**F1-like Properties of an ATPase from the Archaebacterium Halobacterium saccharovorum**

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For F1-ATPases from mitochondria and chloroplasts, tight binding of Mg**2⁺** and ADP without Pi at a catalytic site had been reported as a cause of enzyme inhibition. The time dependence of this inhibition and the effect of various agents on this process have been described (Du, Z., and Boyer, P. D. (1990) Biochemistry 29, 402–407, and references therein). Similar results are now reported for the ATPase from *Halobacterium saccharovorum*. The nonlinear hydrolysis kinetics were modulated by nitrate, azide, sulfite, GTP, ADP in the absence of ATP, or Pi, in characteristic ways, in good analogy with the effects of these agents on F1 enzymes. The similarity to the F1 systems suggests that it is tight ADP binding that is affected. Although these reactions of the *H. saccharovorum* ATPase occurred on different time and concentration scales than those of F1-ATPases, the two systems do not appear to be fundamentally different. The hydrolytic mechanism of the *H. saccharovorum* ATPase thus identifies this enzyme as a member of the F0F1-ATPase family.

It has been suggested by several groups that the ATPases of archaebacteria are distinct from ATPases in eubacteria and in the mitochondria or chloroplasts of eukaryotes (F0F1-type ATPases) but resemble a group of ATPases found in membranes of secretory organelles (the V-type ATPases). The criteria for such a classification have included amino acid composition (Stan-Lotter and Hochstein, 1989), primary structures of enzyme subunits (Dends et al., 1988; Manolson et al., 1988; Inatomi et al., 1988; Sidhoff et al., 1989; Gogarten et al., 1989), immunological cross-reactivity (Mukohata et al., 1987), and the pattern of specific inhibitors of enzyme activity. Azide, an inhibitor of F1-ATPases, was reported to be ineffective with archaebacterial ATPases (Kristjansson and Hochstein, 1989; Nanba and Mukohata, 1987; Lübben and Schäfer, 1987, Konishi et al., 1987), whereas nitrate inhibited V-type and archaebacterial enzymes (Mellman et al., 1986; Bowman and Bowman, 1986; Moriyama and Nelson, 1987; Lübben and Schäfer, 1987; Mukohata and Yoshida, 1987; Schobert and Lanyi, 1989) but not F1-ATPases (Bowman and Bowman, 1986; Kakinuma and Igarashi, 1990).

In a previous report on the ATPase from *Halobacterium saccharovorum* (Schobert and Lanyi, 1989), complex kinetics for ATP hydrolysis were described, which had not been reported before for other archaebacterial or V-type ATPases. Pi was liberated at a nonlinear rate due to partial inhibition of the enzyme that developed within the first few minutes of the assay. The steady-state rate, reached after approximately 5 min, was usually less than 30% of the initial rate. This loss of activity was attributed to a hysteretic effect originating from a conformational change of the protein during the assay. The phenomenon was described with the kinetic parameters for the initial and the final rates, respectively, and with k as the rate constant for the transition. Although competitive product inhibition by high concentrations of ADP (>100 μM) could be excluded as the cause for the nonlinearity, a specific inhibitory effect of low ADP concentrations on the ATPase activity was not considered. Recent investigations and comparisons with the properties of F1-ATPases from mitochondria and chloroplasts (reviewed by Chernyak and Kozlov, 1986; Boyer, 1989) suggested possible effects of tightly bound ADP in the *H. saccharovorum* system. Experiments that follow the line of investigation with mitochondrial and chloroplast ATPases are presented in this report on the *H. saccharovorum* ATPase and draw attention to a compelling similarity between these systems.

**EXPERIMENTAL PROCEDURES**

**ATPase Preparation**—The growth of strain M6 of *Halobacterium saccharovorum*, membrane preparation, and enzyme purification are described elsewhere (Schobert and Lanyi, 1989).

