Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 3004–3008, October 1972

An Adenosine Triphosphate–Phosphate Exchange Catalyzed by a Soluble Enzyme Couple Inhibited by Uncouplers of Oxidative Phosphorylation

(acyl-phosphatase/sulfenyl carboxylate/high-energy intermediate/energy conservation)

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Communicated by Nathan O. Kaplan, August 11, 1972

ABSTRACT **The sulfenic acid form of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), which is an acyl phosphatase, will catalyze an acetyl phosphate–Pi exchange reaction. This exchange reaction is reversibly inhibited by the uncouplers of oxidative phosphorylation, 2,4-dinitrophenol, *m*-Cl carbonylcyanide-phenylhydrazone, pentachlorophenol, and 5-chloro-3-tert-butyl-2′-chloro-4′-nitrosalicylanalide, and is irreversibly inhibited by cyanide and dicumarol. An ATP–Pi exchange reaction similar to that catalyzed by mitochondria can be simulated by a system composed of oxidized glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase (EC 2.7.1.28), 3-phosphoglycerate, ATP, ^{32}Pi, and appropriate cofactors. The ATP–Pi exchange is inhibited by uncouplers of oxidative phosphorylation. Higher concentrations of uncouplers will also inhibit the ATPase reaction catalyzed by the coupled enzyme system. The exchange reactions catalyzed by the sulfenic acid form of glyceraldehyde-3-phosphate are consistent with a sulfenyl carboxylate intermediate. On the basis of these observations, a reaction scheme has been postulated for covalent coupling in oxidative phosphorylation that includes a sulfenyl carboxylate as a nonphosphorylated, high energy intermediate and an acyl phosphate as a phosphorylated, high energy intermediate.**

The specific oxidation of the sulfhydryl group at the active site of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GPD) to a sulfenic acid converts the enzyme to an acyl phosphatase (1–3). The following reaction scheme has been postulated to account for the hydrolytic reaction catalyzed by the sulfenic-acid form of the enzyme (3):

$$\mathrm{ESOH} + \mathrm{R{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}O} \sim \mathrm{PO_3H^-} \rightleftarrows \mathrm{ESO{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}R} + \mathrm{H_2PO_4^-} \quad [1]$$

$$\mathrm{ESO{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}R} + \mathrm{H_2O} \rightarrow \mathrm{ESOH} + \mathrm{RCOO^-} + \mathrm{H^+} \quad [2]$$

The reaction scheme suggests that the conjugate base of the sulfenic acid reacts with the carbonyl carbon of the acyl phosphate substrate to form an enzyme–sulfenyl carboxylate intermediate with the displacement of inorganic phosphate. In the second step of the reaction scheme, the sulfenyl carboxylate reacts with water to release the carboxylate anion and the regenerated sulfenic acid form of the enzyme. The reaction scheme is consistent with C–O bond cleavage, which Park and Koshland have shown to occur during the hydrolysis of acetyl phosphate catalyzed by the oxidized enzyme (4).

Unsuccessful attempts have been made to demonstrate the sulfenyl carboxylate intermediate directly with the use of [^{14}C]acetyl phosphate and the chromophoric substrate analog (2-furyl) acryloyl phosphate (5). If the proposed sulfenyl carboxylate intermediate, which is a mixed anhydride between a sulfenic acid and a carboxylic acid, were to exist, its steady-state concentration would be predictably low in water and would therefore defy direct identification.

To provide indirect evidence for the reaction scheme described by Eqs. **1** and **2**, an investigation was initiated to determine if inorganic phosphate labeled with ^{32}P will exchange into acetyl phosphate in the presence of the sulfenic acid form of GPD. The reduced form of GPD catalyzes an acetyl phosphate–Pi exchange that proceeds through a thiol ester intermediate as described by Eq. **3** (6).

$$\mathrm{ESH} + \mathrm{R{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}O} \sim \mathrm{PO_3H^-} \rightleftarrows \mathrm{ES{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}R} + \mathrm{H_2PO_4^-} \quad [3]$$

Since small amounts of the sulfhydryl form of GPD always are present in our preparations of the oxidized enzyme, reaction conditions were sought that would distinguish between the exchange reaction catalyzed by the contaminating, reduced enzyme and that which might be catalyzed by the oxidized enzyme.

