Purification and Steady-State Kinetics of Adenosine 5'-Pyrophosphate Sulphurylase from Baker's Yeast

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ADP sulphurylase (EC 2.7.7.5) was purified by chromatography on Sephadex G-200 and DEAE-cellulose. The enzyme was assayed by measuring the incorporation of [^{32}P]P_i into ADP in the presence of the substrate for the reverse reaction, adenosine 5'-sulphato-phosphate. In the concentration ranges investigated, by using initial-velocity, product-inhibition and isotope-exchange studies, the data were consistent with a Ping Pong reaction mechanism, with a K_m for adenosine 5'-sulphatophosphate of 1.20 ± 0.08 mM and a K_m for P_i of 4.95 ± 0.15 mM. Competitive substrate inhibition by P_i ($K_i = 11.7 \pm 0.3$ mM) was found. ADP sulphurylase catalyses a sulphate-independent P_i–ADP exchange reaction, the kinetics of which are consistent with the kinetics of the overall reaction, but inconsistent with the assay of Burnell & Anderson [(1973) *Biochem. J.* **133**, 417–428], which is based on a sulphate-dependent P_i–ADP exchange reaction.

ADP sulphurylase (EC 2.7.7.5; ADP–sulphate adenylyltransferase) catalyses the reaction:

$$ADP + SO_4{}^{2-} \rightleftharpoons adenosine\ 5'\text{-sulphatophosphate} + P_i$$

The enzyme was first described in yeast by Robbins & Lipmann (1956) and assayed by following the disappearance of P_i in the presence of adenosine 5'-sulphatophosphate. Grunberg-Manago *et al.* (1966) described an enzyme from yeast that catalysed the incorporation of [^{32}P]P_i into ADP without a requirement for sulphate, in addition to the replacement of the sulphate of adenosine 5'-sulphatophosphate by P_i. They considered that the enzyme could be ADP sulphurylase; however, this requires confirmation (Roy & Trudinger, 1970; De Meio, 1975).

Adams & Nicholas (1972), using a purified enzyme preparation from yeast, showed that the ADP sulphurylase, which replaces inorganic sulphate in adenosine 5'-sulphatophosphate with P_i to give ADP, also catalyses the reversible exchange of [^{32}P]P_i into ADP in the absence of sulphate. On the other hand, Burnell & Anderson (1973) have investigated ADP sulphurylase in plant tissue and yeast by measuring the sulphate-dependent [^{32}P]P_i–ADP exchange. Data in the present paper confirm that the [^{32}P]P_i–ADP exchange catalysed by ADP sulphurylase is sulphate-independent, consistent with the Ping Pong reaction mechanism deduced from kinetic studies.

Materials and Methods

Materials

Adenosine 5'-sulphatophosphate was synthesized by the method of Adams *et al.* (1971). DEAE-cellulose (DE-32) and Whatman 3MM chromatography paper were purchased from W. and R. Balston, (Maidstone, Kent, U.K.). [^{32}P]P_i in dil. HCl was supplied by the Australian Atomic Energy Commission (Lucas Heights, Sydney, N.S.W., Australia). Triethylenetetramine (technical grade) was supplied by Hopkin and Williams, Chadwell Heath, Essex, U.K. All other chemicals were of analytical grade. [^{32}P]ADP was synthesized by the method of Adams & Nicholas (1972). Baker's yeast (*Saccharomyces cerevisiae*) was obtained from Mauri Bros. and Thompson (Adelaide, S. Austral., Australia) and stored at $-15°C$.

Methods

Polyacrylamide-gel electrophoresis was carried out as described by Reid & Bieleski (1968), by using a 7% (w/v) acrylamide gel in 0.375 M-Tris/HCl (pH 8.9), with an electrode buffer of 25 mM-Tris/glycine (pH 8.6). Proteins were stained with Coomassie Blue.

