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Practical Enzymatic Synthesis of Adenosine 5'-O-(3-Thiotriphosphate) (ATP- γ -S)¹

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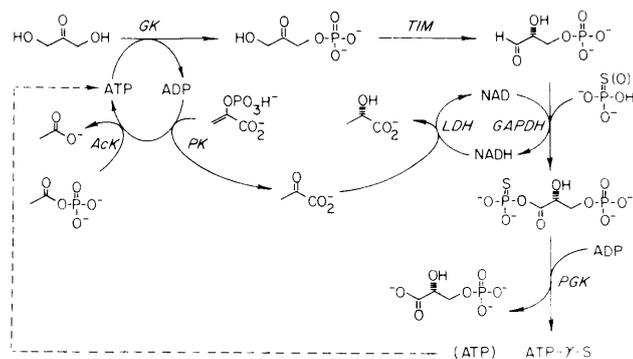
An enzymatic procedure for the synthesis of adenosine 5'-O-(3-thiotriphosphate) (ATP- γ -S) on a 50-mmol scale from dihydroxyacetone, sodium thiophosphate, ADP, and phosphoenol pyruvate is described. The synthesis uses polyacrylamide gel immobilized glycerokinase coupled to a pyruvate kinase catalyzed ATP cofactor regeneration system, and polyacrylamide gel immobilized triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, and phosphoglycerate kinase coupled to a lactate dehydrogenase catalyzed NAD cofactor regeneration system. The ATP- γ -S is purified by adsorption on Dowex 1 and isolated as the sodium or barium salts in ~90% purity.

Adenosine 5'-O-(3-thiotriphosphate) (ATP- γ -S) is an ATP analogue useful in mechanistic enzymology.²⁻¹⁹

ATP- γ -S was first synthesized by Goody and Eckstein by chemical methods.²⁰ It and several isotopically labeled analogues have since been prepared on 0.1-1 mmol scale

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Scheme I. Enzymatic Synthesis of ATP- γ -S^a

^a Abbreviations: GK, glycerokinase; TIM, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; AcK, acetate kinase.

by using enzymatic procedures.²¹ Although ATP- γ -S is commercially available, its high cost precludes its use in practical syntheses. This cost is due partly to the cost of the starting materials used in current syntheses²² and partly to the cumbersome procedures used for its isolation and purification.

We have developed a synthesis of ATP- γ -S applicable to preparations on 50-mmol scale. This synthesis uses a coupled enzyme system comprising PAN-immobilized²³ glycerokinase (GU), pyruvate kinase (PK), triosephosphate isomerase (TIM), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), and lactate dehydrogenase (LDH) (Scheme I). The utility of this system rests on three features. First, the starting material, dihydroxyacetone, is inexpensively available commercially and preparation of dihydroxyacetone phosphate from it is straightforward. Second, the ATP formed in this procedure as a byproduct is readily converted to ATP- γ -S; this conversion facilitates purification and improves the yield of ATP- γ -S. Third, the ultimate purification of ATP- γ -S is accomplished readily (by adsorption on an ion-exchange resin).

Results and Discussion

Synthesis of Dihydroxyacetone Phosphate (DHAP). We have previously described two procedures

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(22) The approximate costs of starting materials (current Sigma prices) are as follows: L- α -glycerophosphate, \$5000/mol; dihydroxyacetone phosphate, \$100 000/mol; D-glyceraldehyde 3-phosphate, \$180 000/mol; D,L-glyceraldehyde 3-phosphate, \$30 000/mol; dihydroxyacetone, \$25/mol.

