ALLOSTERIC INFLUENCE OF ANIONS ON MITOCHONDRIAL ATPase OF YEAST

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1. Introduction

Recently, nonlinear steady-state kinetics of mitochondrial ATPase of beef heart [1,2], rat liver [1,3] and yeast [4] have been reported in various laboratories. In all cases, the existence of catalytic as well as regulatory sites for the binding of magnesium–ATP were suggested. In addition, in case of the yeast enzyme, a differential effect of anions by binding to a regulatory site was indicated [4]. In the course of extensive studies we now observed a strong influence of a variety of anions effecting the regulatory binding site for magnesium–ATP. This anion competition for the regulatory site allows to describe the kinetic data for the ATP-hydrolysis by a simple phenomenological model. Our experiments clearly show that in addition to ATP, anions as allosteric ligands control the affinity of ATP to the site of its hydrolysis in yeast mitochondrial ATPase.

2. Materials and methods

Materials and the preparation of F_1-ATPase by chloroform-extraction procedure were used in this study as described elsewhere [4]. The specific activity of the enzyme was about 150 U/mg. In the kinetic experiments about 1.0 ml of an ammonium-sulfate suspension of the enzyme preparation was centrifuged at 35 000 × *g* for 1 h. The pellet was dissolved in a minimum amount (~ 1.0 ml) of a solution containing 50 mM Tris–SO_4 and 0.5 mM EDTA, pH 8.0, in a 1:1 glycerol/water-mixture and chromatographed on a Sephadex G-50 column (12 × 1 cm^2) equilibrated with the same buffer to remove residual ammonium sulfate. Fractions with ATPase activity were combined and stored at 4°C. In 50% glycerol ATPase is stable for several months. For use stock solutions were further diluted five-fold with glycerol buffer and 20 μl were used in each test.

Initial velocities were determined in the system described elsewhere [5]. The basic mixture contained in 0.4 ml 125 mM HEPPS/KOH, pH 8.0 (HEPPS = *N*-2-hydroxyethylpiperazine-*N'*-2-propane sulfonic acid), 5 mM phosphoenolpyruvate (K^+), 0.75 mM NADH, 50 μg each of pyruvate kinase and lactate dehydrogenase suspended in 50% glycerol. To this mixture were added 0.1 ml of the appropriate salt solution and 0.5 ml of a mixture of ATP-Na_4 and $MgCl_2$, adjusted to pH 8.0, giving the desired concentration of the ATP–magnesium-complex and 1.0 mM of free Mg^{2+} on a 1:1 dilution. A dissociation constant of 0.22 mM for the ATP–magnesium-complex was assumed according to [6]. The ionic strength was

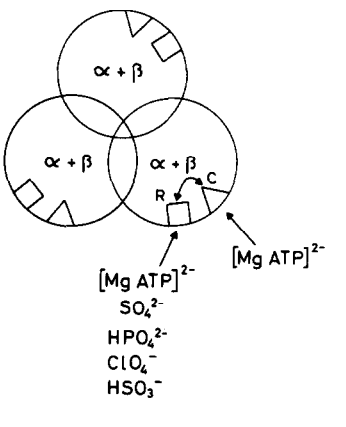

Fig.1. Schematic model of F_1-ATPase

kept constant by addition of HEPPS–K buffer, pH 8.0. Experiments were carried out at 25°C.

Our results were fitted by a curve-fitting program as described elsewhere [7] according to a minimum model (see [1]) assuming two different affinities of one catalytic site (respectively a set of non-interacting catalytic sites) (C) of the trimeric ATPase complex determined by the state (bound or unbound) of a regulatory site (respectively a set of non-interacting regulatory sites) (R) as shown schematically in fig.1.

