HUMAN ERYTHROCYTE AMP: PYROPHOSPHATE PHOSPHORIBOSYLTRANSFERASE (EC 2.4.2.7)

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1. Introduction

The purine ribonucleotides regulate the rate of purine biosynthesis by feedback inhibition of ribosylamine-5-phosphate: pyrophosphate phosphoribosyltransferase (glutamate amidating) (EC 2.4.2.14) which catalyses the first specific step of this biosynthetic pathway. These compounds are formed by phosphoribosyltransferase enzymes acting on free purine bases as well as by *de novo* synthesis and subsequent ribonucleotide interconversions. Orally administered adenine inhibits purine biosynthesis in normal and gouty man [1,2], presumably after its conversion to adenylic acid by AMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7). It has been shown that adenylic acid but not adenine inhibits purified ribosylamine-5-phosphate: pyrophosphate phosphoribosyltransferase (glutamate amidating) (EC 2.4.2.14) [3]. About two thirds of gout patients show excessive *de novo* purine biosynthesis [4] which could reflect an abnormality of AMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7); and with this in view, we report here the results of preliminary observations on the kinetic properties of normal human erythrocyte AMP: pyrophosphate phosphoribosyltransferase.

2. Methods

The enzyme activities of dialyzed centrifuged erythrocyte lysates were determined radiochemically, the cells having been lysed by rapid freezing (at approximately -70^{o}C) and thawing three times. Portions of lysate (0.02 ml) containing 0.08–0.12 mg protein [5] were incubated for 15 minutes at 37^{o} with tris (hydroxymethylaminomethane) buffer (0.1 M, pH 7.45) containing 3×10^{-3} M $MgCl_2$, 5-phosphoribosyl-1-pyrophosphate (PRPP) and [8–^{14}C]-adenine in a total volume of 0.2 ml. The reaction was stopped by adding 5 μmoles of disodium ethylene diamine tetra-acetate (EDTA) and rapid cooling to -15^{o}. The purines and ribonucleotides were separated by co-electrophoresing aliquots of the reaction mixture with 0.1 μmoles of adenylic acid (80 V/cm, Whatman 3MM paper) in borate buffer (0.05 M, pH 9.6), containing ethylene diamine tetra-acetate (0.001 M). The areas which contained adenylic acid were located under an ultraviolet light source (254 nm) cut out, immersed in 15 ml toluene containing 2,5-bis-[5′-t-butylbenzoxazolyl(2′)]-thiophene (0.5 percent W/V), and their radioactivity measured in a liquid scintillation spectrometer.

3. Results and discussion

Preliminary experiments showed that the amount of adenylic acid formed was directly related to the amount of lysate present and to the duration of incubation for as long as 20 min at the concentrations of adenine and PRPP used. The concentration of magnesium ions had little influence on the activity over the range $2–6 \times 10^{-3}$ M, although Mg^{2+} was inhibitory in the range $10–20 \times 10^{-3}$ M. A plot of v (the velocity of adenylic acid formation) against s (the adenine concentration), at constant PRPP concentration was a rectangular hyperbole, but the corresponding plot of v against s (PRPP concentration) at a constant concentration of adenine (5.2×10^{-5} M

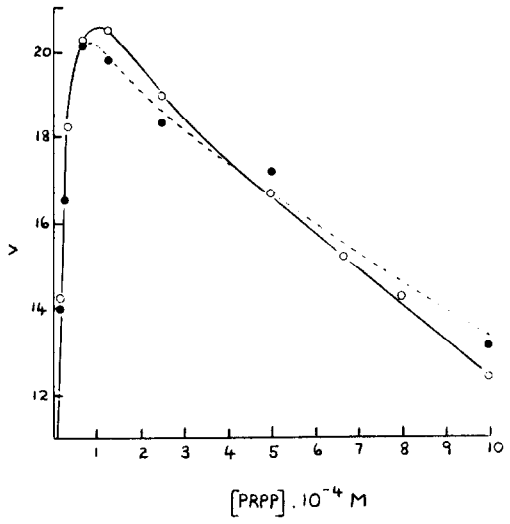

Fig. 1. Plot of reaction velocity (v) expressed as nmole AMP/hr/mg protein against PRPP concentration at two concentrations of adenine. Each study was performed on blood from a different subject. Subject (A) 3.6×10^{-4} M adenine, •----•; subject (B) 5.2×10^{-5} M adenine, o———o.

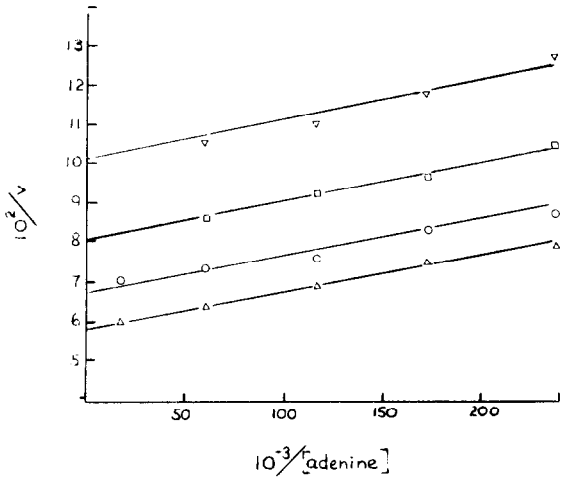

Fig. 2. Subject A(1). Double reciprocal plots of velocity against adenine concentration. PRPP concentrations were held constant at: ▽, 6.5×10^{-6} M; □, 10×10^{-6} M; ○, 20×10^{-6} M; and △, 62.5×10^{-6} M. v is expressed as nmole of AMP/hr/mg protein.

and 3.6×10^{-4} M in each of the two experiments) passed through a maximum value at s = approximately 0.07×10^{-3} M (fig. 1).