(23) Pollak, A.; Blumenfeld, H.; Wax, M.; Baugh, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* 1980, 102, 6324-6336. PAN is a copolymer of acrylamide and N-(acryloyl)succinimide.

that convert dihydroxyacetone (DHA) to DHAP.²⁴ These procedures are based on enzymatic phosphorylation of DHA by ATP with in situ cofactor regeneration (Scheme I) and differ only in the regeneration sequence used for the conversion of ADP to ATP. Both procedures can be carried out on scales of up to several moles, and the product solutions can be used directly in subsequent transformations. The procedure utilizing phosphoenolpyruvate (PEP) and pyruvate kinase (PK) for phosphorylation²⁵ yields a solution of DHAP of low inorganic phosphate content, because PEP is hydrolytically stable at neutral pH. The procedure using AcP as phosphorylating agent²⁶ is more convenient and less expensive than that using PEP. For the synthesis of ATP- γ -S, in which contamination of the reaction mixture by inorganic phosphate leads to ATP, the use of PEP/PK is preferable for two reasons. First, the concentration of inorganic phosphate in the resulting solution of DHAP is considerably lower than that obtained by using AcP. Second, the DHAP solution contains pyruvate, which itself is utilized in the NAD-regeneration system required in a later step of the synthesis of ATP- γ -S. The procedure employing PEP and pyruvate kinase afforded DHAP and pyruvate in 91% and 77% yield, respectively, based on DHA; the final concentration of DHAP was 125 mM and that of inorganic phosphate was 3 mM. This solution was utilized without further purification in the subsequent synthesis of ATP- γ -S.

Synthesis of Adenosine 5'-O-(3-Thiotriphosphate) (ATP- γ -S). The method we outline in Scheme I for the synthesis of ATP- γ -S differs from previous syntheses of this compound in various important practical details but not in fundamental structure. Dihydroxyacetone phosphate was obtained previously by Walseth and Johnson by enzyme-catalyzed oxidation of α -L-glycerol phosphate.²¹ Cassidy and Verrick modified this procedure in their synthesis of ATP- γ -[³⁵S] by substituting labeled thiophosphate for phosphate.²¹ Our synthesis has four distinguishing characteristics. First, we use DHA as starting material. Second, we incorporate into the procedure a method for consuming the ATP formed as a byproduct (by forming DHAP). This modification increases both the purity and yield of the ATP- γ -S (the yield of ATP- γ -S based on ADP is ~80% in this synthesis as compared to a yield of 10% in the procedure of Cassidy and Verrick). Third, we use immobilized enzymes. Immobilization allows the reaction to be run on larger scale and increases the lifetime of the enzymes. Fourth, we use a simple isolation procedure.

Simultaneously with our work Webb²⁷ demonstrated that ATP- γ -S could be obtained on a 0.1-mmol scale from fructose 1,6-diphosphate, sodium thiophosphate, and ADP. Fructose 1,6-diphosphate is a realistic alternative precursor to GAP and DHAP and should have a similar potential as starting material for ATP- γ -S synthesis, provided the modifications suggested in our synthesis are adopted.

The preparation of ATP- γ -S was carried out by addition of sodium thiophosphate and a solution containing DHAP and pyruvate to a mixture of ADP, NAD⁺, EDTA,²⁸ DTT,²⁹ MgCl₂, and four enzymes immobilized in PAN:

(24) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* 1983, 48, 3199-3205.

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(26) Crans, D. C.; Whitesides, G. M. *J. Org. Chem.* 1983, 48, 3130-3132.

(27) Webb, M. R. *Methods Enzymol.* 1982, 87, 301-316.

(28) Small amounts of EDTA were used to chelate trace quantities of heavy metal salt contaminating the commercial preparations of MgCl₂.

TIM, GAPDH, PGK, and LDH. ATP is formed as a by-product in the reaction from 1,3-diphosphoglycerate, which was in turn produced by reaction of 3-phosphoglycerate and inorganic phosphate. Inorganic phosphate is generated by hydrolysis of starting materials and intermediates; it is also present as a contaminant in the solution containing DHAP and pyruvate. Conversion of this ATP to ADP was accomplished in a separate step by addition of DHA and immobilized GK to the solution. This reaction converted ATP to ADP (and DHA to DHAP) in 98% yield. Although ATP- γ -S is also a substrate of GK, it reacts at a significantly slower rate than does ATP. Use of 1 equiv of DHA per mole of ATP limited the extent of reaction of ATP- γ -S to approximately 2% under the reaction conditions. This ADP was, in turn, converted to ATP- γ -S by using the original reaction conditions and enzymes recovered from the first reaction.

