inhibitors of DNA synthesis (*19–23*). It is important to determine whether this conclusion has general validity and whether the regulation of histone gene expression resides at the transcriptional or posttranscriptional level (*24*).

ANGELO M. DELEGEANE
AMY S. LEE*

Department of Biochemistry, University of Southern California School of Medicine, Los Angeles 90033

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* Address correspondence to A.S.L.

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β,γ-Peroxy Analogs of Adenosine and Guanosine Triphosphate: Synthesis and Biological Activity

Abstract. *Extended analogs of adenosine triphosphate (ATP) and guanosine triphosphate (GTP), in which a peroxide bridge replaces the terminal bridge-oxygen of the triphosphate chain, have been synthesized. The ability of* β,γ*-peroxy-ATP to inhibit or substitute for ATP in representative enzyme systems and that of* β,γ*-peroxy-GTP, for GTP in protein synthesis was tested.*

We have introduced the concept of testing the geometrical restrictions of nucleotide binding sites by examining the biological properties of synthetic purine analogs with defined dimensional alterations within their heterocyclic nuclei (*1–3*). Compounds in the first series of these dimensional probes have a purine-like ring system which is laterally extended by 2.4 Å as a result of the introduction of a benzene ring between the terminal imidazole and pyrimidine rings.

Another method of stretching out a nucleotide molecule is by inserting an additional oxygen atom between the central (β) and terminal (γ) phosphorus atoms in the triphosphate chain to form a peroxide linkage (Fig. 1). In such a structure, the peroxydiphosphate moiety, comprising the β and γ atoms, has known geometry as determined by x-ray crystallography of lithium peroxydiphosphate hydrate (*4*). The presence of the peroxide bridge results in the lengthening of the triphosphate chain (*5, 6*) by slightly more than one angstrom unit when maximally extended. The actual distance may vary because of internal rotation in the nonlinear chain (*7*).

Both 5′-adenylyl peroxydiphosphate (AMP-PO_2P) and 5′-guanylyl peroxydiphosphate (GMP-PO_2P) were prepared by a general phosphate coupling procedure (*8*). Reaction of peroxydiphosphate with the appropriate nucleoside 5′-phosphorimidazolidate and subsequent purification by ion-exchange chromatography (*9*) provided AMP-PO_2P and GMP-PO_2P in yields of 60 to 70 percent. The identity and purity of the peroxy nucleotide analogs were verified by chromatographic analysis and standard spectroscopic methods. Their ^{31}P nuclear magnetic resonance spectra differed from those of the normal nucleoside triphosphates ATP and GTP both in chemical shifts (from 85 percent phosphoric acid) and in coupling constants (*J*), as expected. For example, the resonances for the phosphorus atoms adjacent to the peroxide linkage in AMP-PO_2P are shifted about 11 ppm downfield from their positions in the ATP spectrum. A smaller *J* (spin-spin coupling constant) value of 13 Hz was measured between the central and terminal phosphorus atoms of the peroxy analog compared with a value of 20 Hz for ATP. This difference reflects three-bond versus two-bond coupling.

The peroxy analogs have chemical properties that make them suitable for enzyme studies. They are stable in neutral or basic solution, as is peroxydiphosphate (*5, 10*), within the *p*H range used in most enzyme assays. The AMP-PO_2P analog is capable of binding divalent metal cations, a strict requirement for all known nucleotide-dependent phosphate displacement reactions, although the AMP-PO_2P-Mg^{2+} association is about 3 percent of that of the ATP-Mg^{2+} association (*11*). The difference is parallel to the relative association of peroxydiphosphate versus pyrophosphate with Mg^{2+} (*5, 12*). It is also consistent with the finding that stabilization due to chelation is less for a seven-membered ring than for a five- or six-membered ring (*13*). At this time, however, we cannot say whether the cause of the lower association of AMP-PO_2P-Mg^{2+} lies (i) in less pyrophosphate ligation with Mg^{2+} than in ATP-Mg^{2+}, (ii) in lower affinity of the seven-membered ring β,γ-peroxydiphosphate-Mg^{2+} pair, or (iii) in altered affinity of Mg^{2+} for the terminal dianion in the β,γ-peroxy analog (*14, 15*).

The potential usefulness of the peroxy nucleotide analogs as probes of the limitations of enzyme binding and mechanism depends upon their ability to inhibit or substitute for the natural cofactors in enzyme systems. To initiate our studies on the biological reactivities of the peroxy derivatives, we selected enzymes that require ATP as donor of adenylyl, pyrophosphoryl, and phosphoryl groups. The conditions used gave consistent results for both ATP and AMP-PO_2P but not necessarily optimal activity (Table 1). Dithiothreitol (*16*) was added to each assay to prevent oxidation of reactive sulfhydryl groups caused by peroxide release.

Adenylyl transfer reactions, represented here by firefly luciferase and NAD^+ pyrophosphorylase, proceed by nucleophilic attack at the α-phosphorus of ATP with cleavage of the α-P-O linkage and displacement of pyrophosphate (*17*). The peroxy analog of ATP is an acceptable substrate for representatives of this class of enzymes, displaying similar enzyme binding and a reduced rate of catalysis compared with ATP. With luciferase in the presence of luciferin, AMP-PO_2P brought about light emission at the same wavelength, 562 nm, as did ATP. In a test of biological activity with tyrosyl-tRNA synthetase, one of the adenylyl transferases responsible for the activation of transfer RNA's (tRNA) for protein synthesis, AMP-PO_2P was found to be about 50 percent as effective as ATP in the formation of the aminoacyl-

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Table 1. Binding and catalytic activity of AMP-PO_2P relative to ATP (V_{max} = 1.0).

