

## Studies on a Coupled Enzyme Assay for Rate Measurements of ATPase Reactions

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In order to obtain true measurements of initial rates in enzyme reactions involving ATPase, an assay in which the ATPase reaction is coupled to the oxidation of  $\text{NADH}(+\text{H}^+)$  by means of pyruvate kinase, phosphoenolpyruvate and lactate dehydrogenase has been studied in detail.

The kinetically complicated progress curves for the MgATPase reaction found by use of the conventional method for activity measurements are due to inhibition of the MgATPase by ADP and to the fact that the enzyme preparation also contains adenylate kinase.

Based on rate measurements for the individual enzyme reactions entering into the complete coupled system at various concentrations of substrates, enzymes and activators, the conditions for which the rate of  $\text{NADH}(+\text{H}^+)$  oxidation is equal to the rate of the uninhibited ATPase reaction are established.

The quantitative and qualitative kinetic analysis of enzyme reactions generally depend on accurate measurements of initial rates over a wide range of substrate and activator concentrations.\* Preliminary studies on MgATPase from brain microsomes, however, showed that initial rates were difficult to obtain when the conventional measurement of activity was used (increase in inorganic phosphate with time). This was due to product inhibition by ADP; *vide infra*. Moreover, the phosphate method is not sufficiently sensitive for initial rate measurements at low ATP concentrations.

In order to solve these problems a detailed study of the ATPase assay,<sup>1,2</sup> in which the ATPase reaction is coupled to the oxidation of  $\text{NADH}(+\text{H}^+)$  by means of pyruvate kinase, phosphoenolpyruvate and lactate dehydrogenase, was undertaken. Following the general principles pointed out by Bergmeyer,<sup>3</sup> the reactions of this assay have been investigated separately and in combination with the purpose of assessing the experimental limits, within which the rate of  $\text{NADH}(+\text{H}^+)$  oxidation is a true measurement of the rate of the uninhibited ATPase reaction.

\* Non-usual abbreviations: PEP, phosphoenolpyruvate; Py, pyruvate; a suffix t, e.g.  $\text{ATP}_t$ , means total.

## MATERIALS AND METHODS

*Enzymes and other reagents.* The ATPase preparations were obtained from ox brain, as described by Skou and Hilberg.<sup>4</sup> Pyruvate kinase (10 mg/ml 2.2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), lactate dehydrogenase (10 mg/ml 2.2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, rabbit muscle), reduced NAD, disodiumsalt, trihexylammonium phosphoenolpyruvate (PEP) and sodium pyruvate (Py) were purchased from Boehringer, Mannheim, Germany. MgCl<sub>2</sub>·6H<sub>2</sub>O and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> were Merck *p.a.*, Na<sub>2</sub>H<sub>2</sub>ATP and Na<sub>2</sub>ADP (Boehringer) were purified and converted to trissalts by chromatography on Dowex 1-X2, Cl<sup>-</sup> columns (200–400 mesh, Bio Rad Laboratories). Tris-buffers were prepared from TRIZMA-base (Sigma) and HCl. The concentration of nucleotides were determined from the absorbance at 259 nm (absorbance coefficient 15.4 × 10<sup>3</sup> (1 M, 1 cm light path)).

*The Mg-content* of the reagents, enzymes, and reaction mixtures was determined by atomic absorption. ATP was found to contain 0.5–1.5 mol Mg per 100 mol. The other reagents contained very small amounts of Mg, and in all cases contributed less than 10 μM Mg to the assay mixtures. Total Mg in the reaction mixture, calculated as Mg (added) + Mg (from ATP) + Mg (from other reagents) agreed well with that measured directly.

*The ionic strength* was calculated from the known composition of the solution, the Cl<sup>-</sup> concentration of the tris-buffer and ATP solutions, and by assuming that PEP has 3 and ATP 4 negative charges per molecule<sup>5,6</sup> at the pH used.

