Journal of Bioenergetics and Biomembranes (1977) **9**, 283–288

The Effects of Solvent Viscosity on the Kinetic Parameters of Myosin and Heavy Meromyosin ATPase

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Received 15 May 1977

Abstract

The effects of different solvent viscosities on the kinetic parameters of ATP hydrolysis by myosin and heavy meromyosin (HMM) were investigated at high and low ionic strength (i.e., 0.53 and 0.08 M KCl where myosin is polymerized into thick filament). The solvent viscosity was adjusted by the addition of appropriate amounts of sucrose. The maximum rate constants (V_m) for both myosin and HMM decreased monotonically with increasing solvent viscosity at either ionic strength. The Michaelis constants (K_m) for soluble myosin and HMM became minima at a viscosity nearly twice that of the solvent without sucrose, then increased abruptly with increasing solvent viscosity. On the other hand, K_m of polymerized myosin at the low ionic strength decreased monotonically with increasing solvent viscosity. These experimental results are discussed with special reference to Kramers' kinetic theory of a chemically reacting system in viscous media.

Introduction

Although the molecular mechanism of the conversion of ATP energy from a chemical to a mechanical form during muscular contraction is not yet elucidated, it is naturally expected that the energy released by ATP hydrolysis is at first stored in myosin and/or actin molecules before its conversion to mechanical energy. Indeed, Yamada et al. [1] have demonstrated that the rate of heat liberation after mixing of ATP with myosin in a solution was much more retarded than that of acid-labile

orthophosphate liberation. Furthermore, Morita and Yagi [2] have found that a conformational change in the vicinity of a tryptophan residue in heavy meromyosin (HMM) was brought about by the binding of ATP with heavy meromyosin. Besides, the turnover time of myosin and heavy meromyosin ATPase is very slow. This property is probably related closely to the mechanism of energy conversion [3].

Accordingly, it is conceivable that each cyclic process in ATP hydrolysis involves several successive changes in the protein conformation, some of whose rates are significantly affected by the frictional force due to solvent viscosity. Therefore, it is very interesting to investigate the effects of solvent viscosity on the kinetic parameters of ATP hydrolysis by soluble myosin, its thick filament, and its active head (heavy meromyosin) at high and low salt solutions. Thirty-seven years ago, Kramers [4] published a theoretical calculation on chemical reaction rates for particles which perform micro-Brownian motions in a field of force and in a viscous medium. His intention was to elucidate the applicability of the transition state method proposed by Eyring [5] for calculating the rate of chemical reactions. We will use his method to discuss our results.

Materials and Methods

Myosin and HMM were prepared from rabbit skeletal muscle according to the methods of Perry and Szent-Gyorgyi, respectively. The concentrations of myosin and HMM were determined by the method of Lowry using 4.5×10^5 and 3.2×10^5 daltons as the molecular weight, respectively.

ATP was obtained from Sigma Chemical Company. The concentration of ATP was determined using the molar extinction coefficient of $1.54 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, at pH 7.0. Other chemicals were the reagent grades.

The viscosities in solutions were varied by the addition of various amounts of a concentrated sucrose solution. In all experiments of ATP hydrolysis activities, the reaction solutions contained 10 mM $CaCl_2$, 20 mM Tris-maleate buffer (pH 7.0), 0.08 or 0.53 M KCl, and various amounts of sucrose at 25°C. The presence of sucrose in reaction solutions did not cause any specific effect in ATPase activity, except the viscous effect studied. Solvent viscosity of the solutions was measured by an Ostwald viscometer. ATP hydrolysis was started by the additon of 0.5 ml ATP solution, the final concentration of which varied from 0.78 to 4.7 mM. Immediately after addition of ATP, the reaction solution was stirred for 5 min using a Komagome glass pipet. The amounts of orthophosphate liberated were colorimetrically determined by the method of Gmori [6].

