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CONTENTS

ATP ANALOGS†

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CONTENTS

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I. Introduction

Enzymic reactions involving ATP remain at the forefront of unsolved problems in biochemistry. One needs only to recall the fact that we do not understand the fundamental chemistry of oxidative phosphorylation, muscle contraction, or active transport to see that this is true. Moreover, the discovery of the energy charge concept (1,2) has given numerous examples of key enzymes whose activities are controlled by ATP/ADP concentrations. In addition, ATP acts noncovalently to modify the activity of a host of other enzymes. How this occurs is not at all well understood. Of special interest is the fact that ATP may be used to covalently modify key enzymes in order to control their activity (3). Here ATP analogs have been sparingly used and promise to yield unique information about such systems. ATP is listed as a substrate for over 120 enzymes (4), the majority of which are of intense current interest; one thinks immediately of adenylate cyclase, the amino-acyl tRNA synthetases, the RNA polymerases, myosin, and ($Na^+ + K^+$) ATPase of membranes as prime examples.

Recent advances in the synthetic chemistry of nucleotides have led to a series of versatile analogs which have already allowed certain key questions to be answered concerning the role of ATP in the above reactions. The purpose of this review is to point out where each analog has been useful, some of their properties, and where they are likely to be useful in the future. Little will be said about synthetic methods because that is more properly a subject for a separate chapter. Hopefully chemical suppliers will continue to make more and more of these analogs commercially available as knowledge of their usefulness becomes more widespread. Some analogs are easily prepared, even by those unskilled in synthetic methods, and where appropriate this will be pointed out. Nonstandard abbreviations are given in the Appendix.

II. Phosphate Modified Analogs

It is convenient to divide ATP analogs into three categories, each of which represents modifications of either the triphosphate chain, the ri-

bose moiety, or the adenine ring. Because the triphosphate chain is the site of most enzymic action, much attention has been centered here by enzymologists and workers preparing analogs, and this area will be discussed first. Modifications of the ribose and adenine rings on the other hand have often been the result of interest in preparing useful drugs, especially for cancer chemotherapy. After being converted to their triphosphates, many of these analogs are of potential use to the enzymologist. They are discussed later, as well as special applications of ATP analogs acting as affinity labels or as parts of affinity columns.

A. DERIVATIVES WITH PHOSPHORUS-CARBON BONDS

1. General Properties

The first phosphate-modified ATP analog to be prepared was adenylyl methylenediphosphonate (AMP-PCP) in which a $-CH_2-$grouping replaced the β,γ-bridge oxygen of the triphosphate chain (5,6). Meyers and coworkers also prepared the α,β-methylene analog of ADP (AMPCP) (7). Although the synthesis of the related α,β-methylene analog of ATP (AMPCP-P) has never been described, all three of the above methylene analogs have been commercially available for several years. This availability has allowed much wider use of these analogs than would normally occur. Recently, Kenyon and coworkers (8) have prepared the analog in which both the α,β- and the β,γ-bridge oxygens of ATP have been replaced by $-CH_2-$groups but little is known about its reaction with enzymes.

The advantage of phosphonate analogs is the extreme stability of the P-C-P bonds which preclude any enzymic (or accidental) cleavage. The disadvantage of the methylene analogs is that the structural characteristics of the P-C-P grouping are substantially different from the P-O-P grouping they replace so that often these analogs are ineffective as substrates, effectors, or inhibitors. The salient structural parameters for the P-O-P, P-C-P, and P-N-P linkages in tetrasodium pyrophosphate, methylenediphosphonic acid, and tetrasodium imidodiphosphate, respectively, as determined by single-crystal X-ray diffraction, are given in Table I. Structurally, the important differences between these three compounds is centered around the P-X-P linkage. The P-X bond length increases systematically from P-O (1.61 Å) to P-N (1.68 Å) to P-C (1.79 Å), while the P-P distance increases only slightly, 2.92, 3.00, and 3.05 Å, respectively. Of especial interest are the P-X-P bond angles. The P-O-P and P-N-P bond angles are only slightly different, 130°

TABLE I

Comparison of the Geometries of Sodium Pyrophosphate, Sodium Imidodiphosphate, and Methylenediphophonic Acid. The Standard Deviation on the Least-Significant Digit is Given in Parenthesis

Bond	$O(OP_3)_2Na_4 \cdot (H_2O)_{10}$[a]	$HN(PO_3)_2Na_4 \cdot (H_2O)_{10}$[b]	$CH_2(PO_3)_2H_4$[c]
P–X	1.612(5) Å	1.678(5) Å	1.79 Å
P–O (ave)	1.523(4) Å	1.521(7) Å	1.54 Å
P–P	2.925 Å	3.006(3) Å	3.05 Å
P–X–P	130.2(6)°	127.2(5)°	117°

[a] McDonald, W. S., and Cruickshank, D. W. J., *Acta Crystallogr.*, *22*, 43 (1967).