Several compounds that inhibit the acyl phosphatase reaction catalyzed by the oxidized enzyme and that have no effect on the reactions catalyzed by the reduced enzyme were used. Among these are cyanide and uncouplers of oxidative phosphorylation. The sulfenic acid form of GPD catalyzes the exchange of inorganic phosphate labeled with ^{32}P into acetyl phosphate, which is sensitive to uncouplers of oxidative phosphorylation. Furthermore, the sulfenic acid form of GPD coupled to phosphoglycerate kinase (EC 2.7.1.28) will catalyze an ATPase reaction in the presence of 3-phosphoglycerate, which is also sensitive to uncouplers of oxidative phosphorylation. The coupled enzyme system catalyzes an ATP–Pi exchange reaction, which is also inhibited by the uncouplers. The results of this investigation and their implications in terms of an energy conservation mechanism for oxidative phosphorylation are presented in this communication.

MATERIALS AND METHODS

GPD was prepared from rabbit muscle as described by Cori, Slein, and Cori (7) and was oxidized with *o*-iodosobenzoate (3).

The exchange of ^{32}Pi into acetyl phosphate was in reaction mixtures that contained in 2.20 ml of 18 mM Veronal (pH 7.6): 3 mg of oxidized or reduced GPD, 28 μmol of acetyl phosphate, 30 μmol of $K^{32}PO_4$ (about 2×10^4 cpm/μmol), and the inhibitors where indicated. The radioactivity of

Abbreviations: DNP, 2,4-dinitrophenol; *m*Cl-CCPhe, *m*-Cl carbonylcyanide-phenylhydrazone; S-13, 5-chloro-3-tert-butyl-2′-chloro-4′-nitrosalicylanalide: GPD, glyceraldehyde-3-phosphate dehydrogenase.

acetyl phosphate was determined after it was separated from Pi in 0.4-ml samples by calcium precipitation (8) with the following modifications. The acid deproteinization step was omitted. After precipitation with the 3.3% $CaCl_2$ in 33% ethanol reagent, the supernatant was filtered through a membrane filter with a 0.4-μm pore size to completely remove the calcium phosphate. Acetyl phosphate in 0.2-ml samples was assayed with hydroxylamine (8).

The exchange of ^{32}Pi into ATP was in reaction mixtures that contained in 4.0 ml of 18 mM Veronal buffer (pH 7.6): 4 mg of oxidized or reduced GPD, 2.8 μmol of NAD, 0.1 mg of phosphoglycerate kinase, 30 μmol of ATP, 30 μmol or 6 μmol of 3-phosphoglycerate, 150 μmol of $K^{32}PO_4$ (about 5 $\times$ 10^4 cmp/μmol), and the inhibitors where indicated. Samples of the reaction mixtures, 1.0 ml each, were deproteinized with 0.1 ml of 35% perchloric acid. The radioactivity of the ATP was determined after separation of Pi from ATP in 0.5 ml of the deproteinized samples (9).

RESULTS

The Acetyl Phosphate–Pi Exchange Reaction. Table 1 illustrates that the sulfenic-acid form of GPD catalyzes an acetyl phosphate–Pi exchange reaction that is not due to the presence of small amounts of contaminating reduced enzyme. This exchange can be distinguished from the exchange reaction catalyzed by the reduced form of the enzyme by the following criteria. 2,4-Dinitrophenol (DNP), *m*Cl-CCPhe, and cyanide inhibit the exchange reaction catalyzed by the oxidized preparations of the enzyme, while they have no inhibitory effect on the exchange reaction catalyzed by the sulfhydryl form of the enzyme. In addition, the rate of the exchange reaction catalyzed by the sulfenic acid form of the enzyme is faster than the rate of the exchange reaction catalyzed by the sulfhydryl form of the enzyme. This is more clearly demonstrated when the rates of exchange are corrected for the hydrolysis of acetyl phosphate. The exchange reaction catalyzed by the reduced enzyme is not accompanied by acetyl phosphate hydrolysis, while significant acetyl phosphate hydrolysis occurs during the course of the exchange reaction catalyzed by the oxidized enzyme. When the exchange rates are expressed as the increase in the specific radioactivity of the acetyl phosphate remaining in the reaction mixtures with time, the efficiency of the oxidized enzyme in the exchange reaction is accentuated.