*Calculation of [Mg<sup>2+</sup>]* was carried out on an Olivetti Programma 101 by the iterative procedure outlined below for a solution containing Mg, ATP, PEP, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The symbols and equations used are:

M<sub>t</sub> = total Mg concentration, A<sub>t</sub> = total ATP concentration, PEP<sub>t</sub> = total PEP concentration, S<sub>t</sub> = total SO<sub>4</sub> concentration. The concentration of the corresponding complexes are symbolized by MA, MPEP and MS, respectively, and that of the uncomplexed species by M, A, PEP, and S.

$$M_t = M + MA + MP + MS \quad (MA = [MgATP^{2-}] + [MgHATP^-]) \quad (1)$$

$$A_t = A + MA \quad (A = [ATP^{4-}] + [HATP^{3-}]) \quad (2)$$

$$PEP_t = PEP + MPEP \quad (3)$$

$$S_t = S + MS \quad (4)$$

$$\frac{MA}{M \times A} = K_1 \quad (5); \quad \frac{MPEP}{M \times PEP} = K_2 \quad (6); \quad \frac{MS}{M \times S} = K_3 \quad (7)$$

Transformation of eqn. (1) gives

$$M_t = MA + M \left( 1 + \frac{MPEP}{M} + \frac{MS}{M} \right) \quad (8)$$

From (3) and (6) we have

$$\frac{MPEP}{M} = \frac{K_2 PEP_t}{1 + K_2 M} \quad \text{and by analogy} \quad \frac{MS}{M} = \frac{K_3 S_t}{1 + K_3 M}$$

which leads to

$$M_t = MA + M \left( 1 + \frac{K_2 PEP_t}{1 + K_2 M} + \frac{K_3 S_t}{1 + K_3 M} \right) = MA + M \alpha \quad (9)$$

solving (2), (5), and (9) with respect to M gives

$$M = -0.5 \left( \frac{1}{K_1} + \frac{A_t - M_t}{\alpha} \right) + \sqrt{\left[ 0.5 \left( \frac{1}{K_1} + \frac{A_t - M_t}{\alpha} \right) \right]^2 + \frac{M_t}{K_1 \alpha}} \quad (10)$$

A preliminary value for M, M<sub>0</sub>, is now calculated for α = 1. This M<sub>0</sub> is used in the calculation of a first approximation of α, α<sub>1</sub>, by means of which a new value for M, M<sub>1</sub>, is computed from eqn. (10). M<sub>1</sub> is used to form α<sub>2</sub> a.s.o. In most cases, 2 to 3 runs will give 0.999 < M<sub>n</sub>/M<sub>n-1</sub> < 1.00.

Table 1. Apparent stability constants used in the investigation.<sup>7</sup>

Ionic strength M	$K'_{\text{MgATP}}$ $K_1$ (M <sup>-1</sup> )	$K'_{\text{MgPEP}}$ $K_2$ (M <sup>-1</sup> )	$K'_{\text{MgSO}_4}$ $K_3$ (M <sup>-1</sup> )
0.085	18000	200	20
0.14	7000	0	0
0.24	2800	0	0

The values of  $K_1$ ,  $K_2$ , and  $K_3$  used (Table 1) were those determined by the author<sup>7</sup> for conditions similar to those of the present investigation (Tris-buffer, pH 7.4, 37°C).

*Enzyme assays.* The composition of reaction mixtures and the conditions under which the many different assays were carried out are reported under RESULTS AND DISCUSSION. In experiments where NADH(+H<sup>+</sup>) oxidation was measured, the absorbance at 340 nm was recorded continuously on a Zeiss PMQ II spectrophotometer equipped with a thermostated cell house, automatic sample exchanger for six cells, and a Servogor recorder.

In some ATPase experiments inorganic phosphate ( $P_i$ ) liberated was measured by the method of Fiske and SubbaRow.<sup>8</sup>

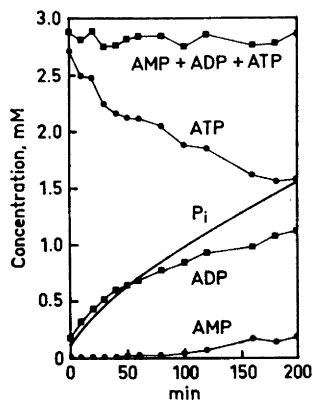
*Thin layer chromatographic* separation of ATP, ADP, and AMP was performed on precoated TLC-plates (Silica Gel F 254, Merck, Germany) with the following solvent system (vol/vol): 1-propanol 36 %, 1-butanol 30 %, 25 % NH<sub>4</sub>OH 10 %, H<sub>2</sub>O 24 %. The ATPase reaction mixture was precipitated with trichloroacetic acid, centrifuged and neutralized with NaOH. 20  $\mu$ l samples were plated, and after development of the chromatogram for about 17 h in a closed chamber the spots were localized in UV-light, cut out and eluted with 1.20 ml H<sub>2</sub>O. The amount of nucleotide was calculated from the absorbance at 259 nm using an absorbance coefficient of  $15.4 \times 10^3$  (1 M, 1 cm light-path). Blanks were obtained from nucleotide-free parts of the TLC-plate.