Preparation of enzyme

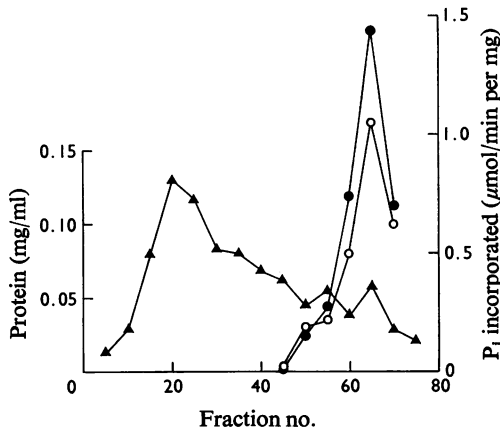Yeast cells were frozen and ground (Leis & Ralph, 1960) into cold 50 mM-Tris/HCl (pH 7.5) containing

Fig. 1. *Chromatography on DEAE-cellulose*
After application of the sample (190ml) the column was washed with 400ml of 50mM-Tris/HCl (pH8.0) containing 5mM-triethylenetetramine, 6.6mM-P_i and 20% (w/v) glycerol, and the enzyme eluted (7.5ml fractions) with a linear gradient of 0–0.4M-KCl in the starting buffer in a total volume of 1 litre. ▲, Protein; O, adenosine 5'-sulphatophosphate-dependent activity; ●, ADP-dependent activity (values $\frac{1}{3}$ of those shown).

5mM-EDTA (sodium salt) and fractionated as described by Adams & Nicholas (1972) to obtain fraction IV. All subsequent operations were carried out at 2°C.

Fraction IV was dialysed overnight against 50mM-Tris/HCl (pH8.0) containing 5mM-triethylenetetramine and 6.6mM-P_i. A sample (15ml) was adjusted to 5% (w/v) sucrose and applied to a column (3cm × 58cm) of Sephadex G-200 equilibrated with the same buffer and also eluted with this buffer. As soon as possible, 2ml of glycerol was added to each fraction (7.5ml) and mixed. The elution profile had two distinct peaks of protein. Fractions containing ADP sulphurylase activity in the second protein peak were combined and loaded on a column (2.4cm × 12.5cm) of DE-32 DEAE-cellulose equilibrated with 50mM-Tris/HCl (pH8.0) containing 5mM-triethylenetetramine, 6.6mM-P_i and 20% (w/v) glycerol. The column was washed with 400ml of the buffer before elution of the enzyme with a linear gradient of 0–0.4M-KCl in a total volume of 1 litre (Fig. 1). The fractions containing ADP sulphurylase were pooled. For kinetic studies, a sample (5ml) of pooled enzyme was desalted by passage through a calibrated column (1.5cm × 25cm) of Sephadex G-25 equilibrated with 50mM-Tris/HCl (pH8.0) containing 5mM-triethylenetetramine. The enzyme was used within 3h of desalting.

Determination of ADP sulphurylase activity

ADP sulphurylase was assayed by measuring the incorporation of [^{32}P]P_i into ADP with either adenosine 5'-sulphatophosphate or ADP as substrate. The assay mixture used during the purification contained 0.3ml of enzyme in buffer [50mM-Tris/HCl (pH8.0), containing 5mM-triethylenetetramine, 6.6 mM-P_i, with or without 20% (w/v) glycerol], 0.1ml of adenosine 5'-sulphatophosphate (or ADP) (5mM) and 0.1ml of [^{32}P]P_i (carrier-free, to give 0.1μCi/μmol). The reaction was started by the addition of the nucleotide, and after incubation for 10min at 30°C the reaction was stopped by addition of 0.1ml of 20% (w/v) trichloroacetic acid. A control for each assay was done by omitting nucleotide and adding trichloroacetic acid before the enzyme. After centrifuging for 10min at 5000g, a 0.2ml sample of the supernatant fraction was analysed for [^{32}P]ADP by a modification of the method of Ochoa (1957).

To the sample (0.2ml) were added 0.3ml of 5M-H_2SO_4 and 1.5ml of 5% (w/v) ammonium molybdate; the solution was mixed thoroughly and left for 2min. Water (3ml) and 2-methylpropan-1-ol (5ml) were added, mixed thoroughly, and left for 3min for phase separation. The 2-methylpropan-1-ol layer was removed and, to ensure the complete removal of [^{32}P]P_i, 5μl of 0.2M-P_i (pH8.0) and 5ml of 2-methyl-propan-1-ol were added, mixed thoroughly, and the 2-methylpropanol-1-ol layer was again removed after phase separation. The aqueous layer (3ml) was added to 7ml of water in a scintillation vial and the [^{32}P]ADP determined by Čerenkov counting in a Packard 3375 Tri-Carb liquid-scintillation spectrometer. A minimum of 1000 counts above background was collected. The channels-ratio technique was used to correct for colour quenching due to carry-over of phosphomolybdate complex. The discriminator settings used were: red channel 20–250; green channel 250–1000; blue channel 20–1000; 50% amplification in each channel. The relative efficiency was determined from a standard plot of the ratio (c.p.m. in red channel/c.p.m. in green channel) against relative efficiency and was 70–85% of an unquenched standard.

