Fourier Transform Phosphorus Magnetic Resonance Study of the Interaction of P-enolpyruvate with the Muscle Pyruvate Kinase-Gadolinium Complex[1]

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SUMMARY

The phosphorus spin-lattice relaxation rates of P-enolpyruvate is enhanced 13 fold in the presence of muscle pyruvate kinase and gadolinium as compared to either enzyme or metal ion alone. In the presence of the enzyme-gadolinium complex the phosphorous relaxation rate decreases as the temperature increases which suggests fast exchange between enzyme-bound and free P-enolpyruvate. Assuming that the longitudinal electron spin relaxation rate of the gadolinium ion dominates the correlation time for the ternary P-enolpyruvate-gadolinium-enzyme complex, analysis of the relaxation rate data via the Solomon-Bloembergen equations yield a 5.2 Å internuclear gadolinium to phosphorus distance.

Pyruvate kinase (EC 2.7.1. 40) catalyzes the phosphorylation of ADP by P-enolpyruvate when activated by Mg^{2+} or Mn^{2+} (1). Recently the spectroscopic and magnetic properties of lanthanide ions have been used to study the muscle pyruvate kinase-metal complex (2). The rare earth ions were shown to be potent inhibitors of the enzymatic reaction. A binary enzyme-gadolinium complex was formed (K_D = 26 μM) which enhanced (ε_b = 12) the water proton relaxation rate when measured at 24.3 MHz. Furthermore, the rare earth ions displaced manganese from the enzyme-manganese complex, which suggests that both metal ions may be binding to the same site on the enzyme.

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In an attempt to understand the effect of rare earth metal ions on the catalytic activity of muscle pyruvate kinase, high resolution proton magnetic resonance and water proton relaxation rate techniques were used to detect ternary complexes formed by addition of ATP or P-enolpyruvate to the gadolinium-pyruvate kinase complex (2,3). The enhancement of the water proton relaxation rate decreased upon addition of ATP to the enzyme-gadolinum complex suggesting formation of a ternary complex (2). However, no change of enhancement was observed upon addition of P-enolpyruvate to the pyruvate kinase-gadolinium complex in contrast to the results observed when manganese was used as the paramagnetic metal ion (2,4,5). These results suggested that the P-enolpyruvate-gadolinium-enzyme ternary complex might not form or that a small decrease in the hydration of the bound gadolinium was compensated by an appropriate change in the correlation time. However, a high resolution proton magnetic resonance study did demonstrate that the vinyl protons of P-enolpyruvate are broadened more in the presence of both gadolinium and pyruvate kinase than in the presence of either alone (3). In this report, ^{31}P nuclear magnetic resonance has been used to demonstrate ternary complex formation as detected by changes in the longitudinal relaxation rate of the phosphorus atom of P-enolpyruvate in the presence of pyruvate kinase and gadolinium.

MATERIALS AND METHODS

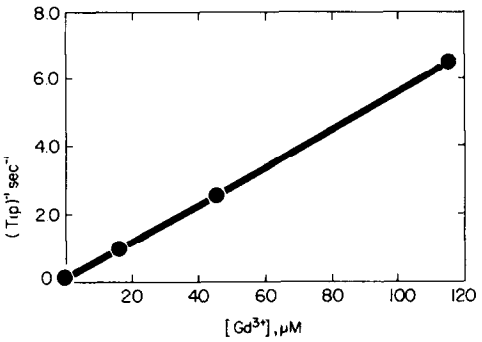
The pyruvate kinase was isolated from fresh rabbit skeletal muscle and recrystallized to a specific activity between 280 and 310 micromoles/min/mg of protein (1,6). The enzyme was stored as an ammonium sulfate suspension at a protein concentration between 10 to 20 mg/ml. The protein concentration was determined by absorbance at 280 nm and using 0.54 ml/mg/cm as the extinction coefficient (7). The catalytic activity of pyruvate kinase was determined by measuring the rate of pyruvate formation in the presence of NADH and excess lactic dehydrogenase with reagents described previously (6). Since lanthanide ions undergo hydrolysis, form insoluble hydroxides and

polynuclear polymers at pH values of 7 to 8 (8), these studies were completed using sodium acetate buffer at pH 5.7 in an attempt to minimize these problems.

The Fourier transform ^{31}P spectra were obtained from a spectrometer operating at 24.3 MHz. The T_1 was measured using the inversion-recovery technique with a π-τ-$\pi/2$ pulse sequence. The free induction decays were accumulated in an NMR ensemble averager prior to Fourier transformation with the Varian 620L/100 computer. At least six different values of τ were used to determine each T_1 value. The recycle delay between pulse sequences was at least 4 times τ null on samples in the absence of paramagnetic ion and at least 6 times τ null on samples containing the paramagnetic ion. For the temperature studies the temperature of the samples was maintained constant ($\pm$ 0.5°C) with a Varian temperature controller, otherwise the ambient temperature was 29°C. The observed enhancement (ε^*) of the P-enolpyruvate phosphorus relaxation rate is defined as $\varepsilon^* = (1/T_{1P}^*)/(1/T_{1p})$; the ratio of the paramagnetic contribution to the relaxation rate in the presence of protein (*) to that in the absence of protein (9). The paramagnetic contribution to the longitudinal relaxation rate is $1/T_{1P} = (1/T_1 - 1/T_{1(o)})$; the observed relaxation rate in the presence and absence (o) of paramagnetic ion.