## RESULTS AND DISCUSSION

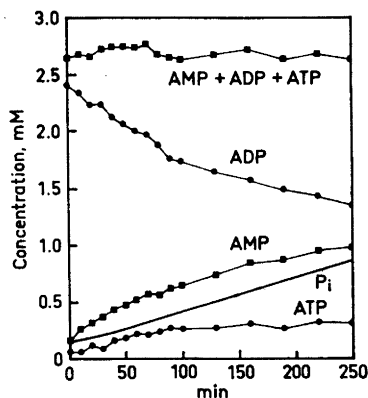
*Demonstration of adenylate kinase and ADP inhibition of the MgATPase in ATPase preparations.* Preliminary efforts to measure the initial rate of the MgATPase reaction from  $P_i$  progress curves revealed a peculiar shape of such curves, as shown in Fig. 1, which also gives the results of further investigations on the stoichiometry of the reaction. It is seen that  $d[P_i]/dt = d[ADP]/dt = -d[ATP]/dt$  at any time during the first 30–40 min, and that there is an appreciable decrease in reaction rate during this period. Thereafter, the rate of  $P_i$  release ( $d[P_i]/dt$ ) becomes approximately constant and greater than  $d[ADP]/dt$  and  $-d[ATP]/dt$ . During this phase AMP is produced.

These observations can be explained by assuming (1) inhibition of the MgATPase by ADP, and (2) the presence of adenylate kinase in the ATPase preparations. The last of these assumptions is strongly supported by the experiment shown in Fig. 2, suggesting a conversion of ADP to AMP and ATP, and a subsequent splitting of ATP to ADP and  $P_i$ . The low rate of  $P_i$  production during the first 10–20 min, where [ADP] is high, points against a direct splitting of ADP to AMP and  $P_i$ , and the results obtained here are thus in excellent agreement with those of Robinson.<sup>9</sup>

The ADP-inhibition has been observed for a large variety of ATPases,<sup>1,10-18</sup> and is demonstrated for our preparations by Fig. 3. The fact that ADP inhibits the reaction and that rather high ADP concentrations tend to give a linear

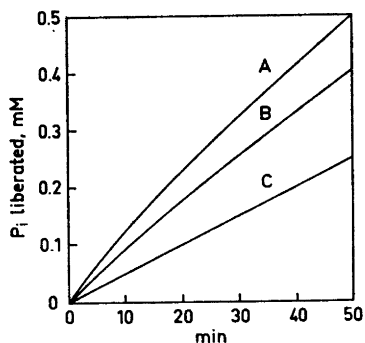


*Fig. 1.* Metabolism of ATP by the brain microsomal ATPase preparation. Reaction mixture: 38  $\mu\text{g}$  ATPase/ml, 2.8 mM ATP, 3.0 mM Mg, 22.5 mM propanediol buffer and 35 mM tris buffer, pH 7.4, temp 37°C. The reaction was started by addition of  $\text{MgCl}_2$  to the preheated mixture and 1 ml samples withdrawn and analyzed at the times indicated.

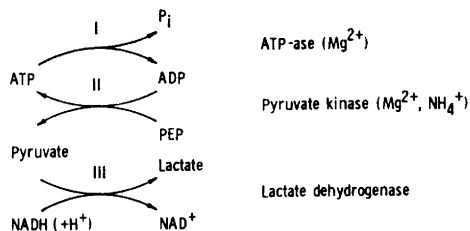


*Fig. 2.* Metabolism of ADP by the brain microsomal ATPase preparation. Experimental conditions as for Fig. 1, but with 2.8 mM ADP instead of ATP.

progress curve confirms the observations in Figs. 1 and 2, and shows that the action of adenylate kinase under certain conditions will result in a steady-state in the system.