[b] Reference 11.

[c] F. M. Lovell, *Abstracts of the American Crystallography Association*, 1964, p. 86, and personal communication (1967).

and 127°, respectively. This contrasts with the much smaller P–C–P angle of 117°. Thus it may be this more acute P–C–P bond angle and the longer P–C bond distance which prevent AMP–PCP from interacting with certain enzymes. (The consequences of the very similar P–N–P and P–O–P bond distances and bond angles are discussed in Section II.C)

Another important difference is that the ionization constant of the last phosphonate hydrogen of AMP-PCP (6) is much smaller than the comparable hydrogen for ATP (pK_a 8.5 versus a pK_a of about 7 for ATP). Thus the net charge on AMP–PCP may be quite different than ATP at pH 7. It should be noted that in the presence of divalent metal ions, the weak proton of AMP–PCP will normally be displaced so the final net charge should be the same. However, the structure of the predominant chelate remains unknown and it may be quite different than that of ATP. At present nothing is known about this possibility. AMP–PCP is known to bind divalent metal ions even more tightly than ATP, even at neutral pH (9) so that if it does not react it is not because of the absence of metal binding. Binding constants of AMP–PCP (as well as other related nucleotides) with Mg^{2+}, Ca^{2+}, and Mn^{2+} are given in Table II. Unfortunately, pK_a values and binding constants are not available for

AMPCP, AMPCPP, or any of the thiophosphate analogs (see Section II.B). A systematic study of the properties of these compounds would be valuable.

2. *Enzyme Reactions*

Table III gives a partial list of interactions of phosphonate ATP analogs with various enzymes. The first enzyme to be studied with AMP–PCP was actomyosin (5). In general, the results were disappointing in that AMP–PCP was not a competitive inhibitor of ATP hydrolysis and did not act to dissociate actomyosin at high ionic strength. Later studies showed the inhibition of myosin by AMP–PCP was complex (10) and probably reflected modifier binding at more than one site on the enzyme. AMP–PCP, in general, is not effective in replacing ATP in muscle studies (5,10).

AMP–PCP, of course, is not a substrate for any of the kinases or any enzyme which splits by the β–γ pyrophosphate linkage of ATP. As a substrate for enzymes which cleave the α–β linkage, AMP–PCP has given mixed results. Simon, Myers, and Mednieks in an early study (17) showed AMP–PCP was 30–50% as effective as ATP as a substrate in the synthesis of RNA catalyzed by DNA-dependent RNA polymerase. However, with various aminoacyl-tRNA synthetases, AMP–PCP may

TABLE II

Summary of Metal Binding Studies of ATP Analogs

Analog	pH	Ka Mg^{2+}	Ka Ca^{2+}	Ka Mn^{2+}	Supporting Electrolyte	Reference
ADP-sulfate	7.4	42	69	—	0.2 *M* KCl	85
ATPγF	8.2	200	242	505	0.1 *M* NaCl	78
AMP–PCP	7.4	12,900	4,800	—	0.1 *M* KCl	9
AMP–PCP	9.2	38,100	13,000	83,000	0.1 *M* KCl	9
AMP–PNP	8.5	38,200	11,800	85,000	0.1 *M* KCl	9
ATP	8.5	14,900	5,400	32,000	0.1 *M* KCl	9
ATP	7.4	4,500	1,800	—	0.1 *M* KCl	9

Conditions: 0.025 *M* Tris·Cl, 25°. All values obtained by the resin competition method.