**ATPase Assay**—The standard assay mixture contained the following in 1.5 ml: 3.5 M KCl, 0.1 M MOPS, pH 7, 3 mM MnSO**4**, 30 μl of ATPas and 10 mM ATP. The final protein concentration was between 12 and 20 μg/ml, depending on the experiment and the age of the preparation. The assay mixture was incubated at 30 °C, and the enzyme reaction was started by the addition of ATP (unless otherwise indicated). A 0.1-ml aliquot was withdrawn at the times shown and processed as described earlier (Schobert and Lanyi, 1989). In some experiments, it was necessary to replace 3.5 M KCl by 4 M NaCl, or 3 mM MnSO**4** by 5 mM MgSO**4**, because of solubility problems with some of the components. Kinetic analysis was as described elsewhere (Schobert and Lanyi, 1989). The data given represent the average of three separate assays. The specific activity of the enzyme was between 0.85 and 1 amol F1/min·mg of protein (initial rates of various preparations).

**Materials**—ATP was from Sigma, and GDP, GTP, phosphoenolpyruvate, pyruvate kinase, and lactate dehydrogenase were from Boehringer Mannheim.

**RESULTS**

**Measurement of Activity with Two Different Methods**—Coupled assay systems containing pyruvate kinase and lactate dehydrogenase have been employed by many groups to follow the rate of ATP cleavage. This system functions as an ADP trap and, at the same time, regenerates the ATP hydrolyzed by the ATPase. The pyruvate kinase/lactate dehydrogenase system could be used in the assays even at the high salt concentrations known to be required by the *H. saccharovorum*.
ATPase for full activity (Kristjansson and Hochstein, 1985). After adjusting the different scales, the measurement of ADP with the coupled assay at 339 nm (Fig. 1A, curve 1) and of P, with malachite green at 650 nm (Fig. 1A, curve 2) yielded curves of very similar shape. Phosphoenolpyruvate and pyruvate kinase were included in the malachite green assay as well (Fig. 1A, curve 2) to provide comparable conditions. The liberated ADP and P, concentrations agreed, within approximately 15%, with the two methods (not shown). The coupled assay method revealed that the ATP solution used for this experiment had contained approximately 0.4% ADP (not shown). A preincubation time of 10–15 min was usually sufficient to consume the endogeneous ADP before the ATPase-dependent reaction was started. Although relatively high concentrations of pyruvate kinase and lactate dehydrogenase were required for maximal coupled activity, the pyruvate kinase/lactate dehydrogenase system could be used as an alternate assay procedure for the H. saccharovorum ATPase. In a second experiment, the effect of an ADP trap on the ATPase kinetics with the malachite green procedure was investigated. Pyruvate kinase was omitted in the first assay (Fig. 1B, curve 1) but included in the second assay under otherwise comparable conditions (Fig. 1B, curve 2). For curve 1, the ADP concentration was approximately 40 μM at time 0 and nearly 80 μM after 14 min, whereas for curve 2, the free ADP concentration was extremely low at all times, due to the ADP trap. The ADP content of ATP at the initiation of the assay (40 μM) did not affect the initial rate of the ATP hydrolysis nor were the steady-state rates significantly different in the two assays. However, the reduction of the free ADP concentration did have a small but reproducible effect on the onset of the time-dependent inhibition, i.e. the rate constant for the transition was reduced. This result suggests that ADP is involved in the turnover-dependent inhibition. Although the ADP trap somewhat delayed this event, it was not able to abolish the inhibition completely. Most assay systems with mitochondrial or chloroplast ATPases showed a similar nonlinear rate of hydrolysis attributed to ADP binding, even with an ADP trap in the reaction mixture (Vasilyeva et al., 1982a, 1982b; Vulfson et al., 1984). This indicates that the ADP affinity of a site on the ATPase is much higher than that of pyruvate kinase, and only loosely bound ADP, which is released into the medium, can be consumed by this enzyme. The existence of a pyruvate kinase inaccessible ADP bound to MF,-ATPase was pointed out by Gresser et al. (1982) and Vasilyeva et al. (1982b).

**The Activating Effect of P,**—The presence of several mM P, or PP, in the assay medium had an activating effect on F,-ATPases from various sources (Carmeli and Lifshitz, 1972; Bar-Zvi and Shavit, 1982; Vulfson et al., 1984; Kalashnikova et al., 1988). The H. saccharovorum ATPase showed similar behavior (Fig. 2). The presence of 20 or 40 mM P, in the coupled assay system did not affect the initial rate, but the onset of the inhibition that developed in time was clearly reduced, and the final rate was increased. The upper concentration for P, was restricted to 40 mM because of the limited solubility of magnesium phosphate in high salt. For the same reason, PP, could not be tested. The pyruvate kinase/lactate dehydrogenase activities were not affected by the phosphate concentrations used in the experiment (not shown).