An assay mixture containing 0.28μmol of [^{32}P]-ADP (0.05μCi/μmol, 90% radiochemical purity) was taken through the 2-methylpropan-1-ol procedure with 104% recovery of radioactivity. When repeated with an assay mixture that contained 0.25μCi of [^{32}P]P_i in addition to the [^{32}P]ADP, a recovery of 106% of the [^{32}P]ADP radioactivity was obtained.

For the kinetic assays, each set of reactant concentrations was used in duplicate, with duplicate controls for each P_i concentration. The duplicates generally agreed to within 5%. Because the enzyme was desalted in a dilute solution in a buffer containing

50 mM-Tris/HCl (pH 8.0) and 5 mM-triethylenetetramine, an appropriate volume (0.2 or 0.3 ml) of this Sephadex G-25 eluate was used in each 0.5 ml reaction mixture. The activity was linear with time up to 10 min and proportional to protein concentration in the range 6.5–39.0 μg/ml. AMP, a possible contaminant of the nucleotide solutions, at a concentration of 1 mM inhibited the adenosine 5'-sulphatophosphate reaction (1 mM-adenosine 5'-sulphatophosphate, 4 mM-P_i) by 13% and the ADP reaction (1 mM-ADP, 4 mM-P_i) by 8%.

Analysis of kinetic data

The nomenclature of Cleland (1963a) is used. The lines in the double-reciprocal plots of the initial velocity against the varied substrate were drawn by fitting the data to eqn. (1), or to eqn. (2) when substrate inhibition was observed.

$$v = V[\mathrm{S}]/(K_m^{app\cdot} + [\mathrm{S}]) \tag{1}$$

$$v = V[\mathrm{S}]/\{K_m^{app\cdot} + [\mathrm{S}] + ([\mathrm{S}]^2/K_i)\} \tag{2}$$

where v is the observed velocity, V is a maximum velocity, S is the varied substrate, $K_m^{app\cdot}$ is a Michaelis constant for S, and K_i is the substrate-inhibition constant. Data were fitted to eqn. (1) by the method of Wilkinson (1961) and to eqn. (2) by the method of Nicholls *et al.* (1974).

For the estimation of kinetic parameters, the complete set of data was fitted to the appropriate equation by the method of Nicholls *et al.* (1974), which uses a least-squares method for a non-linear regression of the required equation. The parameters are expressed as estimate ± standard error of the estimate.

Results

Enzyme purification

The enzyme was purified 42-fold relative to fraction IV of Adams & Nicholas (1972) (Table 1). After Sephadex G-200 chromatography, the enzyme

rapidly loses all activity in the absence of glycerol. In the presence of glycerol the enzyme was stable in the pH range 7.5–9.0. The enzyme after chromatography on DEAE-cellulose was stable, no loss of activity being recorded over 3 months when stored in buffer containing KCl and 20% (w/v) glycerol. Analysis by polyacrylamide-gel electrophoresis of the pooled fractions after DEAE-cellulose chromatography showed one major band with several minor bands of protein. Repeated chromatography on DEAE-cellulose did not result in further significant purification. Attempts to concentrate the combined fractions from the final column invariably led to complete loss of activity.

The specific activity of the enzyme used for kinetic studies as a routine increased 2–3-fold after desalting by passage through Sephadex G-25, as described in the Materials and Methods section. However, the enzyme preparation was now unstable and lost activity exponentially with a half-time of 60 h. A similar increase in activity with adenosine 5'-sulphatophosphate as substrate was observed after the Sephadex G-200 chromatography step (Table 1). The basis of this activation is not clear, though it may reflect an inhibitory effect of salt (KCl or NaCl) in the assay medium, introduced with the enzyme solution. The yield of the Sephadex G-200 chromatography step, based on activity measurements with ADP as substrate, was 85%, indicating that the increase in the ratio adenosine 5'-sulphatophosphate activity/ADP activity (Table 1) was primarily due to the increase in activity with adenosine 5'-sulphatophosphate as substrate.