TABLE III

Reaction of Phosphonate Analogs with Various Enzymes

Enzyme	Analog	Reaction or Effect	Reference
Myosin (skeletal)	AMP–PCP	Inhibitor; competitive with Ca^{2+}, mixed with Mn^{2+}	10
Actomyosin	AMPCP–P	Substrate; dissociates actomyosin at high ionic strength	13
RNA polymerase	AMP–PCP	Substrate; 30–50% as effective as ATP	17
Formyltetrahydrofolate synthetase	AMP–PCP	Competitive inhibitor; binds 8 times tighter than ATP; ($K_i = 10^{-5}$ M) AMPCP–P binds more weakly	21
Aminoacyl tRNA synthetase(s)	AMP–PCP	Either substrate, inhibitor, or without effect, depending on the enzyme	12
D-α-Lysine mutase (B-12 coenzyme dependent)	AMP–PCP AMPCP–P	Both analogs replace ATP as positive allosteric effectors	20
Glutamine synthetase (brain)	AMP–PCP AMPCP–P AMPCP	None will replace ATP or ADP as activators; do not inhibit ATP as substrate	24
ADP/ATP translocase (mitochondria)	AMP–PCP AMPCP–P AMPCP	Replace ATP (ADP) in atractyloside sensitive transport across membrane	25,26
Intestinal 5′-nucleotidase	AMPCP–P AMPCP	Both inhibit enzyme; K_i(AMPCP) 1/500 that of ADP	27
Adenylate cyclase	AMP–PCP	Not a substrate	62
	AMPCP–P	Competitive inhibitor	28
	AMPCP	No effect	
($Na^+ + K^+$) ATPase	AMP–PCP	Does not support Na^+, K^+ transport, prevents ouabain binding	29,31
ATP: L-Methionine *S*-Adenosyltransferase	AMP–PCP	($K_i = 2.2$ mM) Both are competitive inhibitors versus ATP	30
	AMPCP–P	($K_i = 0.55$ mM)	

be a substrate, a competitive inhibitor or have little or no effect (12). These latter findings emphasize the individuality of this class of enzymes. AMP–PCP is not a substrate for adenylate cyclase (62) but it is cleaved by snake-venom phosphodiesterase to AMP and methylene diphosphonate (9). Thus the response of AMP–PCP as a substrate is highly unpredictable and may either reflect the known differences in the structure of the P–C–P linkage as discussed previously or the predominance of a metal chelate of the wrong structure for a given enzyme active site.

Studies with AMPCP-P, the α,β methylene analog, are less common. However, recent studies on the kinetics of its hydrolysis by myosin systems have shown its utility (13). In the myosin-catalyzed hydrolysis of ATP, cleavage of the phospho-anhydride bond occurs much faster than release of the products ADP and Pi (14). Actin is believed to speed up the hydrolysis of ATP by increasing the rate of product release from the myosin surface. With AMPCP-P as substrate, product release is no longer rate limiting, because AMPCP–P is cleaved some 1000 times slower than ATP (13). Thus as would be expected, actin has no effect on this system. The ability of analogs to "freeze" certain states in a complex series of reactions such as muscle contraction is just now being explored and the proper combination of analogs and physical techniques such as low-angle X-ray diffraction (15,16), depolarization of fluorescence (70, 18), and electron microscopy promise to give unique information about such phenomena. This general approach may also be useful in studying the complex steps in oxidative phosphorylation and active transport of ions across membranes if appropriate model systems can be found.

AMP–PCP and AMPCP-P have proven effective as allosteric effectors in a number of systems. For example, ATP, ADP, and other nucleoside diphosphates are known to convert fructose 1,6-diphosphatase into a conformer of low catalytic activity (19). AMP–PCP was the most effective of all nucleotides tested, being active in the micromolar range. AMPCP-P was also effective but neither the ADP analog, AMPCP, nor any of the β-γ methylene analogs of GTP, dATP, dTTP, or UTP were with effect. Likewise, Morley and Stadtman (cf. Table III) have shown that both AMP–PCP and AMPCP-P, like ATP, are positive allosteric modulators of the D-α-lysine mutase from *Clostridium sticklandii* (20).