The model is defined by the following equations:

(i) For the regulatory site:

$$[ES] = \frac{[E]\cdot[S]}{K_{Ds}}$$

$$[EM] = \frac{[E]\cdot[M]}{K_{Dm}}$$

(ii) For the catalytic site:

$$[SE] = \frac{[E]\cdot[S]}{K_m\,(II)}$$

$$[SES] = \frac{[E]\cdot[S]^2}{K_{Ds}\cdot K_m\,(I)}$$

$$[SEM] = \begin{cases} \dfrac{[E]\cdot[S]\cdot[M]}{K_{Dm}\cdot K_m\,(I)} & \text{for } H_mXO_4^{n-} \text{ anions} \\ \dfrac{[E]\cdot[S]\cdot[M]}{K_{Dm}\cdot K_m\,(II)} & \text{for } HSO_3^- \end{cases}$$

The rate of ATP-hydrolysis is described as:

$$v = \frac{V_m\cdot([SE] + [SES] + [SEM])}{[E] + [ES] + [EM] + [SE] + [SES] + [SEM]}$$

where K_m (I) identifies the low-affinity site and K_m (II) the high-affinity site respectively of the enzyme E, XEY is the enzyme complex with X at the catalytic and Y at the regulatory site, S being the substrate and M being the anion.

3. Results

The Lineweaver-Burk plot of fig.2a shows that in the absence of anions such as SO_4^{2-} or HSO_3^- a non-linear relationship is obtained indicating at least two different states of the enzyme. In the presence of sulfate (20 mM), phosphate (20 mM) and perchlorate (50 mM) ions only a low-affinity K_m (I) for magnesium–ATP (0.58 mM) is observed as shown only for sulfate in fig.2b. In contrast, in the presence of sulfite ions (10.0 mM) only a high-affinity K_m (II) (0.042 mM) is found (fig.2c). Mixtures of sulfite and sulfate cause K_m.values between 0.04 mM and 0.4 mM dependent on the amount of sulfite and sulfate in the mixture. This indicates that an ionic equilibrium is involved and sulfite is not acting as a reducing agent.

The interaction of magnesium–ATP and sulfate was tested as shown in fig.3, demonstrating a negligible influence of sulfate on the activity of the enzyme at high magnesium–ATP concentration (fig.3a) and an inhibition of the enzyme activity at low magnesium concentrations (fig.3b). This observa-

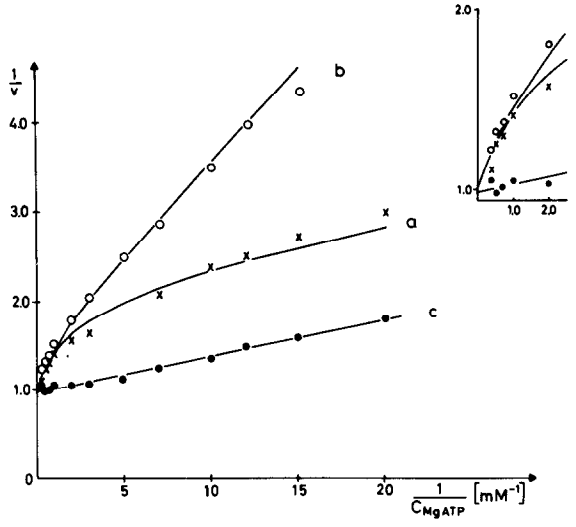

Fig.2. Relationship between ATPase activity, ATP and anion concentration. The lines were calculated according to the model described in the text (a) without added anions, (b) $C_{SO_4^{2-}}$ 20 mM, (c) $C_{HSO_3^-}$ 10 mM (a and b mean relative error 3.7%, a and c mean relative error 2.9%). Parameters for the fit: K_m (I) = 0.58 mM, K_m (II) = 0.042 mM, K_D(R–Mg–ATP) = 0.04 mM, K_D (R–SO_4^{2-} = 3.5 mM, K_D (R–HSO_3^-) = 0.05 mM (R–X, X bound at regulatory site).