The major technical problem in the preparation of ATP- γ -S is that of minimizing the generation of inorganic phosphate. DHAP,³⁰ sodium thiophosphate,³¹ and 1-(thiophospho)-3-phosphoglycerate,³² are all labile compounds that hydrolyze readily to inorganic phosphate. Thus it is difficult to prevent the generation of some ATP. Its formation can, however, be minimized by controlling the rate of addition of DHAP and sodium thiophosphate to the reacting mixture. In addition, use of the appropriate ratio of PGK to GAPDH ($\geq 4:1$) prevents the accumulation of significant amounts of 1-(thiophospho)-3-phosphoglycerate.

Isolation and Purification of ATP- γ -S. The first purification step involves the conversion of contaminating ATP to ADP using the GK/DHA system. Although ATP- γ -S can be separated from both ATP and ADP by chromatography on Dowex 1, yields are higher if the ATP has been converted to ADP prior to chromatography. The second purification step involves the separation of ATP- γ -S from contaminants by adsorption of the crude reaction mixture onto Dowex 1. All of the nucleoside phosphates absorb,³³ but ATP, ADP, and other phosphates can be selectively desorbed. Dowex-1 separation offers a purification procedure that is superior to the cellulose chromatography resins currently employed in ATP- γ -S syntheses: first, the loading capacity of Dowex 1 is larger; second, the separation can be done with high flow rates; third, this type of separation is amenable to large-scale preparations. The crude ATP- γ -S reaction mixture is applied to the Dowex-1 column (CO_3^{2-}); all the phosphates absorb. All contaminants (inorganic phosphate, 3-phosphoglycerate, ADP, ATP, and DHAP) are eluted with a solution of 0.01 M HCl–0.2 M NaCl. Since the nucleosides are labile under acidic conditions, the manipulations are performed at 4 °C.

The desorption of ATP- γ -S from Dowex 1 can be done by two methods: one method affords the barium salt of ATP- γ -S, the other the sodium salt of ATP- γ -S. Isolation

involving the barium salt results in relatively pure ATP- γ -S in high yield. The procedure is convenient and requires fairly small desorption volumes. The ATP- γ -S can be regenerated from the $\text{Ba}_2\text{ATP-}\gamma\text{-S}$ by dissolving the salt in dilute sulfuric acid and removal by filtration of the insoluble precipitate of BaSO_4 .

For convenient use of ATP- γ -S the sodium salt is preferable. It dissolves easily in water, it contains an innocuous cation, and it can be used directly in further enzymatic transformations without removal of the cation. This isolation procedure is, however, more cumbersome than that for the barium salt and results in lower yields of material with lower purity.

Conclusion

This paper describes the best method presently available for the large-scale preparation of ATP- γ -S. This synthesis should be amenable to further scale-up. The synthesis has several advantages. First, it uses readily available starting materials (DHAP, ADP, thiophosphate). Second, this approach provides an effective method of coupling pyruvate, a coproduct in the synthesis of DHAP, to the NAD regeneration system required for the preparation of 1-(thiophospho)-3-phosphoglycerate. Third, the ATP produced as a byproduct can be converted to ATP- γ -S by using a simple additional step. Fourth, purification of ATP- γ -S can be accomplished on a large scale by selective adsorption on Dowex 1.

The major technical problem in this synthesis is that of maintaining a system in which the concentration of inorganic phosphate is minimized. We conclude that although inorganic phosphate cannot be completely eliminated, its concentration in the reaction can be minimized by careful purification of starting materials and by their controlled addition to the reacting solution. Furthermore, the incorporation of the step that consumes ATP and generates additional DHAP provides a way of destroying the contaminating ATP and simultaneously increasing the yield of ATP- γ -S.