An interesting suggestion (21) has been put forth to explain the unusual tight binding of AMP–PCP to the enzyme, formyltetrahydrofolate synthetase (Table III). With this enzyme AMP–PCP is a potent

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competitive inhibitor and binds some 8 times *stronger* than ATP. The related α,β methylene analog, AMPCP-P, is also a competitive inhibitor but it binds more weakly than either ATP or ADP (21). Curthoys and Rabinowitz suggest the tighter binding of AMP–PCP results from the P–C bond mimicking the longer axial P–O bond which would result if the γ phosphate of ATP formed a trigonal-bipyramidal intermediate during the catalytic cycle. Model compounds for trigonal-bipyramidal structures estimate the axial P–O bond to be 1.76 Å, whereas shorter equatorial P–O bonds were 1.60 Å (22). Thus with ATP as substrate the enzyme may use part of its binding energy to strain the γ phosphate into a more reactive conformation to promote catalysis. With AMP–PCP the longer P–C bond and more acute P–C–P bond angle may give a situation in which the enzyme may use some of its binding energy to increase its affinity for the "substrate." The discovery (N. Curthoys, personal communication) that the ATP analog, AMP–PNP (see Section II.C), binds with an affinity intermediate to ATP and AMP–PCP fits this interpretation because the P–N bond is intermediate in length to that of P–O and P–C bonds (see Table I). These authors also suggest that the enzyme, glutamine synthetase, may be analogous to formyltetrahydrofolate synthetase in that both enzymes utilize the energy of ATP to synthesize C–N bonds. Furthermore, studies by Gass and Meister (23) have suggested the γ phosphate of ATP may also be bound in a strained triagonal-bipyramidal conformation by this enzyme. Therefore, by analogy, AMP–PCP should be a potent inhibitor. However, previous studies (24) have shown that neither AMP–PCP nor AMPCP-P, even at millimolar concentration have any effect on this enzyme; hence, the predictive power of this finding appears to be limited and other factors not considered may be important.

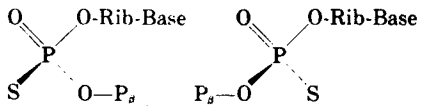
B. THIOPHOSPHATE ANALOGS

1. General Considerations

The substitution of sulfur for phosphate oxygens in nucleotides was first reported by Eckstein who prepared the thiophosphate derivatives of TMP and UMP (32). These mononucleotides were subsequently phosphorylated to yield TTPαS and UTPαS (33) for use in synthesizing artificial polynucleotides (34,35). ATPαS (39) was used later for a similar purpose (35). The advantage in such analogs is that thiophosphate derivatives are generally resistant to cleavage by various phosphatases and

phosphodiesterases while retaining the normal charge of the parent oxygen-containing compounds. Hence such derivatives often are longer lived *in vivo* and, being structurally quite similar, they effectively mimic the action of the parent oxygen compound. This was dramatically demonstrated by the enhanced induction of interferon in human skin fibroblasts and in rabbits by the thiophosphate analog of a copolymer of adenylic and uridylic acids (36).

Analogs have now been made with sulfur replacing nonbridge oxygens in each of the phosphates of ATP (37,38,39). Of these, ATPγS (39) has been most extensively studied (40) and is now commercially available (41). Of particular interest is the fact that the other two thiophosphate ATP analogs, ATPαS and ATPβS, exist as two pairs of diastereomers because the sulfur makes the thiophosphate group asymmetric. This is illustrated below for ATPαS.



The possibility thus exists that certain enzymes will act preferentially on one of the diastereomers allowing the stereochemical pathway of cleavage of α–β P–O–P bond of ATP to be determined. Such an approach using the thiophosphate analogs of cyclic 2′, 3′-UMP has allowed the stereochemistry of its formation and cleavage by pancreatic ribonuclease to be established (42,43,45).

The P–S bond in thiophosphate analogs is normally quite stable at physiological pH values and hence suitable for most enzyme studies. The sulfur is lost, however on prolonged exposure to low pH values (< pH 3). Thiophosphate substituted polynucleotides are thermally stable enough to do normal melting-curve studies of double-helical structures (35).

The substitution of sulfur lowers pK_{a2} of orthophosphate from approximately 7 to 5.75 (46). Thus any thiophosphate analog would be expected to be more negatively charged than ATP at pH 7. This is not likely to be significant except in those cases where it appears as if $HATP^{-3}$ is the normal substrate or effector for an enzyme (see ref. 47 and references therein). Whether the negative charge is on the sulfur or oxygen of ATPγS at pH 7 is not known. Likewise, nothing is known about the metal-binding properties or metal-chelate structures of

thiophosphate analogs. NMR studies of the phosphate chain and its metal complexes could be especially instructive in the latter case.