**GTPase Activity Is Less Inhibited than ATPase Activity**—GTP or ITP is hydrolyzed by MF, and CF, ATPases but with lower initial rates than ATP. The influence of GTP or ITP on the ATPase kinetics was described to be similar to that of P, and the time-dependent inhibition of the enzyme was greatly reduced (Zhou et al., 1988) or even abolished when

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**Fig. 1.** A, comparison of two different assay systems for the ATPase. Curve 1 represents the measurement of ADP released from the enzyme. The assay contained the standard mixture and, in addition, 0.375 mg of phosphoenolpyruvate, 0.3 mg of NADH, 0.06 mg of lactate dehydrogenase, and 0.15 mg of pyruvate kinase. The reaction mixture was incubated for 15 min at 30 °C to obtain a stable base line. ATP hydrolysis was then started by adding ATPase at zero time, and the absorbance decrease was recorded at 339 nm. Curve 2 represents the measurement of P, The assay was as described under curve 1 except that NADH and lactate dehydrogenase were omitted. The reaction mixture was preincubated for 15 min at 30 °C, and ATP hydrolysis was started as before. B, the effect of an ADP trap in the reaction mixture. Assay 1 contained the standard mixture and, in addition, 0.375 mg of phosphoenolpyruvate. After 15 min of preincubation at 30 °C, the reaction was started by adding ATP. Assay 2 was treated as assay 1 but contained, in addition, 0.15 mg of pyruvate kinase. Kinetic parameters were: curve 1, ν, = 0.12 OD/min, ν, = 0.025 OD/min, k = 0.55 min⁻¹; curve 2, ν, = 0.12 OD/min, ν, = 0.025 OD/min, k = 0.44 min⁻¹.

**Fig. 2.** The effect of P, on the ATPase kinetics. The assay contained the standard mixture except that 5 mM MgSO, was used instead of MnSO, and, in addition, 0.375 mg of phosphoenolpyruvate, 0.3 mg of NADH, 0.06 mg of lactate dehydrogenase, and 0.15 mg of pyruvate kinase. The samples contained: 1, no P, added; 2, 20 mM P, and 3, 40 mM P, After 15 min of preincubination at 30 °C, the reaction was started by the addition of ATPase (arrow), and the absorbance decrease at 339 nm was recorded.
these nucleotides were used as the substrate (Vasilyeva et al., 1982a; Vulfson et al., 1984). Fig. 3 shows a similar effect of GTP on the kinetics of the *H. saccharovorum* ATPase. When GTP was used as the substrate, the turnover-dependent inhibition of the enzyme was not completely abolished, but it was significantly less as compared with ATP.

*The Effects of Sulfite, Nitrate, and Azide*—Certain anions, like sulfite or bicarbonate (Nelson et al., 1972; Moyle and Mitchell, 1975), were found to constitute another group of ATPase-activating compounds. The inclusion of several mM sulfite in the reaction mixture reduced the ADP-dependent inhibition of MF, and resulted in linear phosphate liberation curves (Vasilyeva et al., 1982b; Chernyak et al., 1988). This effect could also be observed with the *H. saccharovorum* ATPase, except that much higher concentrations of sulfite were required for a comparable response. Concentrations up to 300 mM sulfite did not affect the initial rate, but drastically increased the steady state rate $v_2$ and reduced the rate of transition (Fig. 4A). However, even 300 mM sulfite was not sufficient to fully abolish the time-dependent inhibition. Since the upper limit of the $P_i$ determination method was rapidly reached with increasing sulfite concentrations, in another experiment the enzyme concentration was reduced by 80%. Under these conditions and with 400 mM sulfite present, the $P_i$ liberation rate was linear up to 14 min (data not shown). $v_2$ never exceeded $v_1$ even at higher concentrations of sulfite (not shown).