TABLE 1. *Differences between the acetyl phosphate–Pi exchange reactions catalyzed by the sulfhydryl and sulfenic-acid forms of GPD*

Enzyme species*	Protein (mg/ml)	Dehydrogenase activity† (μmol NADH/mg per min)	[^{32}P] Acetyl-PO_4 formed (nmol/mg per min)
ESH	1.36§	27.5	22.1
ESH + CN^-‡	1.36	27.5	21.2
ESH + DNP¶	1.36	27.5	27.4
ESH + *m*Cl-CCPhe¶	1.36	27.5	22.3
ESOH	1.36	4.0×10^{-2}	52.4
ESOH + CN^-‡	1.36	5.6×10^{-2}	6.5
ESOH + DNP¶	1.36	7.6×10^{-2}	39.7
ESOH + *m*Cl-CCPhe¶	1.36	5.0×10^{-2}	4.3

* The preparation of the oxidized enzyme (ESOH) and the compositions of the reaction mixtures are described in *Methods*.

† The dehydrogenase activity in each reaction mixture was determined spectrophotometrically (11).

‡ The cyanide concentration in the reaction mixtures was 35 μM.

§ This protein concentration corresponds to an enzyme subunit concentration of 39 μM.

¶ The concentrations of DNP and *m*Cl-CCPhe were 970 μM.

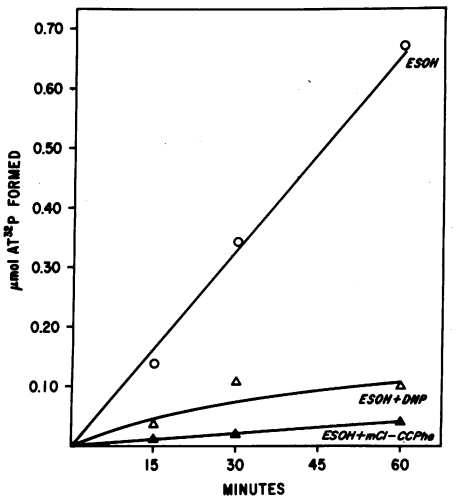


FIG. 1. The contents of the reaction mixtures and the assay for the exchange reaction are described in *Methods*. △, this reaction mixture contained 700 μM DNP. ▲, this reaction mixture contained 700 μM *m*Cl-CCPhe.

Cyanide reacts with the sulfenic acid at the active site of the acyl phosphatase to form a β-thiocyanoalanine residue. The inhibition of the exchange reaction catalyzed by the oxidized enzyme by DNP and *m*Cl-CCPhe is relieved by gel filtration.

The ATP–Pi Exchange Reaction. Fig. 1 shows that the sulfenic-acid form of GPD will catalyze an ATP–Pi exchange reaction in the presence of 3-phosphoglycerate and phosphoglycerate kinase. The reduced form of the enzyme also catalyzes this exchange reaction in the same reaction (10), which differs from the exchange shown in Fig. 1 in two respects. The exchange catalyzed by the oxidized form of the enzyme in the coupled enzyme system is clearly inhibited by fairly low concentrations of DNP and *m*Cl-CCPhe. Cyanide is a stoichiometric inhibitor of this exchange reaction. The ATP–Pi exchange that is catalyzed by the reduced enzyme in the coupled enzyme system is not inhibited by the uncouplers or by cyanide. The enzyme system composed of reduced GPD and phosphoglycerate kinase does not hydrolyze ATP, while the oxidized enzyme works in concert with phosphoglycerate kinase as a potent ATPase, as shown in Fig. 2. The ATPase activity is inhibited by DNP and by *m*Cl-CCPhe.