The possibility of preparing ATP analogs with P–S–P bonds is interesting. However, the largest P–S–P bond angle known is only 110° (48) and this fact coupled with the very long P–S bond distance (2.1 Å) would make P–S–P analogs structurally quite unlike ATP in comparison with the P–N–P and P–C–P analogs (see Table I). In addition, the P–S–P bond is thermodynamically less stable than the nonbridge P–S bond and readily hydrolyzes or rearranges to give P–O–P bonding (48). Hence such P–S–P analogs would appear to be of limited promise at present.

Analogs with P–S–S–P bonds, however, do exist and have been prepared by H_2O_2 oxidation of ATPγS (39,49). The resulting disulfide analog [ATPγS-]$_2$ offers the interesting possibility of labeling thiol groups at ATP binding sites by disulfide exchange (eq. 1).

$$[\text{ATP}\gamma\text{S}-]_2 + \text{HS-Enz} \rightleftharpoons \text{ATP}\gamma\text{S-S-Enz} + \text{ATP}\gamma\text{S} + \text{H}^+ \qquad (1)$$

Preliminary results (49) indicate that (ATPγS–)$_2$ inactivates myosin in the manner illustrated above although it is not known if the analog reacts only at the active site. The apparent weak protection by ATP may mean the analog is reacting at a second site.

2. *Specific Reactions with Enzymes*

As discussed above one of the initial purposes in making thiophosphate analogs was to prepare various thio-substituted polynucleotides. Eckstein and Gindl (35) have shown that DNA-dependent RNA polymerase with a poly-d(A–T) template will effectively copolymerize ATPαS with UTP (or UTPαS), although the rate and extent of the reaction is lower with the sulfur analogs. Neither the formation nor the thermal stability of double-stranded polymers was impaired by the substitution of sulfur on the phosphate groups. The interesting question as to whether both diastereomers of ATPαS were utilized to an equal extent is not known because less than 50% of ATPαS was utilized by the enzyme. However, in the copolymerization of ATP and UTPαS, about 60% of the substrates react indicating that at least both isomers of UTPαS are substrates.

Recent work (F. Eckstein, personal communication) has shown that each pair of diastereomers (designated A and B) of ATPαS and ATPβS

can be prepared enzymatically. For example,

(a) ADPαS (mixed isomers) + phosphoenol pyruvate

$$\xrightarrow[\text{kinase}]{\text{pyruvate}} 50\%\ \text{ATP}\alpha\text{S (A)}$$

(b) ATPαS (mixed isomers) + GDP $\xrightarrow[\text{diphosphate kinase}]{\text{nucleoside}}$ 50% ATPαS (B)

(c) ADPβS (mixed isomers) + phosphoenol pyruvate

$$\xrightarrow[\text{kinase}]{\text{pyruvate}} \text{ATP}\beta\text{S (A)}$$

(d) ADPβS (mixed isomers) + phosphocreatine

$$\xrightarrow[\text{kinase}]{\text{creatine}} \text{ATP}\beta\text{S (B)}$$

Preliminary work has already shown that the (A) isomer of ATPαS is utilized much more rapidly by myokinase than the (B) isomer. Likewise, myosin cleaves ATPβS (A) more rapidly than the (B) isomer.

It would be of considerable interest if the absolute configuration of the various isomers could be determined. In view of the normal difficulty in crystallizing nucleoside triphosphate derivatives this appears unlikely. An alternate possibility would be to diffuse the appropriate analog into a protein crystal which binds triphosphates. With good crystals of known structure and tight, stereospecific binding it may be possible to resolve the relative location of the sulfur and adenosine moieties. Regardless, the usefulness of these analogs seems assured and future work is awaited with interest.