It was previously reported that nitrate acted as an unusual inhibitor of the *H. saccharovorum* ATPase because the initial rate was unaffected, only $v_2$ was reduced, and the rate constant for the transition was increased (Schobert and Lanyi, 1989). Identical behavior was demonstrated for azide inhibition of MF (Vasilyeva et al., 1982b). Furthermore, the inhibition by azide was reduced by sulfite (Vasilyeva et al., 1982b). Sulfite was also able to counteract the nitrate-induced inhibition of the *H. saccharovorum* ATPase (Fig. 4B). On reinvestigating the *H. saccharovorum* ATPase azide was shown to be inhibitory at concentrations of several hundred mM (Fig. 4C). The maximum inhibition of the final rate, however, was rather small and was not increased at azide concentrations higher than 300 mM. The requirement for high concentrations and the relatively small effect were the probable reasons for the inhibitory action of azide being previously missed (Kristjansson and Hochstein, 1985; Nanba and Mukohata, 1987). Nitrate and azide are widely used to distinguish between archaeabacterial and F$_1$-type ATPases. Since nitrate and azide both seem to inhibit the *H. saccharovorum* ATPase and affect the kinetics in the same way, it is apparent that these compounds can be valuable as diagnostic tools but do not identify

![Fig. 3. Comparison of ATPase and GTPase kinetics.](image)

![Fig. 4. A, the effect of sulfite on the ATPase kinetics.](image)
prior to the initiation of the assays was investigated. After such incubations, the initial hydrolytic rate of F1-ATPases was low but recovered in the presence of ATP (Fitin et al., 1979; Vasilyeva et al., 1982a; Vulfson et al., 1984; Guerrero et al., 1990). Fig. 5A shows the effect of preincubating the H. saccharovorum ATPase with MnADP. It is evident that the initial rate is inhibited, but the final rate is not affected. Thus, before the preincubation, the assay curve contained a fast rate followed by inhibition and a slower final rate (Fig. 5A, curve 1), but after lengthy preincubation, it contained an initially very slow rate followed by recovery and the same final rate as before (Fig. 5A, curve 3). A semilogarithmic plot showed that the kinetics of the inhibition of $v_i$ during the preincubation was described by three time constants (Fig. 5B). The concentrations of ADP and Mn$^{2+}$ affected the relative proportions of the three phases. For example, during incubation with 50 $\mu$M ADP and 50 $\mu$M Mn$^{2+}$, 56% of the enzyme was already inhibited after 30 s (Fig. 5B, curve 2). When preincubated with Mn$^{2+}$ alone (up to 3 mM) for 60 min, no inhibition of the ATPase could be detected (not shown). For F1-ATPases, both metal and ADP were necessary for the inhibitory effect (Vasilyeva et al., 1980; Feldman and Boyer, 1985; Chernyak et al., 1988). Incubation with Mg$^{2+}$ alone was effective only with the native enzyme (Moyle and Mitchell, 1975) but did not inhibit the nucleotide-depleted enzyme preparation (Drobinskaya et al., 1985). Since the H. saccharovorum ATPase was not inhibited after preincubation with Mn$^{2+}$, it is concluded that the purification procedure had removed any ADP bound to a catalytic site. On the other hand, preincubation with ADP alone was already effective (Fig. 5B, curve 1), probably because halobacterial proteins are rich in acidic groups with complexed mono- and divalent cations, which can partly substitute for the omitted metal ion. Recent results obtained with CF$_3$ (Guerrero et al., 1990) are consistent with the inhibition occurring with an initial rapid phase followed by slower processes (Fig. 5B).

**Inhibition by GDP in the Absence of ATP**—To compare the inhibitory effects of ADP and GDP under these conditions, the H. saccharovorum ATPase was incubated with 50 $\mu$M GDP and 50 $\mu$M Mn$^{2+}$ but assayed under standard conditions. GDP had an inhibitory effect on the initial rate only, as did ADP. However, the inhibition developed much more slowly and its extent was also less (Fig. 6A, compare with Fig. 5A). A semilogarithmic plot revealed that during GDP preincubation, the time-dependent inhibition of $v_i$ was at least biphasic (compare Fig. 6B with Fig. 5B, curve 2). An inhibition of the initial rate by preincubation with MgGDP could not be seen with MF$_1$ (Drobinskaya et al., 1985), but comparable results have been obtained with CF$_3$ (Guerrero et al., 1990).