Initial-velocity studies

When P_i is varied from 1 to 20 mM at different fixed concentrations of adenosine 5'-sulphatophosphate from 0.1 to 2 mM, a parallel initial-velocity pattern, with substrate inhibition by P_i, was obtained (Fig. 2a). When the initial velocity is plotted with adenosine 5'-sulphatophosphate as the variable substrate, no substrate inhibition by adenosine 5'-

Table 1. *Purification of ADP sulphurylase*
The activity was measured with adenosine 5'-sulphatophosphate (APS) as substrate, as described in the Materials and Methods section. A unit of activity is 1 μmol of P_i incorporated/min. The column headed 'APS activity/ADP activity' is the ratio of the enzyme activity to the P_i–ADP exchange activity, each determined at 1 mM-nucleotide and 4 mM-P_i. Protein was determined by a spectrophotometric method (Layne, 1957).

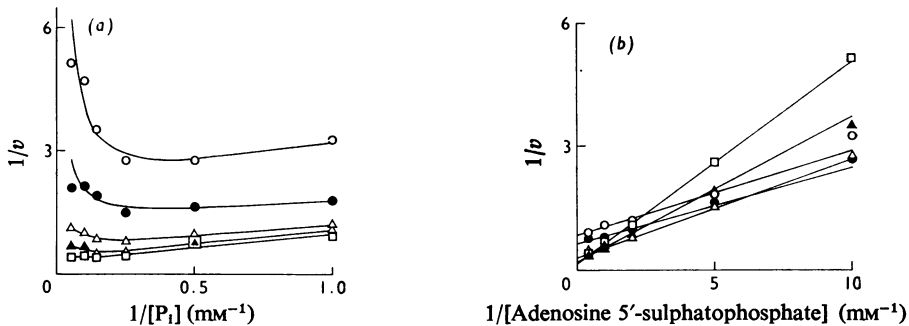| Procedure	Volume (ml)	Protein (mg/ml)	Activity (unit/ml)	Specific activity (unit/mg of protein)	Purification (fold)	Yield (%)	$\dfrac{\text{APS activity}}{\text{ADP activity}}$
Dialysed fraction IV (Adams & Nicholas, 1972)	15	18.5	0.353	0.019	1	100	2.5
Sephadex G-200	138	0.725	0.070	0.096	5	220	5.3
DEAE-cellulose	47	0.066	0.053	0.807	42	57	3.7

Fig. 2. *Kinetics of incorporation of* P_i *into ADP*

(a) Double-reciprocal plot of the rate of P_i incorporation (v, μmol/min per mg) as a function of P_i concentration at fixed concentrations of adenosine 5'-sulphatophosphate: $\bigcirc$, 0.1 mM; $\bullet$, 0.2 mM; $\triangle$, 0.5 mM; $\blacktriangle$, 1.0 mM; $\square$, 2.0 mM. The data at each adenosine 5'-sulphatophosphate concentration were fitted to eqn. (2). The rate of P_i incorporation was measured at 30°C in 30 mM-Tris/HCl (pH 8.0) containing 3 mM-triethylenetetramine. The protein concentration was 32 μg/ml. (b) Double-reciprocal plot of the rate of P_i incorporation (v, μmol/min per mg) as a function of adenosine 5'-sulphatophosphate concentration at fixed concentrations of P_i: $\bigcirc$, 1.0 mM; $\bullet$, 2.0 mM; $\triangle$, 4.0 mM; $\blacktriangle$, 6.6 mM; $\square$, 20 mM. The data at each P_i concentration were fitted to eqn. (1). The results at 10 mM-P_i have been omitted for clarity. Other conditions were as for (a).
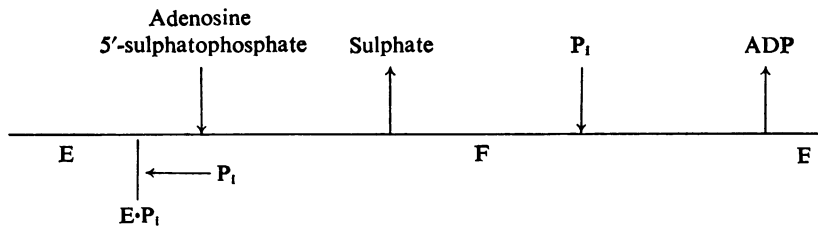
Scheme 1. *Ping Pong mechanism with competitive substrate inhibition by* P_i

The rate equation for this mechanism is given by eqn. (3) (see the text). In this mechanism, F is a stable enzyme intermediate, E·AMP.

