HYPOXANTHINE UPTAKE BY HUMAN ERYTHROCYTES

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1. Introduction

It is well established that the uptake of hypoxanthine by human erythrocytes is mediated by its incorporation into IMP [1]. The synthesis of this nucleotide is dependent on the intracellular availability of 5-phosphoribosyl α-1-pyrophosphate (PRPP) and on the activity of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) [1]. The synthesis of PRPP in erythrocytes incubated in saline glucose medium is strikingly enhanced by increasing P_i content of the incubation medium [1]. The kinetics of the uptake of hypoxanthine by human erythrocytes has not been investigated systematically. Here, evidence is presented that the rate of uptake is a direct function of P_i concentration and is independent of hypoxanthine concentration in the incubation medium suggesting that PRPP synthesis is the rate limiting step in hypoxanthine uptake.

2. Materials and methods

[8-^{14}C]Hypoxanthine (59 Ci/mol) was obtained from Radiochemical Centre, Amersham. All other reagents were high purity commercial samples from Merck AG.

Human erythrocytes were prepared from blood freshly drawn in heparin and washed twice with ice-cold 0.9% NaCl with removal of white cells by aspiration. The red cells were suspended in an equal volume of medium containing 5×10^{-2} M Tris–HCl (pH 7.4), 5×10^{-3} M glucose, $0–10^{-5}$ M [8-^{14}C]-hypoxanthine, $0–1.2 \times 10^{-2}$ M P_i and the appropriate amount of NaCl to give isotonic solution and incubated at 37°C. At the end of the incubation period, the hematocrits were within 1% of the initial values and hemolysis was very little (hemoglobin in the suspending medium, determined as cyanmethemoglobin [2], was <1.5% of the total hemoglobin content of the incubation mixture). At regular intervals samples of the incubation mixture were removed. The medium, separated from the erythrocytes by centrifugation, was employed for liquid scintillation counting and paper chromatography [3]. The packed erythrocytes (0.05 ml) were washed twice in 1 ml cold 0.9% NaCl and the sedimented cells were hemolyzed by addition of 0.45 ml 1 mM EDTA solution (pH 7.4). After protein precipitation [4], samples of the supernatant fluid were employed for paper chromatography [3]. The PRPP content of erythrocytes was determined according to [5]. HGPRT employed for the PRPP assay was purified as in [6].

3. Results and discussion

As fig.1 shows, under the experimental conditions employed, the rate of decrease in radioactivity of the incubation medium was constant with time, a linear function of [P_i], and independent of [hypoxanthine].

Hypoxanthine in the suspending medium and IMP in the erythrocytes were the only radioactive substances present in detectable amount during the incubation period. The amount of incorporated radioactive IMP was always equal, within the experimental error, to the decrease in radioactive hypoxanthine in the suspending medium.

In the absence of hypoxanthine, PRPP content

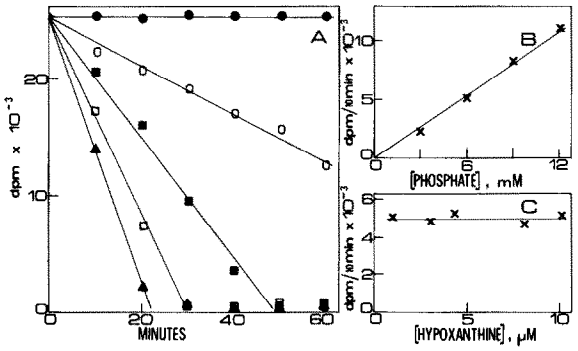

Fig.1. Effect of P_i and hypoxanthine concentration on the rate of decrease in radioactivity of the medium in which human erythrocytes were suspended to a 50% hematocrit. (A) The incubation medium contained: 5×10^{-2} M Tris–HCl (pH 7.4); 5×10^{-3} M glucose; 3×10^{-6} M [8-^{14}C]hypoxanthine; P_i, (•) none, (○) 3×10^{-3} M, (■) 6×10^{-3} M, (□) 9×10^{-3} M, (▲) 12×10^{-3} M; and the appropriate amount of NaCl to give isotonic solution. The incubation was carried out at 37°C. All other conditions were as described in the text. (B) Rate of decrease in radioactivity as a function of $[P_i]$. The experimental conditions were as in A. (C) Rate of decrease in radioactivity as a function of [hypoxanthine]. Phosphate was held constant at 6×10^{-3} M. All other conditions were as in A.

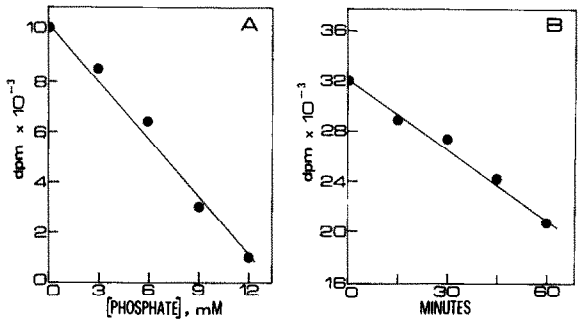

Fig.2. Human erythrocytes were preincubated in a medium containing P_i but not hypoxanthine and transferred into a fresh medium containing [8-^{14}C]hypoxanthine but not P_i. [Tris–HCl], [glucose], [NaCl], pH and incubation temperature were as in fig.1. The radioactivity was measured after 2 min incubation of erythrocytes in the medium containing the labeled purine base. (A) Decrease in radioactivity in the suspending medium as a function of $[P_i]$ at a fixed preincubation time (40 min). Initial [8-^{14}C]hypoxanthine was 5×10^{-6} M. (B) Decrease in radioactivity in the suspending medium as a function of the preincubation time at a fixed $[P_i]$ (9×10^{-3} M). Initial [8-^{14}C]hypoxanthine was 1×10^{-5} M.

of erythrocytes increased with increasing P_i concentration in good agreement with the results in [1,5]. When erythrocytes were preincubated in a medium containing P_i but not hypoxanthine and transferred into a fresh medium containing [8-^{14}C]hypoxanthine but not P_i, the PRPP content of erythrocytes rapidly decreased and the labeled purine base was incorporated within 2 min. The decrease in [8-^{14}C]hypoxanthine concentration in the suspending medium was proportional to the preincubation time at a fixed $[P_i]$ and to $[P_i]$ at a fixed preincubation time (fig.2).

The results presented above lead to the following conclusions:

1. Hypoxanthine is incorporated in human erythrocytes by reacting with intracellular PRPP with formation of IMP.
2. At least two steps can be separated in hypoxanthine uptake. A $[P_i]$-dependent slow step in which PRPP is synthesized and a rapid step in which PRPP reacts with hypoxanthine forming IMP.

References

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