The effect of the concentration of adenine on the initial reaction velocity in the presence of a series of different concentrations ($6.5 - 62.5 \times 10^{-6}$ M), and of the PRPP concentrations in the presence of a series of different adenine concentrations ($4.3 - 66.6 \times 10^{-6}$ M) was investigated. Double reciprocal plots of the velocity against the concentration of one substrate at different fixed concentrations of the second substrate are shown in figs. 2 and 3. There is a direct relationship between V_{max} (the initial velocity at infinite substrate concentration) and K_m (the Michaelis constant) with respect to either substrate and the concentration of the other substrate (fig. 4). The present findings suggest that adenine and PRPP are not present on the enzyme simultaneously, and are in accord with those of Hori and Henderson [6] who studied a partially purified preparation of AMP: phosphoribosyltransferase (EC 2.4.2.7) from Ehrlich ascites tumour cells. These authors represented the mechanism as follows:

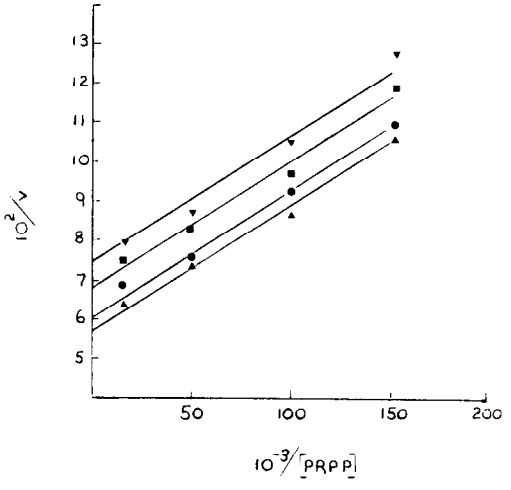

Fig. 3. Subject A(1). Double reciprocal plots of velocity against PRPP concentrations. Adenine concentrations were held constant at: ▼, 4.4×10^{-6} M; ■, 5.8×10^{-6} M; ●, 8.6×10^{-6} M; and ▲, 16.6×10^{-6} M. v is expressed as nmole of AMP/hr/mg protein.

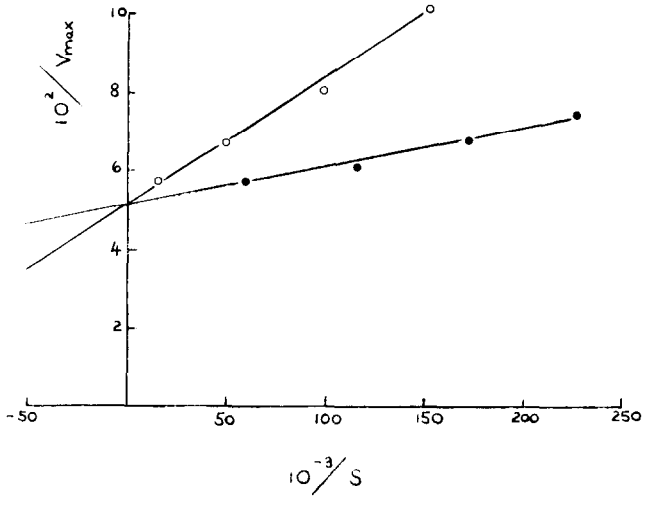

Fig. 4. Secondary double reciprocal plot of maximal velocity, obtained from the y axes of figs. 2 and 3 against adenine concentrations, •, and PRPP concentrations, o. The intercepts on the x axis, i.e. the negative reciprocals of the Michaelis constants for adenine and PRPP, were calculated.

$$E + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ER + PP$$

$$ER + A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EAMP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + AMP$$

where E = free enzyme, P and EP = free and enzyme bound PRPP respectively, ER = enzyme bound ribose-5-P, A = adenine, EAMP = enzyme bound adenylic acid. The values for V_{max} and K_m have been determined graphically for adenine and PRPP respectively as shown in fig. 4, and are presented for three different normal subjects in table 1. The K_m values obtained with the present impure enzyme system agree with those of Hori and Henderson [6] and Atkinson and Murray [7] for partially purified enzyme from Ehrlich ascites tumour cells.

The compound 2-ethylamino-1,3,4-thiadiazole accelerates purine synthesis in man [8,9]. Studies with the present system have shown that this is not due to its having an inhibitory effect on AMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7).

Table 1

Maximal velocity (V_{max}) and Michaelis constants (K_m) for adenine and 5-phosphoribosyl-1-pyrophosphate (PRPP) of normal human erythrocyte AMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7).

Subject	Maximal velocity (V_{max})	Michaelis constant (K_m)	
	(nmole/hr/mg protein)	Adenine (M)	PRPP (M)
A 1	19.6	1.9×10^{-6}	6.3×10^{-6}
2 *	19.0	1.4×10^{-6}	6.5×10^{-6}
B 1	30.3	2.7×10^{-6}	7.6×10^{-6}
2 *	26.3	2.5×10^{-6}	6.4×10^{-6}
C	28.7	2.5×10^{-6}	9.3×10^{-6}

* The second determinations were performed on a fresh sample of blood from the same subject after an interval of approximately one month.

Further investigations with the pure enzyme are needed to establish the mechanism of the reaction with complete certainty.

References

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