Table 2. *Kinetic parameters for ADP sulphurylase*
Constants were obtained by fitting the initial-velocity data with adenosine 5'-sulphatophosphate and P_i as substrates to the rate equation (eqn. 3) for competitive substrate inhibition in a Ping Pong mechanism (Scheme 1). The apparent maximum velocity was $6.55 \pm 0.17 \mu$mol of P_i incorporated/min per mg.

Parameter	Value (mM)
K_a	1.20 ± 0.08
K_b	4.95 ± 0.15
K_i	11.7 ± 0.3

sulphatophosphate was observed; however, the characteristic plot obtained (Fig. 2b) is indicative of competitive substrate inhibition in a Ping Pong mechanism (Cleland, 1970).

The kinetic parameters shown in Table 2 were obtained by fitting the data in Fig. 2(a) to eqn. (3)

for competitive substrate inhibition by B in a Ping Pong mechanism (Scheme 1).

$$v = \frac{V[A][B]}{K_b[A] + K_a[B]\{1 + ([B]/K_i)\} + [A][B]} \quad (3)$$

where A is adenosine 5'-sulphatophosphate, B is P_i, K_a is the Michaelis constant for adenosine 5'-sulphatophosphate, K_b is the Michaelis constant for P_i, and K_i is the dissociation constant of the E·P_i complex.

Product inhibition by sulphate

Sulphate is a poor inhibitor of ADP sulphurylase activity. No inhibition could be observed without increasing the ionic strength, and it is possible that the observed effects described below are due to changes in the ionic strength.

When sulphate was tested with adenosine 5'-sulphatophosphate as the variable substrate at 1 mM-

P_i, i.e. a non-saturating concentration of the fixed substrate, the non-competitive-inhibition pattern shown in Fig. 3 was obtained. The inhibition by
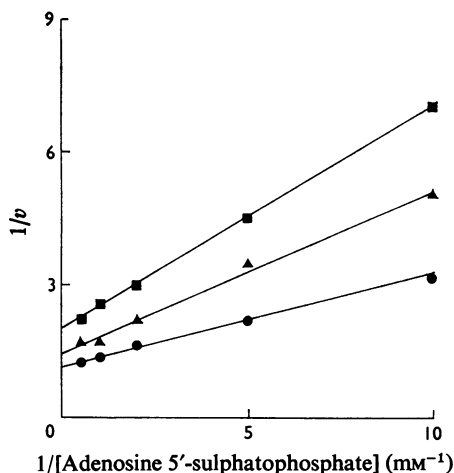

Fig. 3. *Product inhibition of ADP sulphurylase by sulphate*
Double-reciprocal plot of P_i incorporation (v, μmol/min per mg) as a function of adenosine 5'-sulphatophosphate concentration at 1.0mM-P_i and at fixed concentrations of sulphate: ●, zero; ▲, 0.1M; ■, 0.2M. The rate of P_i incorporation was measured at 30°C in 20mM-Tris/HCl (pH8.0) containing 2mM-triethylenetetramine. Protein concentration was 42μg/ml. The data at each sulphate concentration were fitted to eqn. (1). The complete set of data was fitted to the equation for linear non-competitive inhibition (Cleland, 1963b) with the following parameters: V, 0.878$\pm$0.037μmol/min per mg; K_a, 0.172$\pm$0.029mM; $K_{i,slope}$ 296$\pm$116mM; $K_{i,intercept}$, 252$\pm$31mM.

sulphate with P_i as the variable substrate, however, was competitive, whether adenosine 5'-sulphatophosphate was saturating (4mM) or not (0.5mM). At 4mM-adenosine 5'-sulphatophosphate, the K_i for sulphate was 167$\pm$35mM.