Experimental Section

General Methods. Spectrophotometric measurements were performed at 25 °C on a Perkin-Elmer Model 552 spectrophotometer equipped with a constant temperature cell. ³¹P NMR spectra were recorded at 121.5 MHz on a Bruker Model WM 300 spectrometer. NMR samples were prepared in 50 mM HEPES–NaOH, pH 8.0, containing 10 mM EDTA and 20% D_2O internal lock. Chemical shifts for ³¹P NMR are reported relative to external H_3PO_4 . HPLC analyses were carried out on a Waters Associates system equipped with a differential ultraviolet detector operating at 254 nm, using a Waters Radial-PAK SAX column (8 mm \times 10 cm, 10- μm particle size). Elutions were carried out with a solution of 0.7 M ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), pH 4.0 (flow rate 3.0 mL/min). Analytical thin-layer chromatography was performed on polyethylenimine–cellulose plates eluted with 0.75 M potassium phosphate buffer, pH 3.5.

The enzymatic reactions were carried out in three-necked, round-bottomed flasks, which were modified to accommodate a pH electrode. The pH of the reaction mixtures was controlled with a Weston Model 7561 pH controller coupled to an LKB 10200 peristaltic pump. Prior to the addition of the immobilized enzymes, all solutions were deoxygenated with argon by using a gas dispersion tube. The enzymatic reactions were conducted at room temperature under an argon atmosphere unless otherwise specified. At the end of each reaction the enzyme-containing gels were allowed to settle and the supernatant was decanted under positive argon pressure via a cannula. The gels were washed with 50 mM HEPES buffer (pH 7.5) and compacted by centrifugation, and the recovered enzyme activities were assayed.

Materials. Enzymes and biochemicals were obtained from Sigma. Sodium thiophosphate ($\text{Na}_3\text{O}_3\text{PS}\cdot 12\text{H}_2\text{O}$, Alfa) was pu-

(29) DTT was used to maintain a reducing environment for the enzymes, the sodium thiophosphate, and ATP- γ -S (ref 27).

(30) DHAP is not stable at values of pH higher than 6. The half-life of DHAP (10 mM) in triethanolamine buffer (0.1 M, pH 7.0, 23 °C) is 65 h.

(31) Sodium thiophosphate in solution readily decomposes to hydrogen sulfide and phosphate although the anhydrous trisodium salt is stable. The half-life time for hydrolysis of sodium thiophosphate to phosphate is 20 h under the reaction conditions used for the synthesis of ATP- γ -S (room temperature pH 7.0–7.5). Commercial preparations of sodium thiophosphate contain large (20–50%) quantities of inorganic phosphate and therefore must be purified prior to use in this synthesis (ref 1). Yasuda, S. U.; Lambert, J. L. *J. Am. Chem. Soc.* **1954**, *76*, 5356.

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(33) Hurlbert, R. B. *Methods Enzymol.* **1957**, *3*, 785–805. Cohn, W. E.; Carter, C. E. *J. Am. Chem. Soc.* **1950**, *72*, 4273–4275.

rified by the method of Yasuda and Lambert.³¹ Pre-coated TLC PEI-cellulose F sheets were purchased from MCB. Dowex 1 \times 8 (200–400 mesh) was purchased from Bio-Rad. PAN was prepared as previously described.²³

Assay Methods.³⁴ Enzymes and biochemicals were assayed according to procedures of Bergmeyer et al.³⁵

Enzyme Immobilizations. Immobilizations of enzymes in PAN gel were carried out following the procedures described previously²³ for glyceraldehyde-3-phosphate dehydrogenase [EC 1.2.1.12],³⁶ phosphoglycerate kinase [EC 2.7.2.3], lactate dehydrogenase [EC 1.1.1.27], glycerokinase [EC 2.7.1.30], and pyruvate kinase [EC 2.7.1.40]. Triosephosphate isomerase [EC 5.3.1.1] (TIM) was immobilized as follows: PAN-1000 (1 g) was dissolved in 0.3 M Hepes buffer (4 mL, pH 7.6) containing MgCl₂ (15 mM), DHAP (10 mM), and GAP (10 mM). The enzyme solution (0.25 mL, 1000 U) was added, followed by 0.5 M TET (0.8 mL). The gel that formed was allowed to stand for 60 min and was ground and washed as described.²³ The immobilization yield was 36%.