*Fig. 3.* Inhibition of MgATPase by ADP. Reaction mixture, all curves: 38  $\mu\text{g}$  ATPase/ml, 2.25 mM ATP, 22.5 mM propanediol buffer, 28 mM tris-buffer, pH 7.4, temp. 37°C. A: 3 mM  $\text{MgCl}_2$ , 0 mM ADP. B: 3 mM  $\text{MgCl}_2$ , 0.38 mM ADP. C: 6 mM  $\text{MgCl}_2$ , 2.3 mM ADP.



*Fig. 4.* Reactions, enzymes and activators involved in the coupled ATPase assay.

*Recycling of ADP by pyruvate kinase and PEP.* It is obvious that progress curves of the kind shown above, which are similar to those observed by Selwyn<sup>15</sup> and Nakamura *et al.*,<sup>16</sup> are unsuited for measurements of initial rate of the uninhibited MgATPase reaction. However, rapid resynthesis of ATP from ADP by means of pyruvate kinase and PEP produces linear curves (experiments not shown). This system has several advantages:

ADP is removed, which diminishes or probably excludes the ADP-inhibition and rules out any possible complicating effects from the adenylate kinase reaction. Furthermore, continuous resynthesis of ATP might convert initial rates to steady state rates and make measurements at low ATP concentrations possible. Lastly it provides a possibility of continuous rate measurement by further coupling of the system with lactate dehydrogenase and reduced NAD.

The aim of the experiments reported below was to assess the experimental limits within which the rate of NADH(+H<sup>+</sup>) oxidation is a true measurement of the rate of the uninhibited ATPase reaction. The reaction scheme for the coupled assay is given in Fig. 4, and in the following the three reactions are considered separately and in combination.

*Reaction III:* Pyruvate + NADH + H<sup>+</sup> → lactate + NAD<sup>+</sup>

Studies on this reaction were all performed at 37°C, pH 7.4, ionic strength 0.14 M (tris-buffer), 120 μM NADH(+H<sup>+</sup>) (initial) and 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. A typical experiment is given in Fig. 5. The absorbance at 340 nm was recorded until more than 98 % of the pyruvate added (50–100 μM) had been converted to lactate. From this curve  $V_{III} = -d[NADH]/dt = -d[Py]/dt$  was measured at various pyruvate concentrations, [Py]. Plots of  $V_{III}$  versus [Py] (Fig. 6) revealed that  $V_{III}$  was proportional to [Py] and the lactate dehydrogenase concentration, [LDH], provided [Py] is less than about 30 μM. The highest value for [LDH] in these experiments was 4 μg/ml since at higher LDH-concentrations, reaction III was too rapid to be measured:

$$V_{III} (\mu\text{M}/\text{min}) = 1.35[\text{LDH}] (\mu\text{g}/\text{ml})[\text{Py}] (\mu\text{M}) \quad (11)$$

The constant 1.35 was not influenced by the addition of 4 mM Mg, 4 mM ATP, 1 mM PEP, 40 μg pyruvate kinase/ml or combinations of these.

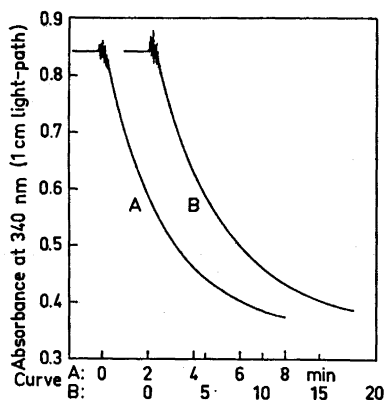
$V_{III}$  is not influenced by the decrease in NADH(+H<sup>+</sup>) concentration, the final concentration being higher than 5  $K_m$  for NADH(+H<sup>+</sup>).<sup>19</sup>