When compared to the rate of the acetyl phosphate–Pi exchange reaction catalyzed by the oxidized enzyme, as shown in Table 1, the rate of the ATP-Pi exchange catalyzed by

TABLE 2. *The effect of uncouplers on acetyl phosphate–Pi exchange and acetyl phosphate hydrolysis*

Uncoupler	Molar excess*	% Inhibition ^{32}P exchange†	% Inhibition hydrolysis‡	Reversible by gel filtration§
DNP	25	62	10	Yes
*m*Cl-CCPhe	25	94	88	Yes
*m*Cl-CCPhe	10	85	70	Yes
*m*Cl-CCPhe	5	58	7	Yes
Dicumarol	5	92	94	No
Penta-Cl-Phenol	10	84	40	Yes
S-13	?¶	83	30	Yes

* The concentration of oxidized enzyme subunit was 28 μM.

† The inhibition of the exchange is based on the initial rate of increase in specific radioactivity of the acetyl phosphate in the reaction mixtures, (μg-atoms ^{32}P per μmol acetyl phosphate) per mg of ESOH per min.

‡ The inhibition of the hydrolysis of acetyl phosphate is based on the initial rate of acetyl phosphate hydrolyzed, Δ μmol of acetyl phosphate per mg ESOH per min.

§ Reversible by gel filtration means that the acetyl phosphate activity returned upon removing the uncoupler by gel filtration on Sephadex G-25.

¶ S-13 was present as a saturated solution.

the coupled enzyme system shown in Fig. 1 is slow. Adenine nucleotides, especially ATP, inhibit the acyl phosphatase activity catalyzed by oxidized GPD; the inhibition can be partly relieved by the addition of NAD (11).

The Differential Effect of Uncouplers on the Hydrolytic and Exchange Reactions. Examination of Figs. 1 and 2 reveals that the ATP–Pi exchange reaction catalyzed by oxidized GPD and phosphoglycerate kinase is more sensitive to inhibition by DNP than is the ATPase reaction catalyzed by the same enzyme. The experimental values shown in Figs. 1 and 2 were obtained from the same reaction mixtures. The observation that the rate of formation of AT^{32}P is linear while the rate of ATP hydrolysis decreases with time in the same reaction mixtures is reproducible. This effect may be related to the differential inhibition of the acyl phosphatase reaction by the various adenine nucleotides (11), the concentrations of which are changing during the course of a given reaction.

Since the coupled enzyme is complicated by the inhibition of oxidized GPD by the adenine nucleotides present, the differential effect of various uncouplers on the exchange and hydrolytic reactions catalyzed by oxidized GPD was studied in the acetyl phosphate system (Table 2). The acetyl phosphate–Pi exchange is more sensitive to uncouplers than is the hydrolysis of acetyl phosphate for all of the uncouplers examined with the exception of dicumarol. The inhibition exerted by the uncouplers is relieved by gel filtration, again with the exception of dicumarol. This suggests that dicumarol covalently modifies the acyl phosphatase. The reactivation of the dehydrogenase activity by dithiothreitol is prevented when dicumarol is added to oxidized GPD. This suggests that dicumarol might oxidize the sulfenic acid at the active site of the acyl phosphatase to a sulfinic or sulfonic acid.

The differential effect of the uncouplers on the exchange and hydrolytic reactions is concentration-dependent, as is illustrated for *m*Cl-CCPhe in Table 2. At a 25-fold molar excess, the uncoupler nearly completely inhibits both the exchange reaction and the hydrolytic reaction. When *m*Cl-CCPhe is present at a 5-fold molar excess the hydrolytic reaction is barely inhibited while the exchange reaction is inhibited by 58%. This suggests that the uncouplers compete with Pi at low concentrations and react with the sulfenic acid of the enzyme at high concentrations.