Dihydroxyacetone Phosphate (DHAP). To an 800-mL solution containing dihydroxyacetone (9.9 g, 0.11 mol), ATP (2 mmol), MgCl₂ (5 mmol), and PEP (K⁺PEP⁻, 20.6 g, 0.1 mol),²⁵ pH 7.0, was added PAN-immobilized GK (500 U) and PK (700 U). The mixture was stirred at room temperature under argon. Enzymatic analysis indicated that the reaction was complete in 7 h. After recovery of the enzyme-containing gels, the solution was treated with acid-washed charcoal (10 g) and filtered. The resulting solution (containing 0.10 mol of DHAP, 91% yield, and 85 mmol of pyruvate, 77% yield) was used directly in the synthesis of ATP- γ -S described below. The recovered enzyme activities (calculated as a percentage of starting activities) were as follows: GK, 82%; PK, 80%. The turnover numbers (mol products/mol enzyme) for the enzymes were GK, 4×10^6 , and PK, 3×10^6 .

Adenosine 5'-O-(3-Thiotriphosphate) (ATP- γ -S). A 1-L solution containing ADP³⁷ (80 mmol), pyruvate (25 mmol), MgCl₂ (20 mmol), EDTA (8 mmol), NAD³⁸ (8 mmol), and DTT (20 mmol) was deoxygenated with a stream of argon and adjusted to pH 7.5 with NaOH. Immobilized triosephosphate isomerase (TIM, 100 U), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 200 U), phosphoglycerate kinase (PGK, 700 U), and lactate dehydrogenase (LDH, 400 U) were added to this solution. A solution (800 mL) containing DHAP (0.1 mol) and pyruvate (85 mmol) was added to the stirred reaction in ten portions over 3 days. Trisodium monothiophosphate (80 mmol) was added separately in eight portions over 5 days; each fraction was dissolved in 30 mL of deoxygenated, doubly distilled H₂O before addition. The reaction was conducted at pH 7.5 with occasional adjustment of pH with a few drops of 2 N NaOH. The course of the reaction was followed by ³¹P NMR, HPLC, and enzymatic assays for DHAP, ADP, ATP, and ATP- γ -S. After 3 days, 46 mmol of ATP- γ -S had been formed (57% yield based on ADP added). The polyacrylamide gels were allowed to settle and the supernatant was decanted. The resulting solution contained ATP- γ -S (46 mmol), ATP (26 mmol), ADP (8 mmol), DHAP (24 mmol), and 3-PG (72 mmol), as well as thiophosphate and inorganic phosphate. The immobilized enzymes were recovered and used in the formation of additional ATP- γ -S from regenerated ADP, as described in the following section. After these transformations the

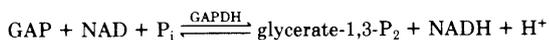
(34) Assays were carried out at 25 °C. One unit (U) of enzymatic activity is defined as the amount of enzyme that catalyzes the formation of one μ mol of product per min at 25 °C.

(35) Bergmeyer, H. U. "Methods of Enzymatic Analysis"; Verlag Chemie: New York, 1974.

(36) The glyceraldehyde-3-phosphate dehydrogenase should be that from muscle, since AMP, ADP, and ATP inhibit the enzyme from yeast. Yang, S. T.; Deal, W. C. *Biochemistry* 1969, 8, 2814–2820.

(37) The ADP should be completely free of heavy metals. Vanadium in particular causes catalytic decomposition of ATP- γ -S. M. Cohn, personal communication.