Initial-velocity studies of the [^{32}P]P_i–ADP exchange reaction

When P_i is varied from 0.2 to 10mM at different fixed concentrations of ADP from 0.4 to 1.5mM, a parallel initial-velocity pattern with substrate inhibition by P_i was obtained (Fig. 4a). From the parameters for the data at each ADP concentration, the intercept and slope of the asymptote in the low-substrate range may be calculated. The replot of intercepts was linear, and the slopes differ by less than the sum of their standard errors. When the data are plotted with ADP as the variable substrate, an apparently parallel plot (slopes differ by less than the sum of their standard errors) was obtained (Fig. 4b). However, at the higher P_i concentrations the slope tended to increase, indicating substrate inhibition by P_i, as seen in Fig. 4(a), and as found for the overall reaction.

The kinetic parameters shown in Table 3 were obtained by fitting the data in Fig. 4(a) to eqn. (4), the rate equation for P_i–ADP exchange in Scheme 1.

$$v^* = \frac{V_{exchange}[B][Q]}{K_{1b}[Q]+K_{1q}[B]\{1+([B]/K_i)\}+[B][Q]} \quad (4)$$

where v^* is the initial velocity of the exchange reaction, $V_{exchange}$ is the maximum velocity of the exchange reaction, B is P_i, Q is ADP, K_{1b} is the dissociation constant of the F·P_i complex, K_{1q} is the
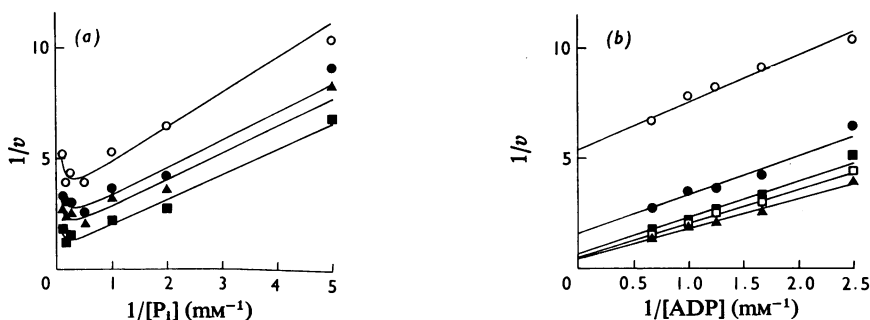

Fig. 4. *Kinetics of the P_i–ADP exchange reaction*
(a) Double-reciprocal plot of the rate of P_i incorporation (v, μmol/min per mg) as a function of P_i concentration at fixed concentrations of ADP: ○, 0.4mM; ●, 0.6mM; ▲, 0.8mM; ■, 1.5mM. The data at each ADP concentration were fitted to eqn. (2). Data at 1mM-ADP have been omitted for clarity. Other conditions are as for Fig. 2. (b) Double-reciprocal plot of the rate of P_i incorporation (v, μmol/min per mg) as a function of ADP concentration at fixed concentrations of P_i: ○, 0.2mM; ●, 0.5mM; ▲, 2.0mM; □, 4.0mM: ■, 10.0mM. The data at each P_i concentration were fitted to eqn. (1). Results at 1mM-P_i have been omitted for clarity. Other conditions are as for Fig. 2.

Table 3. *Kinetic parameters for the P_i-ADP exchange reaction*

Constants were obtained by fitting the data for P_i–ADP exchange to the rate equation (eqn. 4) for P_i–ADP exchange in the Ping Pong mechanism with competitive substrate inhibition (Scheme 1). The apparent maximum velocity was $37.6 \pm 1.5 \mu$mol of P_i incorporated/min per mg.

Parameter	Value (mM)
K_{1b}	45.1 ± 0.7
K_{1q}	42.9 ± 1.5
K_1	11.7 ± 0.2

dissociation constant of the E·ADP complex, and K_1 is the dissociation constant of the E·P_i complex.

Effect of ionic strength on the [^{32}P]P_i–ADP exchange

Increasing the ionic strength inhibited the [^{32}P]P_i–ADP exchange rate. However, the degree of inhibition depended on the salt. When the exchange was measured under the standard conditions, but at 1 mM-ADP and 1 mM-P_i, 0.3 M-KCl caused a 75% inhibition, whereas 0.1 M-K_2SO_4 inhibited by only 52%.

Discussion

A 42-fold purification of ADP sulphurylase has been achieved, which, considered together with the purification by Adams & Nicholas (1972), represents an overall purification of 12000-fold from the initial extract. The ratio adenosine 5'-sulphatophosphate-dependent activity/ADP-dependent activity remains constant throughout the purification, in particular throughout the elution profile of the enzyme from the DEAE-cellulose chromatography (Fig. 1), indicating that the two activities are not separable by this technique.