Results and Discussion

The rates of ATP hydrolysis by myosin and HMM in the presence of various ATP concentrations (from 0.78 to 4.7 mM) were measured in solutions at two different KCl concentrations (0.08 and 0.53 M) and at various sucrose concentrations. From the Lineweaver–Burk plot for the system with a sucrose concentration, the maximum rate constant(V_m)and Michaelis constant(K_m)were estimated. Both V_m and K_m for each myosin or HMM system were significantly changed by the addition of sucrose to vary the viscosity. It was confirmed by some preliminary experiments that substitution of sucrose with glycerol did not cause any significant change in the relationship between the kinetic parameters of ATP hydrolysis and solvent viscosity. However, it is considered that a reagent grade of glycerol is usually contaminated with aldehyde. Thus, systematic experiments on the viscosity effect were carried out by the use of sucrose alone. Of course, reelimination of sucrose in myosin or HMM solutions by dialysis made certain that sucrose did not cause any irreversible effect on ATPase activity. V_m for both myosin and HMM in the low and high ionic solutions decreased monotonically with increasing solvent viscosity. The turnover times of myosin and HMM ATPase which are inversely proportional to V_m are plotted against the solvent viscosity in Fig. 1. It is generally considered that the increase in solvent viscosity of an enzyme solution due to the addition of sucrose or other viscous medium brings about a decrease in the enzyme activity if the enzyme action involves a gross change in some conformations of the enzyme.

One of the main aims in this work is to analyze and compare these data with special reference to Kramers' theory. Kramers [4] has calculated theoretically the influence of solvent viscosity on chemical reaction rate of a particle in a viscous medium. According to his diffusion model of chemical reactions, the particle which is originally caught in a potential hole A and which is subjected to the irregular forces of a surrounding medium in temperature equilibrium and to an external field of force, can escape to another potential hole B in the course of time by passing over a potential barrier C. In principle, his model can at least be applicable to any enzyme, if the external field of force in his model is reinterpreted as the conformational energy barrier in the active site of the enzyme. The chemical reaction rate by the particle is expressed as

$$R = \nu_A \eta / 2\pi\nu_C \left\{ \left[\tfrac{1}{4} + (2\pi\nu_C/\eta)^2 \right]^{1/2} - \tfrac{1}{2} \right\} e^{-Q/kT}$$

where η is the solvent viscosity, and ν_c and ν_A are the frequencies of the harmonic oscillation of the particle in the vicinity of the potential barrier C and of the potential hole A, respectively. Q is the potential difference

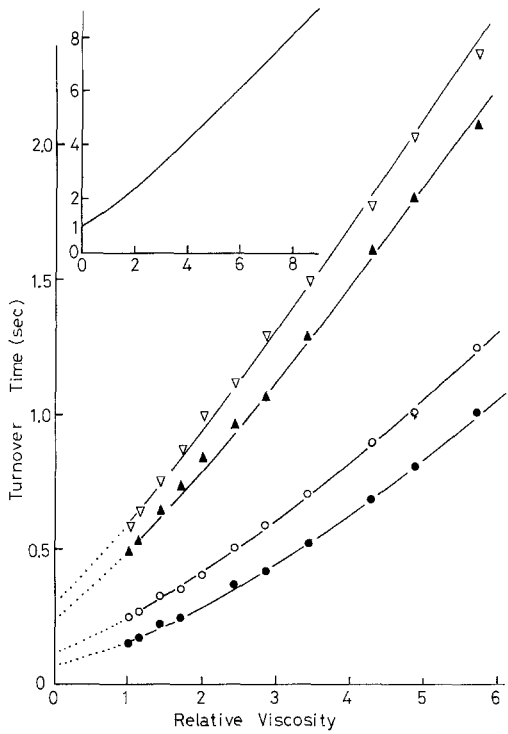

Figure 1. Effects of relative solvent viscosity on the turnover time of ATP hydrolysis by myosin and HMM in low (0.08 M) and high (0.53 M) KCl solutions. The relative solvent viscosity was varied by the addition of various amounts of sucrose (the relative solvent viscosity in the absence of sucrose was taken as the unit). The turnover time was calculated from V_m and molecular weights of myosin and HMM. All solutions for the experiments contained 10 mM $CaCl_2$, 20 mM Tris–maleate buffer (pH 7.0), and various amounts of sucrose at 25°C. △, Myosin in 0.53 M KCl; ○, myosin in 0.08 M KCl; ▲, HMM in 0.53 M KCl; ●, HMM in 0.08 M KCl. The insert illustrates turnover time versus viscosity derived from Kramers' theoretical equation [4].

