Kinetics of the H^+-ATPase in chromatophores from *Rhodospirillum rubrum*

Effect of the electrical transmembrane potential on the rate constants

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The effect of the electrical potential on the H^+-ATPase of *Rhodospirillum rubrum* is examined. It is shown that the forward reaction rate (ATP synthesis) is increased by a factor of 10 during illumination while the reversed rate is only slightly decreased. This indicates that the electrical potential across the membrane affects the rate constants mainly by increasing the forward rate constants rather than decreasing the reversed rate constants in order to go from net hydrolysis to net synthesis.

Rhodospirillum rubrum *H^+-ATPase* *Membrane potential* *Enzyme kinetics* *Photophosphorylation* *Protonmotive force*

1. INTRODUCTION

The H^+-ATPase in chloroplasts, chromatophores and mitochondria has been described as an enzyme complex containing one integral proton channeling part (F_0) and one external catalytic part with ATPase activity (F_1).

According to the chemiosmotic theory [1], ATP synthesis is driven by an electrochemical gradient of protons across the membrane, to which reactions of the H^+-ATPase are coupled. The overall reaction can be written:

$$ADP + P_i + nH^+_{in} = ATP + nH^+_{out}$$

The equilibrium constant K_{eq} is:

$$K_{eq}(\Delta\psi) = [ATP][H^+]_{out}/[ADP][P_i][H^+]_{in}$$

or, as recently pointed out [2]:

$$K_{eq}(\Delta\psi) = \Sigma k_f/\Sigma k_r \text{ (f = forward, r = reversed)}$$

If the equilibrium constant is dependent on the electrical potential, the rate constants must also be potential-dependent [2]. It cannot be known a priori whether it is the forward or the reversed rate constants (or both) that are dependent on the electrical potential. The aim of our investigation has been to obtain an answer to this question.

2. MATERIALS AND METHODS

R. rubrum (strain S-1) was grown and harvested as in [3], with the exception that the cells were suspended in 0.2 M glycylglycine buffer (pH 7.4) and broken in a Ribi Cell Fractionator.

The phosphorylating activity of the chromatophores was determined to be about 10 μmol ATP/μmol Bchl per min. Synthesis of ATP was carried out in a 1-ml reaction mixture containing the following components: Tris–HCl (pH 7.5), 0.1 M; Na-succinate, 0.2 mM; MgCl, 10 mM; $^{32}P_i$, 2 mM (5500 dpm/μmol); ADP, 2 mM; ATP, 2 mM and chromatophores corresponding to 15 μM Bchl.

The concentration of ATP was selected to give no more than maximum 10% decrease or increase in the concentrations during the experiments. This was done to avoid any considerable changes in product inhibition during the experiments.

In the hydrolysis experiments the reaction medium was as described above with the following

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exception: [γ-^{32}P]ATP 2 mM (5500 dpm/μmol), and with no ADP and no labeled P_i present.

The reactions were started by the addition of the phosphorylation substrates to the sample. After 10 min the reactions were stopped by addition of trichloroacetic acid, giving a final trichloroacetic acid concentration of 5%. Illumination was performed by two 100 W tungsten lamps with a $CuSO_4$ solution as an IR-filter, to avoid heating the sample, in front of the reaction tubes.

ATP and P_i were separated in a two-phase system as in [4,5] with the exception that toluene was used instead of benzene. To collapse the electrochemical gradient, FCCP (2 μM) or Triton X-100 (0.5 vol%) was added where indicated. In the blanks, trichloroacetic acid was added before the substrate.

The ratio between labeled and unlabeled components was kept small (see above) to ensure that only very small amounts of radioactivity would be lost in undesirable reversed reactions.

Radioactive ATP and P_i were obtained from Amersham International.

3. RESULTS AND DISCUSSION

The forward reaction (ATP synthesis) was measured by determination of [^{32}P]ATP formation from ADP and $^{32}P_i$, while the reversed reaction (ATP hydrolysis) was measured by determination of $^{32}P_i$ formed from hydrolysis of [^{32}P]ATP.

In table 1 it can be seen that the rate of ATP formation is increased 10 times during illumination, while the rate of ATP hydrolysis is just decreased by about 20%. This shows that it is mainly the forward rate constants which are affected by the electrical potential across the membrane.

Table 1

Rates of synthesis and hydrolysis of ATP under different conditions

Conditions	μmol ATP formed/10 min per μmol Bchl	μmol P_i formed/10 min per μmol Bchl
Light	120 ± 5	40 ± 3
Dark	12 ± 2	48 ± 8
Light + FCCP	15 ± 2	n.d.
Dark + FCCP	0 ± 2	16 ± 2
Triton X-100	0 ± 2	16 ± 2

To calculate the factor by which the equilibrium constant (and thereby the ratio of the rate constants) is changed, we must know the transmembrane electrical potential and the number of protons needed for synthesis of one molecule of ATP.

The relation between the two equilibrium constants is [6]:

$$K_{eq}'/K_{eq} = 10^{(\Delta\psi(mV)/(RT\ \ln 10/nF)}$$

From this equation it can be calculated that with a transmembrane potential of 100 mV and using 3 protons per ATP synthesized the equilibrium constant would be shifted by a factor of about 10^5.

It is difficult to compare the rates under illumination with the rates under uncoupled conditions. This is due to the fact that the enzyme becomes inactivated at low values of the electrochemical gradient [7–9]. The electrochemical gradient can still be high in the dark because of hydrolysis of ATP present. Therefore, the rate of hydrolysis is higher in the dark compared with the illuminated rate and decreased when the electrochemical gradient is lowered by addition of FCCP or Triton X-100 (table 1), reflecting an inactivation of the enzyme. It has been shown [7] that addition of small amounts of FCCP (< 1 μM) results in stimulation of ATP hydrolysis while addition of higher amounts leads to a decrease of ATP hydrolysis.

An interesting difference between the H^+-ATPases of *R. rubrum* and *Rhodopseudomonas capsulata* is that the rate of hydrolysis becomes lower and higher, respectively, upon illumination, compared with the same rates in the dark [8]. It indicates that the magnitude of the electrochemical gradient necessary for activation of the enzyme is different in these two organisms.

Our present experimental results show that it is mainly the rate constants of the synthesis which are increased when an electrical potential is applied across the membrane.

It has been reported [10] that in F_1 from mitochondria, the rate-limiting step in ATP synthesis is the release of bound ATP. It thus seems reasonable to assume that also in the experiments reported here, the main part of the contribution by the potential difference across the membrane

would result in an increase of the rate of releasing bound ATP (and thereby achieve an increased rate of the overall ATP synthesis).

We have recently suggested [2] that the localisation of the catalytic site within the potential gradient (i.e., within the membrane) determines whether it is the forward or reversed rate constants which are influenced by the electrical potential. For an enzyme with its catalytic site outside this potential gradient on the side that becomes negative under energization, it is the forward rate constants which would be increased. This applies to the present case. If, instead, the F_1 were located outside the potential field on the side that becomes positive under energization, the effect of the potential would be a decrease in the reversed rate constants (in this case corresponding to hydrolysis). The overall reaction rate would therefore also be much lower.

Furthermore, it was suggested [2] that the losses of free energy are much lower when increasing the forward rate constants than decreasing the reversed rate constants. The data show that this organism has evolved to synthesize ATP in an energy-economical way.

Our theoretical consideration of the importance of the membrane topology appears to have gained the necessary experimental support with the data presented here. To ascertain the generality of these findings, similar investigations with other energy-converting membrane systems would be highly desirable.

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