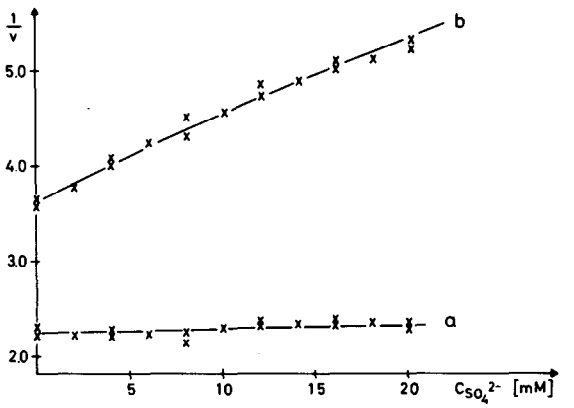

Fig.3. Variation of ATPase activity with anion concentration. The lines were calculated according to the model described in the text a) C_{MgATP} = 0.47 mM, b) C_{MgATP} = 0.074 mM (a and b mean relative error: 1.5%) Parameters for the fit K_m (I) = 0.58 mM, K_m (II) = 0.042 mM, K_D (R–MgATP) = 0.04 mM, K_D (R–SO_4^{2-}) = 4.1 mM (R–X, X bound at regulatory site).

tion points to the non-cooperativity of the anion binding sites (see below) and excludes a simple competitive mechanism of the anion influence upon the hydrolytic sites of the enzyme. This observation is further supported by the data summarized in table 1 for sulfite, demonstrating the increase of the enzyme activity with increasing sulfite concentrations.

The application of a simple model describing the activity states of the enzyme by two conformation states of each α- and β-subunit of the enzyme as independent units, defined by a high- and low-affinity K_m, leads to a good fit of the experimental data as given in the drawn line of the figures. Here the

Table 1
ATPase activity at various HSO_3^- concentrations

HSO_3^- (mM)	Relative activity $v = v/V_m$
0.0	0.46
1.0	0.65
2.5	0.76
5.0	0.82

$V_m \approx 150\ \mu mol.min^{-1}.\ mg^{-1}$

Concentration MgATP = 0.2 mM

conformation-states of the enzyme are simply determined by the occupancy of the regulatory site with the anions involved. It is interesting to note that sulfite ions have a positive, whereas others have a negative, effect on the reaction-rate (see below). According to the model magnesium–ATP and some anions of the type $H_mXO_4^{n-}$ can bind at (a) regulatory site(s) causing a low-affinity of the catalytic site(s) for magnesium–ATP. When HSO_3^- is bound at the regulatory site, or when this site is empty, the high-affinity state of the catalytic site is observed.

From the subunit stoichiometry [4] (the catalytic and regulatory sites are assumed on α- and β-subunits [8]) at least three catalytic and regulatory sites are expected. The linearity (r^2 = 0.98) of the Lineweaver-Burk plot in fig.2c in the presence of high sulfite (10 mM) indicates that there is no significant cooperativity between catalytic sites at concentrations between 0.05 mM and 3.0 mM. The excellent fit of the data shown in fig.3 indicates that no cooperativity between regulatory sites needs to be assumed.

The influence of anions on a regulatory site of ATPase can be considered as a result of the ionic structures involved. The sites seem to be accessible for anions of the type R–O–$XO_3H_m^{n-}$ with an X–O bond-length of about 1.45 Å. Ions similar in size to that of phosphate ($A_{x=0}$ = 1.4–1.6 Å) such as sulfate ($A_{x=0}$ = 1.52 Å), perchlorate ($A_{x=0}$ = 1.48 Å) and ATP are fitting to this site where the latter one could bind with its terminal-phosphate group. In each case the binding results in a decrease of affinity of the catalytic site for ATP-binding. Arsenate with larger ionic size ($A_{x=0}$ = 1.78 Å) show no effect on the affinity for magnesium-ATP. In contrast sulfite ions with a different ionic structure might be bound at the regulatory site in competition towards sulfate stabilizing the catalytic site in its high-affinity state.

The anion effect of soluble ATPase can also be observed in its membrane-bound form as OS–ATPase, or in case of submitochondrial particles [4]. One principle feature of this anion effect is, that it does not affect the maximum activity of the enzyme, an observation, which has also been reported elsewhere [9]. Also it is interesting to note that phenomenologically the anion effect appears with a Hill coefficient in the order of n_H = 0.5. Thus, under physiological conditions of ATP-synthesis the binding of phosphate

and ADP to the regulatory site, or of competitive ions, might promote the release of newly synthesized ATP, supporting a suggestion recently proposed [10].

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