A partial summary of the interactions of thiophosphate analogs with various enzymes is given in Table IV. Of particular interest is the reaction of ATPγS with myosin. Trentham and coworkers (40,40a,50) have studied the transient kinetics of myosin and its proteolytic subfragments using stopped-flow (and quenched-flow) techniques with a variety of ATP analogs. They prepared analogs modified both in the purine ring [e.g., replacement of adenosine by 6-thioinosine, 6-thioguanosine, or formycin (see Section IV)] and in the triphosphate chain [substitution by γ thiophosphate or by a β–γ–NH linkage (see Section II.C)]. The purine-substituted analogs were used because their fluorescent properties change markedly on binding to the enzyme. Substitution of sulfur in the

TABLE IV

Properties of Thiophosphate Adenine Nucleotide Analogs with Various Enzymes

Enzyme and Source	Compounds	Reference
Alkaline phosphatase (*E. coli*)	ADPβS; competitive inhibitor, $K_i = 6.6 \times 10^{-5}$ ATPγS; inhibitor	39
DNA-dependent RNA-Polymerase (*E. coli*)	ATPγS; substrate; $K_m = 3.8 \times 10^{-5}$ UTPγS; substrate ATPαS; substrate	35
Myosin ATPase (Rabbit)	ATPγS; substrate with modified properties	40
Methionyl-tRNA synthetase (Yeast)	ATPγS; substrate; $K_m = 6 \times 10^{-4}$	37
Phenylalanyl-tRNA synthetase (*E. coli*)	ATPγS; substrate ATPαS; competitive inhibitor	37
DNA-Polymerase II	dATPγS; substrate	37
Snake-venom phosphodiesterase	ATPγS; substrate	37
Polynucleotide Phosphorylase (Microc. lysodeicticus)	ADPβS; substrate.	37
C–C–A pyrophosphorylase (Yeast)	ATPαS; substrate	44
Hexokinase	ATPαS; Substrate, $K_m = 3.6 \times 10^{-4}$ ATPγS; no reaction	38
ATP/ADP Translocase (Mitochondria)	ATPαS, ATPγS, ADPαS, ADPβS; all translocated	38

γ phosphate of ATP (ATPγS), thio-GTP (thio-GTPγS), or thio-ITP (called inappropriately "thioATPγS") allowed various kinetic intermediates to be delineated. They postulated (40) the simplest kinetic scheme for myosin cleavage of ATP which would accommodate all the known facts at that time as

$$M + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M^*\cdot ATP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} M^*\cdot ADP\cdot Pi \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$$

$$M\cdot ADP\cdot Pi \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} M\cdot ADP + Pi \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} M + ADP$$

The complexes marked * indicate intermediates with enhanced fluorescence. That the first fluorescence change, that is, M · ATP*, represents the binding of ATP rather than a subsequent cleavage step was shown conclusively by the use of ATPγS; thus, ATPγS induces the same fluorescence change on binding to the enzyme as ATP but it is cleaved at only 0.15% the rate of ATP. This low cleavage rate also means the steady-state complex is M* · ATPγS rather than M* · ADP · Pi as appears to occur with myosin and ATP. This change in rate constants (i.e., k_2 and k_{-2} above) allows different intermediate states to predominate and to be discerned by fast-reaction techniques. Although the above reaction scheme is now known to be oversimplified (40a) the utility of analogs with modified purine rings to give enhanced fluorescence or chromophoric changes possibly combined with modified triphosphate chains to give altered rates of cleavage should be a technique widely used in future studies of ATP requiring systems.

Recent studies have been made of the specificity of the translocation of ADP and ATP thiophosphate analogs and their interaction with the ATP-synthetase complex with mitochondria (38). In general, all the thiophosphate analogs of ATP and ADP are translocated at least as judged by their atractyloside sensitive binding to mitochondria. Neither ADPαS nor ADPβS are phosphorylated in oxidative phosphorylation but are potent inhibitors of state-3 respiration indicating a firm binding to the ATP-synthetase complex (38). Likewise ATPαS and ATPγS are potent inhibitors of ATP-driven reverse-electron transport indicating the high degree of specificity of the characteristic reactions of oxidative phosphorylation.

The specificity of the two kinases most extensively studied, that is, adenylate kinase and nucleoside diphosphate kinase, is less restrictive (Table V). Thus AMPαS with ATP and AMP with ATPαS form substrate pairs for adenylate kinase. A detailed study of the reactivity of AMPαS with adenylate kinase and other enzymes has been published (51). ADPαS is, in turn, a substrate for nucleoside diphosphate kinase whereas ADPβS is not, or at best, a very poor substrate. Only if an ATP regenerating system is added will ADPβS be significantly phosphorylated (38). This latter finding points out the necessity *not* to regard negative results with these or other analogs as definitive because most experiments have been done using limited variations in reaction conditions. A good example of this is the recent study of the suitability of various

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