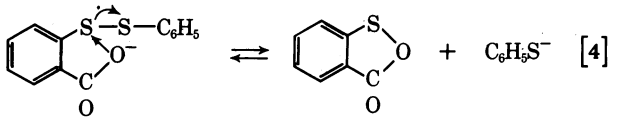
DISCUSSION

The results presented have established that the sulfenic acid form of GPD catalyzes an acyl phosphate–Pi exchange reaction. This exchange reaction is consistent with, but does not prove, the existence of a sulfenyl carboxylate intermediate. When the reactions catalyzed by the sulfenic acid form of GPD and those catalyzed by the sulfhydryl form of GPD are compared, and are viewed in terms of the modification of the active site that accompanies oxidation, an intuitive argument can be made in favor of the sulfenyl carboxylate mechanism. The active site of the acyl phosphatase differs from that of the transferase (the acyl phosphate–Pi exchange reaction) by an oxygen atom. This additional oxygen atom should not alter the acyl phosphate binding properties of the enzyme. However, the conjugate base of the sulfenic acid should be a potent nucleophile capable of reacting with the carbonyl carbon of the bound acyl phosphate substrate to form the proposed sulfenyl carboxylate intermediate by the displacement of phosphate. The sulfenyl carboxylate intermediate, which is a mixed anhydride, should react with water rapidly. On the other hand, the covalent intermediate in the transferase reaction catalyzed by the reduced enzyme is a thiol ester, which should be intrinsically more stable than the sulfenyl carboxylate, and is not hydrolyzed rapidly.

The ATP–Pi exchange catalyzed by the oxidized GPD–phosphoglycerate kinase couple, which is sensitive to uncouplers of oxidative phosphorylation, can be considered as a model for one of the partial reactions of oxidative phosphorylation (12). If this exchange reaction proceeds through the proposed sulfenyl carboxylate intermediate, the coupled enzyme system should catalyze the $H_2O \rightarrow$ Pi oxygen exchange and the $H_2O \rightarrow$ ATP oxygen exchange, two other partial reactions of oxidative phosphorylation (12, 13). The acyl phosphatase reaction proceeds with C–O bond cleavage (4). Therefore, the carboxyl oxygens of 3-phosphoglycerate should equilibrate with the oxygen of water in the coupled enzyme system. The subsequent phosphorylation of the labeled 3-phosphoglycerate followed by the hydrolysis of the labeled 1,3-diphosphoglycerate would lead to the formation of Pi containing oxygen ultimately derived from water. Since the Pi $\rightarrow$ ATP exchange has been shown to occur, the $H_2O \rightarrow$ ATP oxygen exchange would be a direct consequence of the $H_2O \rightarrow$ Pi oxygen exchange.

Sulfenyl carboxylates have been implicated recently as intermediates in the disproportionation of unsymmetrical *o*-carboxyphenyl disulfides (14) and in the overoxidation of β-carboxymercaptans by iodine (15–17). The disproportionation of 2-(phenyldithio)benzoic acid to phenyldisulfide and 2,2′-dithiobenzoic acid depends on pH in a manner that suggests that the *o*-carboxyl group participates in the reaction (14). It was postulated (14) that the attack of the carboxylate anion on the proximal sulfur of the disulfide initiates the dis-

proportionation by displacing thiophenylate. This results in the formation of a 5-membered cyclic sulfenyl carboxylate intermediate as shown by Eq. **4**. That the corresponding *meta*



[4]

isomer undergoes a much slower disproportionation and that the disproportionation is concentration independent is part of the evidence that supports this reaction scheme. Evidence for the synthesis of the cyclic sulfenyl carboxylate has been presented (14).

The overoxidation of β-carboxymercaptans has been studied by Danehy and his colleagues (15–17). The normal oxidation of thiols by iodine proceeds with the stoichiometry shown in Eq. **5**, in which the disulfide is the reaction product.

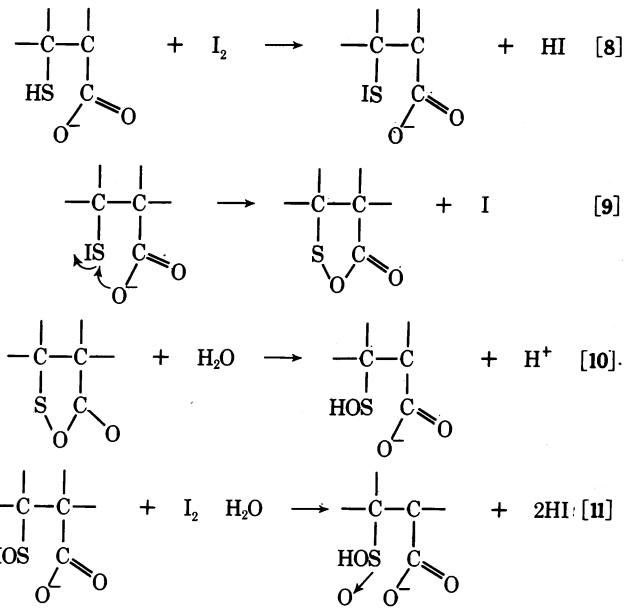
$$2\ RSH + I_2 \rightarrow RSSR + 2I^- \qquad [5]$$