**Sulfite and Nitrate Do Not Affect the Inhibition that Develops during Incubation with MnADP**—Concentrated enzyme solution was incubated for 15 min with 50 $\mu$M ADP and 50 $\mu$M Mn$^{2+}$. An aliquot was used to measure enzyme activity, with a 25-fold dilution of the enzyme in the assay mixture. Fig. 7A (curve 1) shows the control. When 200 $\mu$M sulfite or 20 mM nitrate was included in the assay mixture but not in the preincubation, the initial rate was not significantly changed, but the recovery rate was affected, and the final rate was greatly increased (sulfite, Fig. 7A, curve 2) or decreased (nitrate, Fig. 7A, curve 3). Similar findings were reported for MF$_1$ by Chernyak et al. (1988) and by Kalashnikova et al. (1988). The second question was whether sulfite or nitrate affected the preincubation. When 200 $\mu$M sulfite was added to the preincubation mixture, the resulting concentration in the assay mix was 8 mM. The control experiment did not contain sulfite in the preincubation mixture but contained 8 mM sulfite in the assay mix. It is evident from Fig. 7B that the two curves are virtually identical (curve 1, 2). A comparable experiment was conducted with 20 mM nitrate included in the preincubation mixture and 0.8 mM nitrate added to the assay mix of the control sample. Again, no difference in enzyme activity between the two samples could be detected (Fig. 7B, curve 3, 4). This result clearly indicates that the presence of sulfite in the preincubation mixture did not protect against this type of ADP inhibition nor did nitrate enhance the inhibitory effect. Instead, these compounds were only effective during catalytic turnover. In a similar investigation, bicarbonate (Guerrero et al., 1990) and sulfite (Du and Boyer, 1990) had no effect on CF$_3$ when present during incubation with MgADP.

**Fig. 5. A**, the effect of incubation with ADP on the ATPase kinetics. The assay contained the standard mixture. Prior to the initiation of the assay by adding MnATP, the mixtures were incubated with 50 $\mu$M ADP and 50 $\mu$M MnSO$_4$ for: 1, 0 min; 2, 0.5 min; and 3, 15 min. The kinetic parameters were: curve 1, $v_i=0.136$ OD/mrnin, $v_0=0.029$ OD/min, $k_1=0.65$ min$^{-1}$; curve 2, $v_i=0.062$ OD/min, $v_0=0.029$ OD/min, $k_1=0.42$ min$^{-1}$. B, decrease of the initial rate of ATP hydrolysis as a function of the incubation period with ADP. The ATPase was incubated with different concentrations of ADP and MnSO$_4$ for the indicated periods of time, and the assay was started by the addition of MnATP as described under Fig. 5A. The incubation conditions were: 1, 50 $\mu$M ADP; 2, 50 $\mu$M ADP and 50 $\mu$M MnSO$_4$; and 3, 5 $\mu$M ADP and 5 $\mu$M MnSO$_4$. The initial rate at 0-min incubation was set as 100%. The $y$ axis represents a logarithmic scale. The relative proportions (%) and time constants (min) for the three phases were: curve 1, 46 and 0.65, 24 and 6.2, 30 and 91.2; curve 2, 56 and 0.46, 32 and 12.7, 12 and 70,000; curve 3, 15 and 0.73, 40 and 6.3, 45 and 139.
every 1000 turnovers. During the assay, a steady-state rate is
obtained with 50 pM GDP and 50 pM MnSO₄ for the indicated time
periods, and the activities were assayed by the addition of MnATP
as described under Fig. 5A. The initial rate at 0-min incubation was
set as 100%. The kinetic parameters were: curve 1, \( v_1 = 0.090 \) OD/
min, \( v_2 = 0.0235 \) OD/min, \( k = 0.53 \) min⁻¹; curve 2, \( v_1 = 0.005 \) OD/
min, \( v_2 = 0.019 \) OD/min, \( k = 0.35 \) min⁻¹; curve 3, \( v_1 = 0.048 \) OD/
min, \( v_2 = 0.019 \) OD/min, \( k = 0.3 \) min⁻¹. B, inhibition of the initial rate of ATP hydrolysis as a function of the incubation time with GDP. The ATPase was incubated with 50 pM GDP and 50 pM MnSO₄ for the indicated time periods, and the activities were assayed by the addition of MnATP as described under Fig. 5A. The initial rate at 0-min incubation was set as 100%. The y axis represents a logarithmic scale. The relative proportions (%) and time constants (min) for the phases were: 54 and 6.1, 46 and infinite.

