleosides and/or nucleotides are involved in phosphate transfer reactions. This approach is based on the concept that a DNOP is a multi-, actually bi-, substrate analogue for two-substrate reactions which proceed via a Random or Ordered Sequential Bi Bi kinetic pathway, through a ternary enzyme complex, thus combining the structural elements of the two substrates with relative orientations similar to that prevailing in the ternary enzyme complex. For example, thymidine kinase must possess binding sites for both dT and ATP, so that an analogue such as dTp_nA (with $n \geqslant 3$) might be expected to inhibit by binding to, and bridging, both substrate binding sites, and furnish information about the geometry of the active sites. An excellent illustration is adenylate kinase [8], which is inhibited competitively by Ap_5A with a $K_i \sim 10^{-9}$ M, but much less so by Ap_4A ($K_i \sim 10^{-5}$ M) and Ap_6A ($K_i \sim 10^{-7}$ M). Kinetic studies, widely applied to such systems, including nucleoside kinases and deoxynucleotidyl transferase, may be supplemented by use of DNOPs with fluorogenic nucleoside residues, which are also good substrates for DNOP hydrolases [9], as well as by isosteric and isopolar phosphonate analogues, DNOPs have also proven highly effective affinity ligands for both resolution, and purification, of closely related nucleoside kinase species; and the use of a series of such ligands confirmed the existence of distinct active sites for two deoxynucleoside kinases in a single protein molecule [10].

The foregoing, in turn, has stimulated the development of improved novel procedures for the chemical synthesis of DNOPs [11, 12, 13], as well as a variety of methods for their fractionation and isolation, including two-dimensional TLC [4].

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