*Reaction II:* ADP + PEP → ATP + pyruvate

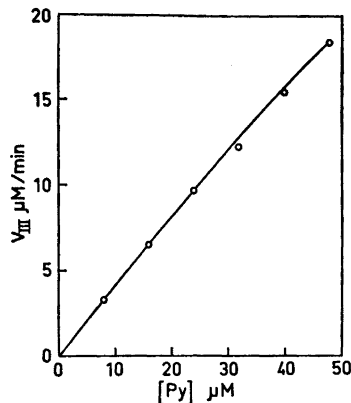
Reaction II was studied by means of coupling to reaction III under the following conditions: temp. 37°C, pH 7.4, ionic strength 0.085, 0.14, or 0.24 M (tris-buffer), 120 μM NADH(+H<sup>+</sup>), 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 mM PEP, 33.3 μg lactate dehydrogenase/ml, 33.3 μg pyruvate kinase/ml. The initial ADP concentration varied between 50 and 100 μM, and ATP and Mg were added to produce concentrations of 0.1–4 mM ATP<sub>i</sub> and 0.03–0.6 mM Mg<sub>t</sub>. In the experiments reported below (an example is given in Fig. 5), [Mg<sup>2+</sup>] never exceeded 60 μM.

For this system Fig. 4 gives

$$d[\text{Py}]/dt = V_{II} - V_{III} \quad (12)$$



*Fig. 5.* Progress curves (NADH(+H<sup>+</sup>) oxidation) for reaction III (curve A) and reaction II+III (curve B) (cf. Fig. 4) at 0.14 M ionic strength, pH=7.4, temp. 37°C. Experimental conditions, curve A: 120 μM NADH(+H<sup>+</sup>), 75 μM pyruvate, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.33 μg lactate dehydrogenase/ml. Curve B: 120 μM NADH(+H<sup>+</sup>), 2.1 mM ATP, 57 μM ADP added, 0.2 mM Mg, 1.1 mM PEP, 33 μg pyruvate kinase/ml and 33 μg lactate dehydrogenase/ml. The reactions were started by addition of lactate dehydrogenase (A) or pyruvate kinase (B).



*Fig. 6.* Rate of reaction III ( $V_{III}$ ) as a function of pyruvate concentration, [Py]. The data were obtained from the experiment shown in Fig. 5, curve A, assuming that [Py] is zero when the absorbance reaches 0.350.

and assuming that relation (11) holds for 33.3 μg lactate dehydrogenase/ml we have:

$$\frac{d[Py]}{dt} = \frac{dV_{III}}{dt} \frac{1}{45} = V_{II} - V_{III}$$

from which

$$V_{II} = V_{III} + \frac{1}{45} \cdot \frac{dV_{III}}{dt} \quad (13)$$

The experiments showed that  $\frac{1}{45} \frac{dV_{III}}{dt} < 0.01 V_{III}$  under the conditions mentioned here, as well as for the following experiments, so that for all practical purposes  $V_{II} = V_{III}$ .  $V_{III}$  was always lower than 40 μM/min, which means that the concentration of pyruvate in this system never exceeded 1 μM (cf. eqn. (11)).

Experiments, like that illustrated by curve B, Fig. 5, showed that  $V_{II}$  was proportional to  $[ADP_t]$  for  $[ADP_t] < 50 \mu\text{M}$ , and that the proportionality factor was dependent on the concentration of free magnesium,  $[Mg^{2+}]$ :

$$V_{II} (\mu\text{M}/\text{min}) = f[Mg^{2+}][ADP_t] (\mu\text{M}) \quad (14)$$

Studies in which the pyruvate kinase concentration was varied up to 33.3  $\mu\text{g}/\text{ml}$  furthermore showed that  $f[\text{Mg}^{2+}]$  was proportional to the pyruvate kinase concentration (the concentration of  $\text{NH}_4^+$ , which activates pyruvate kinase,<sup>20</sup> was constant = 30 mequiv./l).