(38) The equilibrium for the reaction catalyzed by GAPDH with inorganic phosphate as substrate lies far in the direction of GAP formation,



$K_{\text{eq}} = 6.7 \times 10^{-8}$; Cori, C. F.; Velick, S. F.; Cori, G. T. *Biochim. Biophys. Acta* 1950, 4, 160–169. A high concentration of NAD⁺ is essential to drive the formation of 1-(thiophospho)-3-phosphoglycerate and lactate.

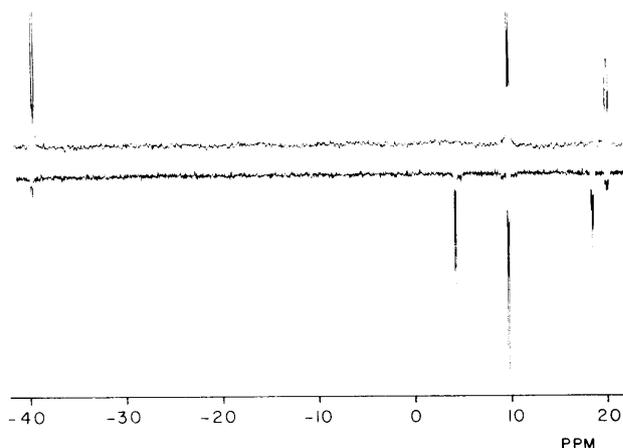


Figure 1. (Upper) ³¹P NMR spectrum of ATP- γ -S, 50 mM, 20% in D₂O, pH 8.0, 500 transients, recorded on a Bruker 121.5-MHz NMR. (Lower) ³¹P NMR of ATP- γ -S and ATP under similar conditions.

recovered enzyme activities were as follows (expressed as percentage of starting activities): TIM, 90%; GAPDH, 40%; PGK, 86%; LDH, 74%. The turnover numbers for the enzymes in this reaction were: TIM, 2×10^7 ; GAPDH, 3×10^6 ; PGK, 2×10^6 ; LDH, 2×10^6 .

Selective Conversion of ATP to ADP. To the solution obtained above (containing 46 mmol of ATP- γ -S, 26 mmol of ATP, 18 mmol of DHAP, and 8 mmol of ADP) was added dihydroxyacetone (DHA, 26 mmol). Immobilized GK (500 U) was added, and the mixture was kept under argon with stirring. The reaction was complete in 2 h. Enzymatic and HPLC analyses indicated that DHA and ATP had been converted to DHAP and ADP in 98% yield. The solution was separated from the enzyme-containing polyacrylamide gels by decantation. The resulting solution contained ATP- γ -S (45 mmol), ATP (0.5 mmol), ADP (34 mmol), and DHAP (50 mmol), as well as 3-PG and inorganic phosphate. This solution was added to the suspension of PAN-immobilized GAPDH, PGK, TIM, and LDH recovered from the first ATP- γ -S reactor (vide supra). After 2 days the reaction mixture was worked up to yield a solution containing ATP- γ -S (66 mmol, 83% yield based on added ADP), ATP (9 mmol), ADP (5 mmol), DHAP (8 mmol), glycerate 3-phosphate, thiophosphate, and inorganic phosphate (as determined by enzymatic assay, ³¹P[¹H] NMR, and HPLC analyses).

Isolation and Purification of ATP- γ -S. Isolation was accomplished by two procedures in which only the desorption from Dowex 1 and the precipitation steps differ. The crude ATP- γ -S reaction mixture was treated with glycerokinase and DHA to convert most of the ATP present to ADP before separation on Dowex-1. This removal of ATP facilitates purification on Dowex-1 and increases isolated yields of ATP- γ -S, since the separation of ADP and ATP- γ -S is simpler than that of ATP and ATP- γ -S.