Enzyme	ATP K_m (mM)	AMP-PO_2P K_m (mM)	AMP-PO_2P V_{max}	AMP-PO_2P K_i (mM)
Adenylyl transferases				
Firefly luciferase	0.050	0.066	0.15	
NAD^+ pyrophosphorylase	0.200	0.294	0.20	
Pyrophosphoryl transferase PRPP synthetase	0.038			0.033
Phosphoryl transferases				
Hexokinase	0.10			0.09
Phosphofructokinase	0.08			0.20
Phosphoglycerate kinase	0.50			0.40

tRNA (*18*). Thus, the active sites of these adenylyl transfer enzymes tolerate the AMP-PO_2P-Mg^{2+} complex, which has a different chelation pattern than does ATP with the metal ion. The compensatory expectation is that the peroxydiphosphate anion is a better leaving group than the pyrophosphate anion (on the basis of relative acidities of the conjugate acids) (*5, 10, 12*).

Nucleophilic substitution at the β-phosphorus of ATP, as in pyrophosphoryl transfer, is less common in nature than substitutions at the α- or γ-phosphorus (*19*). An example of an enzyme that catalyzes such a reaction is 1-pyrophosphoryl-ribose-5-phosphate synthetase (*20*), which brings about pyrophosphoryl transfer from ATP to ribose 5-phosphate with cleavage of the α,β-phosphoanhydride bridge in ATP, and for which ATP is the required donor. The β,γ-peroxy analog was not a substrate but was a potent competitive inhibitor with PRPP synthetase (Table 1). The peroxy linkage in AMP-PO_2P evidently renders the β-phosphorus sterically less susceptible to nucleophilic attack by the substrate in the cofactor complex with Mg^{2+} ion and the enzyme.

Phosphoryl transfer from ATP is catalyzed by numerous kinases. The γ-phosphorus undergoes an enzyme-catalyzed nucleophilic attack by the acceptor molecule in an associative mechanism (*15, 19*). Substitution of AMP-PO_2P for ATP with a variety of kinases resulted in significant decreases in activity, with the phosphate displacement reactions occurring at a rate no greater than 1 to 3 percent of ATP. Depressed kinase activity in these cases (Table 1) may be the result of one or more factors. First, phosphate displacement reactions are known to be sensitive to the basicity of the leaving group (*21*). The departing AMP-POO anion is a poorer leaving group than the corresponding ADP anion, which would lead to a slowing of the reaction (*10*). Second, the weakly Mg^{2+}-chelated AMP-PO_2P may be misaligned in the catalytic pocket so that the particular substrate and the reactive substituents on the enzyme can no longer attain proper orientation for normal efficiency.

Further studies revealed that for each kinase examined, the β,γ-peroxy-ATP served as an effective competitive inhibitor of phosphoryl transfer. Accordingly, AMP-PO_2P may prove useful in defining enzyme-nucleotide interactions that are dependent on the binding of ATP or an analog rather than on the transfer of its terminal phosphate. Analogs in which no P-O bond exists between β- and γ-phosphorus atoms, such as AMP-PCH_2P and AMP-PNHP, and which closely mimic ATP in structure but are not susceptible to hydrolysis, have found widespread use in enzymology (*22*).

While GTP is not a cofactor for as many different enzymatic conversions as is ATP, it is an essential cofactor in protein biosynthesis. In protein synthesizing systems obtained from *Escherichia coli* and from wheat germ that function well in the presence of GTP, the analog GMP-PO_2P (Fig. 1) was substituted. In both prokaryotic and eukaryotic systems, this β,γ-peroxy-GTP served as an inhibitor of ribosomal-dependent peptide synthesis, with activity analogous to that of GMP-PCH_2P (*23*).

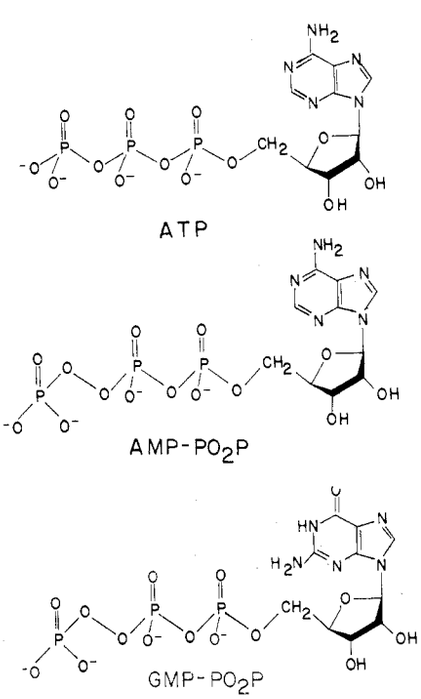

Fig. 1. Comparison of geometric structures of ATP, AMP-PO_2P, and GMP-PO_2P.

With the initial syntheses of AMP-PO_2P and GMP-PO_2P reported here, the pilot biochemical results suggest that nucleoside triphosphate analogs with peroxy modifications in the triphosphate chain may find application through their activity or inhibition in nucleotide-requiring enzyme systems. It is also possible that their photolytic homolytic scission (*24*) with enzyme systems may be controlled to provide binding information.

MARY S. ROSENDAHL
NELSON J. LEONARD*
Departments of Chemistry
and *Biochemistry,*
School of Chemical Sciences,
University of Illinois, Urbana 61801

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* Address correspondence to N.J.L.

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