The anomolous oxidation of β-carboxymercaptans by iodine leads to the corresponding sulfinic and sulfonic acids with the stoichiometry described by Eqs. **6** and **7**.

$$R(COO^-)CH_2SH + 2I_2 + 2H_2O \rightarrow R(COO^-)CH_2SO_2H + 4HI \qquad [6]$$

$$R(COO^-)CH_2SH + 3I_2 + 3H_2O \rightarrow R(COO^-)CH_2SO_3H + 6HI \qquad [7]$$

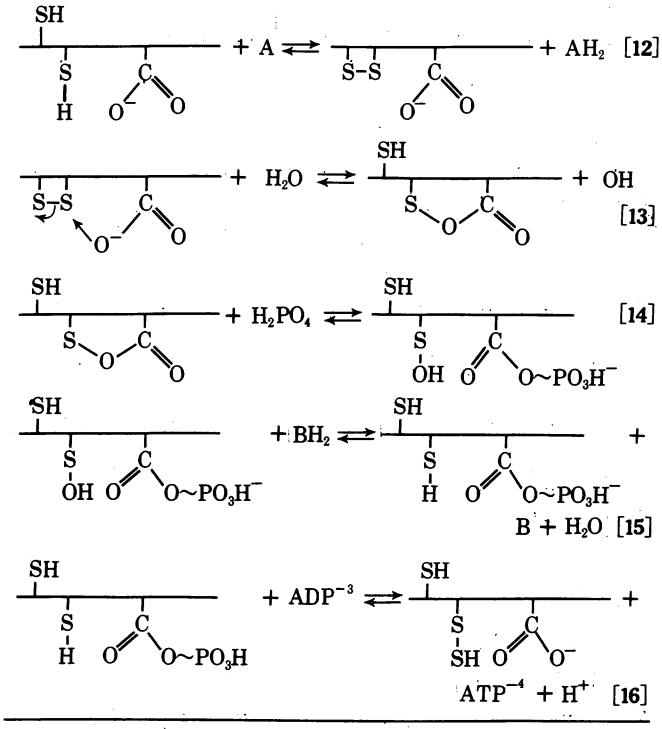
The reaction scheme illustrated by Eqs. **8–11** has been postu-



lated to account for the anomolous oxidation of β-carboxymercaptans by iodine.

The reaction scheme suggests that a sulfenyl iodide is the first reaction intermediate in the oxidation. The carboxylate anion then displaces iodide to form a cyclic, 5-membered sulfenyl carboxylate, which is then hydrolyzed to the sulfenic acid. The sulfenic acid is then oxidized by additional iodine to the sulfinic and sulfonic oxidation states. Kinetic and product analyses as a function of pH have provided experimental support for this reaction scheme (15–17).

The formation of a sulfenyl carboxylate can be accommodated by a reaction scheme similar to the one postulated by Slater in 1953 (18) to describe energy conservation in respiratory electron transport. This scheme is illustrated by Eqs. **12–17**.



$$A + BH_2 + ADP^{-3} + H_2PO_4^- \rightleftarrows AH_2 + B + ATP^{-4} + H^+ + OH^- \qquad [17]$$

Eq. **12** suggests that the oxidation of a sulfhydryl group on an electron carrier to a disulfide provides an electrophilic center for a neighboring carboxylate anion. The carboxylate anion reacts with the disulfide to form a sulfenyl carboxylate as shown in Eq. **13**. The sulfenyl carboxylate then reacts with Pi to form an acyl phosphate and a sulfenic acid, as shown in Eq. **14**. The sulfenic acid is then reduced to a sulfhydryl group in a second electron-transfer step. In the final step in the reaction sequence the acyl phosphate donates metaphosphate to ADP to form ATP. This regenerates the electron carrier in its original state. In this scheme the sulfenyl carboxylate is a nonphosphorylated high-energy intermediate, X~C, and the carboxyl phosphate is a phosphorylated high-energy intermediate, X~P (18). It is interesting to note that Cross and Boyer have labeled a mitochondrial protein with ^{32}Pi that has a pH-hydrolysis profile characteristic of acyl phosphates (19). The ^{32}P-labeled protein is discharged by DNP and ADP suggesting that it is an intermediate in energy conservation.