Glycerokinase Reaction. To the reaction mixture (containing 66 mmol of ATP- γ -S, 9 mmol of ATP, and 5 mmol of ADP) was added DHA (8.5 mmol) and immobilized GK (500 U). The reaction was complete after stirring for 2 h under argon. The GK-containing gel was separated from the solution containing ATP- γ -S (66 mmol), ATP (1 mmol), ADP (13 mmol), DHAP, phosphoglycerate, and inorganic phosphate. This reaction mixture (2.8 L) was then divided into two aliquots and the ATP- γ -S isolated from each according to the following procedures.

ATP- γ -S, Barium Salt. The first half of the glycerokinase-treated reaction mixture (1.4 L containing 33 mmol of ATP- γ -S) was passed through Dowex 1 (900 g, 200–400 mesh, CO₃²⁻ form, supported in a 1-L filter) and washed with 4 L of 0.01–0.2 M NaCl to remove inorganic phosphate, phosphoglycerate, ADP, ATP, and other impurities. ATP- γ -S was then desorbed by washing the resin with 1.2 L of 0.02 M HCl–0.8 M NaCl. This solution was mixed with BaCl₂ (120 mmol, 29.2 g) and the ATP- γ -S barium salt was precipitated with ethanol (1.5 L). After drying, the isolated solid (24.7 g) contained 92% by weight Ba₂ATP- γ -S (28.4 mmol, 86% isolated yield, 71% overall yield based on ADP), 1% by weight Ba₂ATP, and 1% by weight BaADP (determined by

HPLC): ^{31}P [^1H] NMR δ -34.5 (d, P_γ), 10.4 (d, P_α), 22.2 (dd, P_β); $J_{\text{P}_\alpha\text{-P}_\beta} = 19.4$ Hz, $J_{\text{P}_\gamma\text{-P}_\alpha} = 28.8$ Hz (Figure 1). The NMR spectrum (Figure 1) is indistinguishable from a commercial sample of ATP- γ -S; the chemical shifts and the P-P coupling constants are in good agreement with literature values.³⁹

ATP- γ -S, Tetrasodium Salt. The second half of the glycerokinase-treated reaction mixture (1.4 L, 33 mmol of ATP- γ -S) was adsorbed on Dowex 1 (900 g, 200-400 mesh, CO_3^{2-} form, supported in a 1-L filter) and washed as above with 4 L of 0.01 M HCl-0.2 M NaCl. The resin was washed with an additional 2 L of doubly distilled water, before the ATP- γ -S was desorbed by washing with 4 L of saturated aqueous $(\text{NH}_4)_2\text{CO}_3$ (~ 2 M). Most of the $(\text{NH}_4)_2\text{CO}_3$ was removed by addition of Dowex-50 W (H^+ form, ~ 2000 g) until the pH reached 3-4 at 0 $^\circ\text{C}$. The mixture was filtered to separate the resin and the pH adjusted

immediately to 7.5 by addition of NaOH (2 M). Most of the yellow color present in the solution was removed by passing the solution through charcoal (only ~ 25 g of acidic charcoal, since ATP- γ -S adsorbs on charcoal), and the solution was concentrated at reduced pressure to a final volume of ~ 500 mL. Addition of 3 L of ice-cold acetone followed by filtration afforded 15.8 g of solid containing 83% by weight ATP- γ -S, tetrasodium salt (21.3 mmol, 65% isolated yield, 53% overall reaction based on ADP), <1% by weight ATP tetrasodium salt and 8% by weight ADP disodium salt (determined by HPLC).

Registry No. ATP- γ -S, 35094-46-3; DHA, 96-26-4; DHAP, 57-04-5; ADP, 58-64-0; ATP- γ -S \cdot 2Ba, 88453-51-4; ATP- γ -S \cdot 4Na, 88453-52-5; GAPDH, 9001-50-7; PGK, 9001-83-6; LDH, 9001-60-9; GK, 9030-66-4; PK, 9001-59-6; TIM, 9023-78-3; ATP, 56-65-5; NAD, 53-84-9; D-glyceraldehyde-3-phosphate, 591-57-1; phosphorothioic acid, 13598-51-1; 1-(thiophospho)-3-phospho-D-glycerate, 88548-22-5.

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