**DISCUSSION**

It is generally agreed that F₆F₅-ATPases contain six nucleotide binding sites per enzyme (Boyer, 1989). Three are located at noncatalytic sites, at the interfaces of the α and β subunits. The filling of these sites is required for hydrolytic activity, and they remain occupied during catalysis. Their role is not yet completely clear. They most likely exert a control function (Boyer, 1989; Milgrom et al., 1990). The other three binding sites are catalytic sites, on each β subunit. There is accumulating evidence that inhibition of the ATPase is caused by binding of ADP, without an accompanying P, to at least one catalytic site (Abbott et al., 1984; Feldman and Boyer, 1985; Drobinskaya et al., 1985; Zhou et al., 1988).

Three different modes of inhibition by ADP have been described for F₆F₅-ATPases from chloroplasts, mitochondria, or eubacteria. 1) A fraction of ADP which is produced during hydrolysis of ATP remains bound to the enzyme when P, and ADP are not released concomitantly (Chernyak and Kozlov, 1986; Boyer, 1989). This event occurs approximately once every 1000 turnovers. During the assay, a steady-state rate is reached, which represents a dynamic equilibrium between the rate of the formation and the rate of the decay of this ADP-enzyme complex (Chernyak and Kozlov, 1986). Thus, the initial rate is the true activity of the enzyme, while the steady-state rate reflects the fraction of active enzyme species and is only an apparent activity. 2) When the ATPase is incubated with very low (e.g. equimolar) concentrations of MgADP in the absence of ATP, a complex is formed similar to that described in 1) above. With this type of inhibition, the initial rate is affected, but the steady-state rate remains related to the fraction of active enzyme species (Chernyak and Kozlov, 1986). In other words, upon initiation of the assay by adding ATP, ADP is slowly released from another catalytic site (Adolfson and Moudrianakis, 1976; Harris et al., 1978; Fitin et al., 1979; Drobinskaya et al., 1985) but then replaced by an ADP produced during ATP hydrolysis as described in 1) above. The ADP causing these types of inhibition is usually referred to as "tightly bound," because the affinity of the F₆F₅-ATPase for this nucleotide is very high under the conditions described, and it does not readily exchange with medium ADP (Harris et al., 1978). Guerrero et al. (1990) emphasize the role of Mg²⁺, and they attribute the time-dependent inhibition or activation of the CF₅-ATPase to a slow binding or release of Mg²⁺. ADP and Mg²⁺ can be bound or released independently, but they are both required for an inhibitory effect (Guerrero...
et al., 1990). 3) The usual product inhibition is of the classic competitive type, by concentrations of ADP ≥ 70 μM (Cantley and Hammes, 1975). Both initial and steady-state rates are affected to the same extent, because ADP competes with ATP for occupying the same active site from the very beginning of the assay.

The ATPase inhibition induced by tightly bound MgADP (case 1 above) is affected by the presence of certain compounds in the assay mixture. The characteristic of these activators or inhibitors is that they do not interfere with the initial rate of the enzyme but affect only the steady-state rate and the rate of the transition. Most likely, they do not really activate or inhibit in the classic way but change the relative proportions of the inactive and the active species during steady state (Moyle and Mitchell, 1975). The mechanisms of their actions are not yet fully understood and await further clarification. Agents which change this proportion in favor of the active species are P1, PP1, GTP, sulfite, and bicarbonate. Since ADP remains tightly bound after the departure of P1 from the enzyme (Chernyak and Kozlov, 1986; Boyer, 1989), the presence of low mM concentrations of P1 (or PP1) seems to cause a reversal of this process, and after P1 binding, both ADP and P1 can be released together to restore the free enzyme (Carmeli and Lifshitz, 1972; Bar-Zvi and Shavit, 1982; Kalashnikova et al., 1988). Sulfite is believed to release tightly bound ADP (Vasilyeva et al., 1982b), whereas GDP as the product of GTP hydrolysis does not form tight complexes with the enzyme (Bar-Zvi and Shavit, 1982; Drobinskaya et al., 1985). A compound that increases the proportion of the inactive species is azide. It acts as an antagonist to sulfite, most likely by stabilizing the ADP-enzyme complex (Vasilyeva et al., 1982b).