The partial reactions of oxidative phosphorylation, the ATP–Pi exchange (12), the Pi–H_2O oxygen exchange (13), and the ATP–H_2O oxygen exchange (13), as well as the uncoupler-stimulated ATPase, can be accommodated by the sulfenyl carboxylate intermediate. The dynamic equilibria described by Eqs. **14–16**, which are analogous to the reaction sequence that we postulate to account for the ATP–Pi exchange catalyzed by oxidized GPD and phosphoglycerate kinase, provide a means for the equilibration of Pi with the γ phosphate of ATP. The dynamic equilibria described by Eqs. **13** and **14** allow for the equilibration of the carboxyl oxygen on the hypothetical electron carrier with oxygen

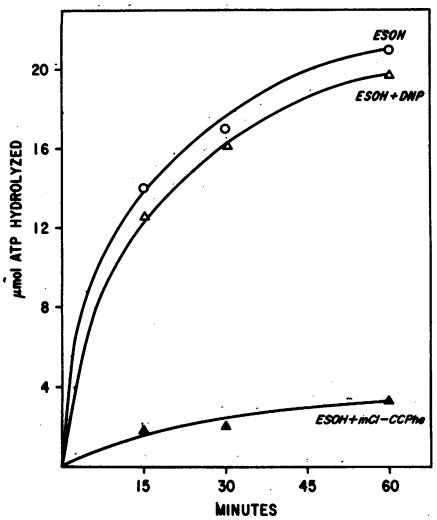


FIG. 2. The contents of the reaction mixtures and the assay of ATPase are described in *Methods*. △, this reaction mixture contained 700 μM DNP. ▲, this reaction mixture contained 700 μM *m*Cl-CCPhe.

derived from water. The reverse of the reactions described by Eqs. **14–16** leads to the exchange of oxygen from water to Pi, which requires the participation of ATP. Since the reaction scheme can account for the ATP–Pi exchange and for the Pi–H_2O exchange, it also must account for the ATP–H_2O oxygen exchange.

The different sensitivity of the ATP–Pi exchange and the ATPase reactions catalyzed by the oxidized GPD–phosphoglycerate kinase coupled enzymes is analogous to the effect of the concentration of uncouplers on the mitochondrial ATPase and respiration. Both respiration and the mitochondrial ATPase are stimulated by low concentrations of uncouplers that abolish ATP formation. Higher concentrations of uncouplers inhibit the ATPase activity and respiration (19, 20). These effects can be accommodated by the sulfenyl carboxylate mechanism that we have postulated. At low concentrations, the uncouplers would compete with Pi for the sulfenyl carboxylate in Eq. **14** to form an unstable carboxyl–uncoupler derivative. This would stimulate an ATPase activity that is described by the reversal of Eqs. **14–16**. At higher concentrations, the uncouplers would react with the sulfenic acid on the hypothetical electron carrier to form sulfenyl–uncoupler derivatives, and thus inhibit the ATPase and respiration.

Sulfenyl phosphate derivatives have been postulated as intermediates to explain the formation of ATP in other model systems. Bauerlein, Klingenfuss, and Wieland have demonstrated the synthesis of ATP in a nonenzymatic system consisting of the *t*-butyl-ammonium salts of ADP and Pi in anhydrous pyridine that use mercapto carboxylic acids or their disulfides as mediators and iodine or hemin compounds as oxidants (22). They propose that sulfenyl derivatives are formed in oxidative reactions which then react with Pi in nucleophilic displacement reactions to form sulfenyl phosphates. The phosphorous of the sulfenyl phosphate is then postulated to react with ADP to form ATP. Painter and Hunter have postulated a sulfenyl phosphate intermediate to explain the formation of ATP from ADP and Pi in a system that they describe as reduction of cytochrome *c* by reduced glutathione, catalyzed by oxidized glutathione (23). The mechanism proposed by Painter and Hunter to describe ATP formation in their model system has been disputed on two counts (24, 25). Massey, Williams, and Palmer have presented experimental evidence that persulfides that contaminate oxidized glutathione are responsible for the reduction of cytochrome *c* (24). Bauerlein has presented evidence that formation of ATP in the system described by Painter and Hunter is due to an adenylate kinase type of reaction (25). The formation of a sulfenyl phosphate intermediate in the acyl phosphatase reaction catalyzed by oxidized GPD is not consistent with C–O bond cleavage, which has been demonstrated for this reaction by Park and Koshland (4).