RESULTS AND DISCUSSION

Addition of gadolinium to a solution containing 22 mg/ml pyruvate kinase 0.1 M P-enolpyruvate, and 1.0 M sodium acetate buffer, pH 5.7, results in a linear increase in the observed longitudinal relaxation rate of the phosphorus atom of P-enolpyruvate (Fig. 1). This corresponds to a molar relaxivity of 56,000 M^{-1} sec^{-1}. In the absence of pyruvate kinase, addition of gadolinium also results in a linear increase in the observed relaxation rate, and a molar relaxation of 4,300 M^{-1} sec^{-1} is obtained, thus, there is an observed enhancement of $\sim$ 13 in the longitudinal relaxation rate of the phosphorus of P-enolpyruvate in the presence of enzyme as compared to

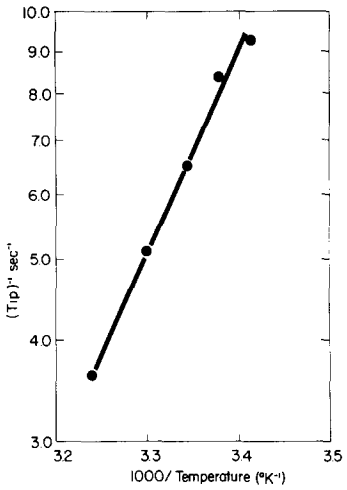


1. Effect of gadolinium on the ^{31}P longitudinal relaxation rate of P-enolpyruvate in the presence of muscle pyruvate kinase. The reaction mixtures contained 22.5 mg/ml pyruvate kinase, 0.1 M P-enolpyruvate 1.0 M sodium acetate buffer, pH 5.7, and the noted concentration of gadolinium. Temp. 29°C.

absence of protein. This result suggests that a ternary complex consisting of P-enolpyruvate, muscle pyruvate kinase, and gadolinium is formed.

The effect of temperature on the longitudinal phosphorus relaxation rate of P-enolpyruvate in a solution containing 36.4 mg/ml pyruvate kinase, 0.12 M P-enolpyruvate, 0.12 mM gadolinium, and 1.0 M sodium acetate buffer, pH 5.7, is illustrated in Fig. 2. As the temperature is increased the value of $(T^*_{1P})^{-1}$ decreases and indicates that the relaxation process is not limited by chemical exchange between the bulk P-enolpyruvate and P-enolpyruvate bound to the enzyme. The temperature data and the large enhancement suggest that the phosphorus relaxation rates are the result of a first coordination sphere process ($T_{1m} > \tau_m$).

For a first coordination sphere process one can estimate the distance (r) between the phosphorus of the P-enolpyruvate and the gadolinium ion in the ternary, enzyme-gadolinium-P-enolpyruvate, complex as shown below. Swift and Connick (10) and Luz and Meiboom (11) modified the Block equations to develop expressions which relate changes in the nuclear magnetic resonance spectrum of a ligand molecule exchanging between the bulk solution and the primary coordination sphere of a paramagnetic ion. The relaxation rates



2. Effect of temperature on the ^{31}P longitudinal relaxation rate of the phosphorus of P-enolpyruvate in the presence of muscle pyruvate kinase and gadolinium. The reaction mixture contained 36.4 mg/ml pyruvate kinase, 1.0 M sodium acetate buffer, pH 5.7, 0.12 mM gadolinium and 0.12 M P-enolpyruvate.

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of the ligand in the bound state are given by the Solomon-Bloembergen equations (12,13), equation 2 is the longitudinal relaxation rate expression. To evaluate equations 1 and 2, the hyperfine term in equation 2 may be ignored (14)

$$(T_{1P*})_b^{-1} = p*q*/(T_{1M*} + \tau_{m*}) \qquad (1)$$

$$\frac{1}{T_{1M}} = \frac{2S(S+1)}{15r^6}[g\beta\gamma_I\hbar]^2\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_s^2\tau_c^2}\right) + \frac{2S(S+1)A^2}{3\hbar^2}\left(\frac{\tau_e}{1+\omega_s^2\tau_e^2}\right) \qquad (2)$$

and the following values are used: q = 1, p = paramagnetic metal ion concentration/ligand concentration; S = 7/2, $\beta = 8.795 \times 10^6$ rad/sec-G, $\hbar = 1.054 \times 10^{-27}$ erg sec, g = 2.0., $\gamma_I = 10.83 \times 10^3$ rad/sec-G, $\omega_I = 1.526 \times 10^8$ rad/sec, $\omega_s = 2.462 \times 10^{11}$ rad/sec, and $\tau_c* = \tau_s$ (Gd) = 0.5 nsec (15). From these values and the data in Fig. 1 or the data at 29°C in Fig. 2, a value of r = 5.2 Å

is obtained for the internuclear P-enolpyruvate phosphorus to gadolinium distance in the ternary complex. These data suggest that the phosphorus of P-enolpyruvate does not form a first coordination spere complex with the enzyme bound gadolinium ion. P-enolpyruvate is reported to induce a conformational change in the magnesium pyruvate kinase complex (16). If a P-enolpyruvate induced conformational change is required to activate the enzyme and to observe a decrease in water proton relaxation rates, the large size of the lanthanide ions may prevent such a conformational change. This would lead to an inactive enzyme and explain the previous observation of no change in the water proton relaxation upon addition of P-enolpyruvate to muscle pyruvate and gadolinium.

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