In the concentration ranges studied, ADP sulphurylase appears to have a Ping Pong mechanism involving the formation of a stable enzyme intermediate, possibly E·AMP, with an additional dead-end E·P_i complex as indicated in Scheme 1.

Thus a parallel initial-velocity pattern is obtained with adenosine 5'-sulphatophosphate as the varied substrate, whereas when P_i is varied the typical pattern for competitive substrate inhibition in a Ping Pong mechanism is obtained. The sulphate inhibitions, though possibly influenced by ionic-strength effects, are consistent with a Ping Pong mechanism. In addition, a P_i–ADP exchange reaction, in the absence of either sulphate or adenosine 5'-sulphatophosphate, is observed, which excludes a purely sequential mechanism.

The kinetics of the P_i–ADP exchange reaction are consistent with the mechanism in Scheme 1. Thus substrate inhibition by P_i is observed in an apparently parallel reciprocal plot. The agreement between the parameters determined from the exchange reaction and the overall reaction is good. Thus K_1, the dissociation constant for the E·P_i complex estimated from the overall reaction (Table 2), is identical with the value obtained from the exchange reaction (Table 3). The maximum velocity for the overall reaction estimated from the exchange data [$V_{overall} = V_{exchange} (K_b/K_{1b}) = 4.13 \mu$mol/min per mg] is in satisfactory agreement with the value of 6.55μmol/min per mg (Table 2).

That the sulphate–adenosine 5'-sulphatophosphate exchange reaction was not observed by Adams & Nicholas (1972) may be accounted for primarily by the high dissociation constant for sulphate (K_{1p}) found in the present study. K_{1p} is given directly by the competitive inhibition constant obtained when P_i is the varied substrate at saturating adenosine 5'-sulphatophosphate (Segel, 1975) and has the value 167 ± 35 mM. In a Ping Pong mechanism, the rate of the sulphate–adenosine 5'-sulphatophosphate exchange reaction is related to the maximum velocity for the overall reaction in Scheme 1 by eqn. (5):

$$v = \frac{V(K_{1a}/K_a)[A][P]}{K_{1a}[P] + K_{1p}[A] + [A][P]} \quad (5)$$

where K_{1a} is the dissociation constant of the E·adenosine 5'-sulphatophosphate complex, K_{1p} is the dissociation constant for the F·sulphate complex, and K_a is the Michaelis constant for adenosine 5'-sulphatophosphate. K_{1a} can be obtained from the value of $K_{1,slope}$ for the non-competitive inhibition by sulphate when adenosine 5'-sulphatophosphate is varied at 1 mM-P_i, since $K_{1,slope} = K_{1p}K_a[B]/(K_{1a}K_b)$ (Segel, 1975). K_{1a} is found to be 0.137 ± 0.037 mM. For the conditions used by Adams & Nicholas (1972) and the parameters reported here, the calculated exchange rate is less than 1% of the maximum velocity of the overall reaction.

In the presence of catalytic amounts of sulphate, the observation of a P_i–ADP exchange reaction would not allow us to exclude a sequential mechanism. However, it is unlikely that the enzyme preparation contains sulphate, as it has been chromatographed twice and the sulphate-inhibition data indicate a high dissociation constant for sulphate. Sulphate is not a normal contaminant of ADP or P_i.

The greater inhibition of the P_i–ADP exchange rate by KCl as compared with K_2SO_4 agrees with the work of Burnell & Anderson (1973). This observation, together with the conclusion that the reaction mechanism of ADP sulphurylase may be Ping Pong, is inconsistent with the use of an assay for ADP sulphurylase based on a sulphate-dependent P_i–ADP

exchange reaction, as used by Burnell & Anderson (1973).

At adenosine 5'-sulphatophosphate concentrations below 0.1 mM, non-linear double-reciprocal plots similar to those reported by Adams & Nicholas (1972) were obtained, possibly indicating a different reaction mechanism at low adenosine 5'-sulphato-phosphate concentrations. However, the mechanism proposed here appears to support the conclusion that the enzyme studied by Grunberg-Manago *et al.* (1966) was, in fact, ADP sulphurylase.

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