The reaction scheme illustrated by Eqs. **12–16** is in its simplest form. For instance, the sulfhydryl groups and carboxyl group could reside on separate proteins and the final step of the sequence described by Eq. **16** could require an additional protein factor that is sensitive to oligomycin. By modifications such as these the basic reaction sequence could accommodate the coupling factors isolated by Racker and his associates and by Sanadi and his associates that have been reviewed recently by Racker (26).

This work was supported by a research grant from the Public Health Service, National Institute of General Medical Sciences (GM-16,979) and by an American Cancer Society Institutional Grant (IN-93A). L. V. B. is a recipient of a study grant from the National Science Development Board of the Philippines.

1. Ehring, R. & Colowick, S. P. (1969) *J. Biol. Chem.* **244**, 4589–4599.
2. Parker, D. J. & Allison, W. S. (1969) *J. Biol. Chem.* **244**, 180–189.
3. Allison, W. S. & Connors, M. J. (1970) *Arch. Biochem. Biophys.* **136**, 383–391.
4. Park, J. H. & Koshland, D. E. (1958) *J. Biol. Chem.* **233**, 986–990.
5. Malhotra, O. P. & Bernhard, S. A. (1968) *J. Biol. Chem.* **243**, 1243–1252.
6. Harting, J. & Velick, S. F. (1954) *J. Biol. Chem.* **207**, 867–878.
7. Cori, G. T., Slein, M. W. & Cori, G. C. (1948) *J. Biol. Chem.* **173**, 605–618.
8. Lipmann, F. & Tuttle, L. C. (1945) *J. Biol. Chem.* **159**, 21–28.
9. Pullman, M. E. (1967) *Methods Enzymol.* **10**, 57–60.
10. Glynn, I. M. & Chappell, J. B. (1964) *Biochem. J.* **90**, 147–149.
11. Francis, S. H., Meriwether, B. P. & Park, J. H. (1971) *J. Biol. Chem.* **246**, 5433–5441.
12. Boyer, P. O., Falcone, A. B. & Harrison, W. H. (1954) *Nature* **174**, 401–402.
13. Cohn, M. (1953) *J. Biol. Chem.* **201**, 735–750.
14. Field, L., Giles, P. M. & Tuleen, D. L. (1971) *J. Org. Chem.* **36**, 623–626.
15. Danehy, J. P. & Oester, M. Y. (1967) *J. Org. Chem.* **32**, 1491–1495.
16. Danehy, J. P., Doherty, B. T. & Egan, C. P. (1971) *J. Org. Chem.* **36**, 2525–2530.
17. Danehy, J. P., Egan, C. P. & Switalski, J. (1971) *J. Org. Chem.* **36**, 2530–2534.
18. Slater, E. C. (1953) *Nature* **172**, 975–978.
19. Cross, R. L. & Boyer, P. D. (1971) *Fed. Proc.* **30**, 1245.
20. Hemker, H. C. (1964) *Biochim. Biophys. Acta* **81**, 1–8.
21. Van Dam, K. (1967) *Biochim. Biophys. Acta* **131**, 407–411.
22. Bauerlein, E., Klingenfuss, M. & Wieland, T. (1971) *Eur. J. Biochem.* **24**, 308–312.
23. Painter, A. A. & Hunter, F. E. (1970) *Biochem. Biophys. Res. Commun.* **40**, 360–395.
24. Massey, V., Williams, C. H. & Palmer, G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 730–738.
25. Bauerlein, E. (1972) *Biochem. Biophys. Res. Commun.* **47**, 1088–1092.
26. Racker, E. (1972) *Amer. Sci.* **60**, 56–63.