The comparison of all these effects which have been reported for F1-ATPases with the properties of the H. saccharovorum ATPase establishes striking similarities between these systems. Characteristic effects that are related to the inhibition of the ATPase by tightly bound ADP could be produced. GTP, P1, sulfite, nitrate, and azide had comparable effects on the turnover-dependent inhibition of the H. saccharovorum ATPase. In the F1-ATPases, azide is the stronger inhibitor, while in the H. saccharovorum ATPase, nitrate is more effective. However, the inhibitory mechanisms of these anions appear to be the same. The inhibition by incubation with MnADP prior to the assay and the subsequent activation after MnATP addition were similar time-dependent processes. There also seemed to be some differences. With the H. saccharovorum ATPase, all the effects occur on a different scale with respect to time and concentration. In assays with the eukaryotic enzymes, the initial rate lasted for only tens of seconds, and the steady-state rate was attained after 1 or 2 min (Vasilyeva et al., 1982a, 1982b; Kalashnikova et al., 1988; Zhou et al., 1988), whereas the H. saccharovorum ATPase required approximately 5 min to reach this state (Fig. 1A). The steady-state rate of the MF1 was approximately 40–60% of the initial rate (Vasilyeva et al., 1982a, 1982b; Kalashnikova et al., 1988), but the H. saccharovorum ATPase showed a stronger inhibition with a steady-state rate (denoted as v2) of 30% or less, relative to the initial rate (denoted as v1). A discrepancy became obvious also for the inhibition that develops after incubation with low MnADP concentrations. In contrast to the eukaryotic enzymes, no inhibition could be seen at very low ADP concentrations (e.g. ADP:enzyme = 1:1) with the H. saccharovorum ATPase. In this system, an approximately 50-fold excess of ADP had to be used to observe any effect (not shown). Furthermore, less than 1 min of incubation time was required for inhibition or the subsequent activation of the MF1 enzyme upon addition of ATP (Drobinskaya et al., 1985), but several minutes were required with the H. saccharovorum ATPase (Figs. 5A and 7A). This difference in reactivity also explains why there is partial inhibition after GDP preincubation (Fig. 6A), while the reactivation of the MF1-ATPase after GDP incubation occurred too fast to be resolved with the assay method (Drobinskaya et al., 1985). However, recent findings with CF1-ATPase showed a time scale that was closer to our result, and comparative studies indicated several differences between CF1 and MF1 (Guerrero et al., 1990). The presence of a few mM sulfite in the assay mixture was already sufficient to abolish the turnover-dependent ADP inhibition and to obtain a linear hydrolysis rate (Vasilyeva et al., 1982b), but with the H. saccharovorum enzyme, only 300–400 mM sulfite produced a comparable effect (Fig. 4A). Similarly, in this system, 300 mM azide was required for inhibition, and only a very small effect was seen, as opposed to 100 μM and a substantial inhibition with MF1 (Vasilyeva et al., 1982b). The sensitivity toward nitrate cannot be compared, because F1-ATPases are not inhibited by this anion (Bowman and Bowman, 1986; Kakunima and Igarashi, 1990), but, in principle at least, nitrate in the H. saccharovorum system acts similarly to azide in the eukaryotic systems.

At present, we cannot provide a satisfactory explanation for the differences in affinity and reactivity between the F1-ATPases and the H. saccharovorum enzyme. One important factor is certainly the halophilism of the latter, because charged substrates are in competition with the high salt concentration of the medium. However, the differences outlined above do not appear to be fundamental. The results demonstrate that the H. saccharovorum ATPase is a principally different from F1-ATPases but shows a striking similarity in its behavior toward tightly bound ADP and various anionic effectors. On this basis, the H. saccharovorum ATPase should not be considered a separate class of ATPases but a member of the F1-ATPase family. This is consistent with the fact that an F1 analogous quaternary structure has been described for the archaeabacterium Sulfolobus acidocaldarius (Lübben et al., 1988). Several groups have pointed out the similarities in the primary structures of vacuolar and F1-ATPases (Zimniak et al., 1988; Bowman et al., 1988, 1989), and 3O exchange measurements revealed analogous catalytic mechanisms (Kasho and Boyer, 1990). On the other hand, vacuolar and archaeabacterial ATPases are known to be closely related. When the primary structure of the H. saccharovorum ATPase becomes available, there will most likely be differences from the F1-ATPases, due to the halophilic nature of the protein, and similarities due to common enzymatic properties. It appears that as more and more criteria are considered, the borders between the ATPase groups begin to blur.

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REFERENCES


H. saccharovorum ATPase