between A and C. At very low solvent viscosity, R becomes $\nu_A e^{-Q/kT}$, which corresponds to the formulas of the transition state method derived by Eyring [5]. For sufficiently high viscosity, R is approximated as $\nu_A/(2\pi\nu_C/\eta)\, e^{-Q/kT}$. Instead of R, therefore, the turnover time, which is inversely proportional to R, is more easily dealt with as a function of solvent viscosity. In the insert of Fig. 1 is illustrated the profile of the turnover time versus $\eta/2\pi\nu_C$ where for the sake of convenience the ordinate is taken arbitrarily. At very high solvent viscosity, the turn over time is nearly proportional to the solvent viscosity. At intermediate values of solvent viscosity, the function of turnover time is concave to upward. The gross features of the experimental results shown in Fig. 1 resemble the theoretical curve of turnover time in the insert.

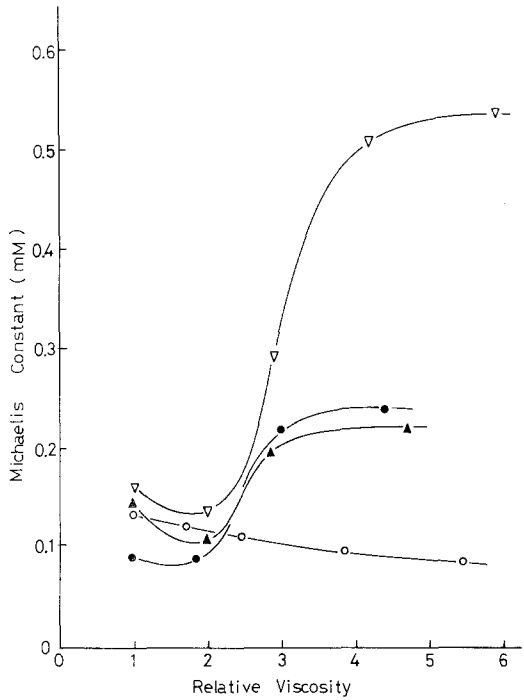

Figure 2. Effects of relative solvent viscosity on the Michaelis constant (K_m) of ATP hydrolysis by myosin and HMM. All conditions and symbols are the same as those in Fig. 1.

The Michaelis constants (K_m) for myosin and HMM obtained from Lineweaver–Burk plots are shown as functions of relative solvent viscosity (see Fig. 2). K_m for HMM and soluble myosin changed in a complicated way with increasing solvent viscosity. All K_m except that of polymerized myosin became minima at about 2 units of relative viscosity, and then increased with relative viscosity. In the case of polymerized myosin in the 0.08 M KCl solutions, K_m decreased monotonically with increasing relative solvent viscosity. This distinct property of K_m for polymerized myosin may be directly or indirectly related to the mechanical work-generating function of thick filament in striated muscle. It is also possible to suppose intuitively that an increase of solvent viscosity in myosin solution might correspond to an increase of tension in muscle fiber. In this respect also, the fact that the binding affinity of ATP with myosin and HMM at about 2 units of relative viscosity is stronger than that at 1 unit of relative viscosity (i.e., in the absence of sucrose) is of great interest.

Damjanovich et al. [7] recently studied the effect of different glycerol concentrations on the activity of phosphorylase *b*. According to their experiments, the addition of glycerol did not change the binding of glucose-1-phosphate but did decrease the binding of AMP.

Acknowledgments

This study was aided partly by grants from the Muscular Dystrophy Association and the Ministry of Education Science and Culture of Japan.

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