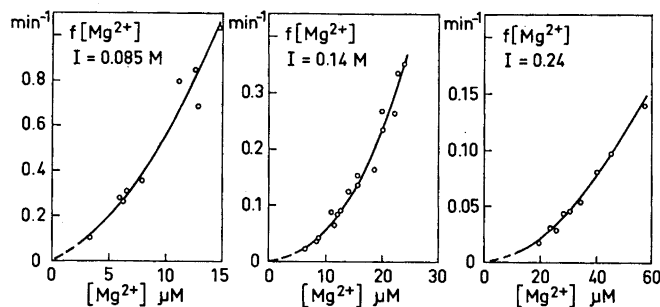


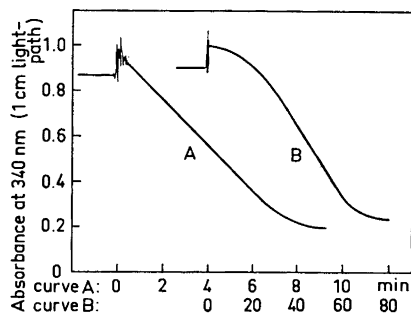
Fig. 7. Relationship between  $f[\text{Mg}^{2+}]$  (cf. eqn. (14)) and  $[\text{Mg}^{2+}]$ . The pyruvate kinase concentration was 33.3  $\mu\text{g}/\text{ml}$ . The other experimental conditions and methods for measuring  $f[\text{Mg}^{2+}]$  and calculation of  $[\text{Mg}^{2+}]$  are given in the text (Reaction II).

In Fig. 7 the values obtained for  $f[\text{Mg}^{2+}]$ , pyruvate kinase concentration = 33.3  $\mu\text{g}/\text{ml}$ , are given as a function of  $\text{Mg}^{2+}$ -concentration at three ionic strengths. It should be noted that for a given  $\text{Mg}^{2+}$ -concentration,  $f[\text{Mg}^{2+}]$  was independent of  $[\text{ATP}]_t$ . This is not shown in Fig. 7, where all  $\text{ATP}_t$ -concentrations, ranging from 0.1 to 4 mM have the same symbol. This observation, which is in agreement with the studies of Wood,<sup>21</sup> greatly facilitates the evaluation of the kinetics of the complete system, as described below.

#### Reaction I + II + III

As mentioned above,  $V_{\text{III}}$  is always equal to  $V_{\text{II}}$  for the experimental conditions reported, which means that studies on the "total" system can be reduced to studies of reaction I + II. Two typical experiments are illustrated by Fig. 8.

Fig. 8. Progress curves (NADH(+H<sup>+</sup>) oxidation) for the coupled ATPase assay, reaction I + II + III (Fig. 4), at two different Mg-concentrations. Experimental conditions, both curves: 40  $\mu\text{g}$  ATPase/ml, 1 mM ATP, 1.1 mM PEP, 120  $\mu\text{M}$  NADH, 33.3  $\mu\text{g}$  pyruvate kinase/ml, 33.3  $\mu\text{g}$  lactate dehydrogenase/ml, ionic strength 0.14 M (tris-buffer), pH 7.38, temp. 37°C. Both reactions were started at zero time by addition of ATPase.  $[\text{Mg}]_t$  was 1.0 mM (A) and 0.063 mM (B), corresponding to  $[\text{Mg}^{2+}]$  being 300  $\mu\text{M}$  (A) and 8.5  $\mu\text{M}$  (B).



Generally, it can be stated that the initial ADP concentration is very low. The only ADP added is that contaminating the ATP preparation (0.5–1 mol %), and this ADP is removed by reaction II + III before reaction I is started with the addition of ATPase to the system. Thereafter, measurement of  $V_{II}$  and calculation of  $[Mg^{2+}]$  provide the concentration of  $ADP_t$  at any point of the experiment by means of Fig. 7 and eqn. (14). The system may now be described by the following equations, assuming  $[ADP_t] = 0$  at  $t = 0$  (point of addition of ATPase):

$$d[ADP_t]/dt = V_I - V_{II} = V_I - f[Mg^{2+}] [ADP_t] \quad (15)$$

which for steady state ( $d[ADP_t]/dt = 0$ ,  $V_I = V_{II}$ ) gives

$$[ADP_t]^{st.st.} = V_I/f[Mg^{2+}] \quad (16)$$

Since it is the rate of the uninhibited ATPase reaction that is wanted, it is important that  $[ADP_t]^{st.st.}$  in any case is so low that it does not influence  $V_I$ . According to eqn. (16)  $[ADP_t]^{st.st.}$  at constant  $[Mg^{2+}]$  may be decreased by decreasing the ATPase concentration (decreasing  $V_I$ ) or by increasing the pyruvate kinase concentration (increasing  $f[Mg^{2+}]$ ). Experiments along this line showed that  $V_I$  was independent of  $[ADP_t]$  when  $[ADP_t]$  was less than about 5 % of  $[ATP_t]$ . Under these conditions eqn. (15) can be integrated to give  $[ADP_t]$  as a function of time:

$$[ADP_t] = \frac{V_I}{f[Mg^{2+}]} [1 - \exp(-f[Mg^{2+}] t)] \quad (17)$$

or

$$V_{II} = V_I [1 - \exp(-f[Mg^{2+}] t)] \quad (18)$$

From this equation it is apparent that the time necessary to give  $V_{II} = 0.5 V_I$  is

$$t_{0.5} = \frac{f[Mg^{2+}]}{\ln 2} = \frac{0.693}{f[Mg^{2+}]} \text{ min}$$

$$\text{After } 6 t_{0.5} = 4.2/f[Mg^{2+}] \text{ min, } V_{II} = 0.98 V_I.$$

At this point or later  $V_I$  can thus be obtained from the experimental curves. For the examples given in Fig. 8,  $6 t_{0.5}$  is about 40 sec (curve A) and about 100 min (curve B).

In the extreme cases where  $f[Mg^{2+}]$  is so low that steady state is not reached within the time taken to use all the  $NADH(+H^+)$  in the system (curve B, Fig. 8),  $V_I$  was obtained from eqn. (18) by measurement of  $V_{II}$  as a function of time, and subsequent curve-fitting by means of an analog computer. Hereby it was possible with reasonable accuracy to find both  $V_I$  and  $f[Mg^{2+}]$  for a number of different experimental conditions. The good agreement between  $f[Mg^{2+}]$  obtained in this way and  $f[Mg^{2+}]$  obtained under similar conditions but without ATPase in the system [see Reaction II (p. 2721) and Fig. 7] is illustrated by Fig. 9, and supports the validity of eqn. (18) and the underlying model for the total system.



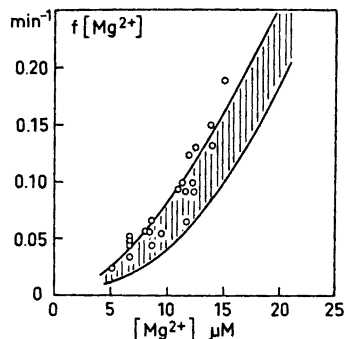


Fig. 9. Comparison between  $f[\text{Mg}^{2+}]$  obtained by curve fitting of non steady state experiments with reaction I+II+III ( $\circ$ ) (see eqn. (18) and the text), and  $f[\text{Mg}^{2+}]$  from studies on reaction II+III (area between the two curves (cf. Fig. 7,  $I = 0.14\text{M}$ )).

In order to exclude inhibition of the ATPase by PEP the latter was increased from 1 mM to 5.4 mM in a number of experiments where  $[\text{ATP}_t] = 0.10$  mM and  $[\text{Mg}_t] = 0.05$  to 0.3 mM. At ionic strength 0.23 M or 0.14 M, the change in PEP-concentration was without effect on the reaction rate, whereas an increase in  $[\text{PEP}]$  from 1 to 5.4 mM lowered the rate by about 5 % at  $I = 0.085$  M. In this case the effect of PEP is readily explained by its slight but significant ability to complex  $\text{Mg}^{2+}$  (Ref. 7). Under similar conditions, an increase in the  $(\text{NH}_4)_2\text{SO}_4$  concentration from 7.5 to 30 mM likewise only had a small lowering effect on the reaction rate at  $I = 0.085$  M and again the effect could be explained by the removal of  $\text{Mg}^{2+}$  from the solution by  $\text{SO}_4^{2-}$  (Ref. 7).

#### CONCLUSION

In conclusion it may be stated that the coupled ATPase assay investigated in this work will provide accurate and reproducible initial rates under a wide range of experimental conditions. The fact that it can be used at low concentrations of  $\text{Mg}^{2+}$  and  $\text{ATP}_t$  and that ADP-inhibition can be excluded makes the method a valuable tool in studies on the kinetics of  $\text{MgATPase}$ . The results of such studies will be published in a later paper from this laboratory.

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