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INHIBITION OF OROTATE PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE-5′-PHOSPHATE DECARBOXYLASE OF HUMAN ERYTHROCYTES BY PURINE AND PYRIMIDINE NUCLEOTIDES

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Absract—Orotidine-5′-phosphate decarboxylase of human hemolysates exhibits triphasic kinetics with K_m values of 33, 1.7 and 0.082 μM. Inhibition of this enzyme at low OMP concentrations (< 3 μM) by several naturally occurring purine and pyrimidine nucleotides was investigated. No significant inhibition was observed with IMP, GMP, TMP, ADP, and TTP at 5 mM. Inhibition constants for CMP, AMP, and dAMP were 31 μM, 0.11 mM and 0.21 mM, respectively. The results are discussed in relation to inhibition by nucleotides of orotate phosphoribosyltransferase, previously measured with a method which depends on orotidine-5′-phosphate decarboxylase activity.

INTRODUCTION

Orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate decarboxylase (EC 4.1.1.23) catalyze the conversion of orotic acid to UMP in *de novo* pyrimidine biosynthesis:

$$\text{orotate} + \text{PRPP} \overset{Mg^{2+}}{\rightleftharpoons} \text{OMP} + PP_i$$
$$\text{OMP} \longrightarrow \text{UMP} + CO_2.$$

ODC activity is inhibited by its product UMP [1–3] and by ribonucleotides of several purine and pyrimidine analogues, e.g. azauridine [4, 5]; allopurinol [3, 5–7] and oxipurinol [5–7]. Conflicting evidence has been presented with respect to inhibition of ODC by naturally occurring nucleotides (see Discussion). We demonstrated that OPRT activity in human hemolysates is also inhibited by its product, OMP. The inhibition was competitive with respect to PRPP [7]. Furthermore, several purine and pyrimidine nucleotides appeared to be inhibitors of OPRT activity without affecting ODC activity [7]. Traut and Jones [8] have criticized this latter conclusion. Since OPRT activity was assayed by measuring the release of $^{14}CO_2$ from [carboxyl—^{14}C]-orotic acid, the assay was dependent on the ODC activity, and inhibition of ODC activity would be mistakenly interpreted as inhibition of OPRT activity. Prior to testing the effects of nucleotides on OPRT activity their non-interference with the ODC reaction was ascertained at 0.1 mM OMP [7]. During the OPRT assay OMP concentration is however much lower and inhibition of ODC by nucleotides might be significant [8]. We therefore decided to investigate in detail the inhibition of ODC activity in human hemolysates by purine and pyrimidine nucleotides at low concentrations of OMP (< 3 μM). Special attention was given to the possibility that derivatives of adenosine (AMP, ADP, ATP, dAMP) inhibit the conversion of orotic acid to UMP. Inhibition of OPRT [9] or ODC [8] by AMP has been suggested as the mechanism responsible for inhibition of cell growth by adenosine. Deficiencies of enzymes of adenosine catabolism (adenosine deaminase deficiency [10] and purine nucleoside phosphorylase deficiency [11]) are associated with immunodeficiencies.

MATERIALS AND METHODS

The procedures for measuring the activities of OPRT and ODC in human hemolysates using carboxyl—^{14}C-labeled substrates were described before [7]. Freshly prepared hemolysates were used for all experiments. ODC was assayed at 0.2–3 μM substrate concentration with [carboxyl—^{14}C]orotidine-5′-monophosphate of high specific activity (37 mCi/m-mol). All inhibitors tested were present at a final concentration of 5 mM except for CMP which was present at 2.5 mM. Care was taken to maintain the pH at 7.4 when nucleotides were present. Radioactivity in samples of [carboxyl—^{14}C]orotic acid and [carboxyl—^{14}C]orotidine-5′-monophosphate was measured in the presence of 0.2 ml of hydroxide of Hyamine (Packard) to trap any $^{14}CO_2$ released into the scintillation fluid.

RESULTS

At OMP concentrations below 3 μM a K_m value of 0.082 ±0.024 μM (mean ± S.D., 8 determinations) was found. All compounds previously reported as inhibitors of OPRT in hemolysates [7], and in addition dAMP, were tested for inhibition of ODC at 2 μM OMP (Table 1). Only CMP, AMP and dAMP caused a significant inhibition, which was competitive with respect to OMP. Figure 1 illustrates the inhibition by CMP. The K_i value for CMP was lower than for AMP

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Abbreviations used: OPRT: orotate phosphoribosyltransferase; ODC: orotidine-5′-phosphate decarboxylase; PRPP: phosphoribosylpyrophosphate; and OMP: orotidine-5′-phosphate.

Table 1. Inhibition of erythrocyte orotidine-5′-phosphate decarboxylase at low concentration of OMP

Competitive inhibitor	K_i value
CMP	31 ± 4 μM
AMP	0.11 ± 0.03 mM
dAMP	0.21 ± 0.04 mM

The OMP concentration ranged from 0.2–3 μM. The K_m value for OMP was 0.082 ± 0.024 μM (mean ± S.D. of 8 determinations). K_i values are the mean ± S.D. of 3–6 determinations. The following compounds were not inhibitory when tested at a final concentration of 5 mM and an OMP concentration of 2 μM; GMP, IMP, TMP, ADP, ATP, TTP.

or dAMP (Table 1). PRPP at 1 mM did not affect the activity of ODC or the inhibition of ODC by CMP, AMP, and dAMP.

At a high concentration of PRPP (0.8 mM) OPRT activity was inhibited by TTP but not by AMP, ADP, IMP or TMP and only slightly (< 15 per cent) by ATP or GMP. At a non-saturating concentration of PRPP (0.2 mM) AMP, ADP, ATP, IMP, GMP, TMP and TTP inhibited OPRT activity as earlier reported [7]. The amount of $^{14}CO_2$ measured in the assay of OPRT activity with [carboxyl–^{14}C]orotic acid could not be increased under these inhibitory conditions by a prolongation of the second incubation period after injection of EDTA. Since EDTA injection only stops the OPRT reaction by complexing the Mg^{2+} ions, but does not affect ODC activity, a prolongation of this second incubation period would relieve inhibition of ODC activity, which could decrease $^{14}CO_2$ production. This was not the case, which indicates that the inhibition is on the level of OPRT, not on that of ODC.

DISCUSSION

Previously we found bimodal kinetics for the ODC enzyme in human hemolysates, with K_m values of 33 and 1.7 μM [7]. At low concentrations of OMP (< 3 μM) we now found a third K_m value of 0.082 μM. Brown *et al.* [12] demonstrated that purified ODC from human erythrocytes exists in three forms, corresponding to monomer, dimer and tetramer, with K_m values of 25, 3 and 0.6 μM, respectively. It appears therefore that the K_m values of 33, 1.7 and 0.082 μM measured in crude hemolysates correspond to monomer, dimer and tetramer forms of the enzyme. The difference in K_m values between purified enzyme and crude hemolysate may be related to the purification procedure. The enzyme from human liver was also found to exist in monomer, dimer and tetramer forms [13]. The kinetic properties of the individual forms appeared identical, possibly as a result of interconversion during the assay. The K_m value was 0.12 μM [13]. Fyfe *et al.* [6] found biphasic kinetics for the ODC enzyme from rat liver with $K_m$values of 4 and 1 μM. Two K_m values (4.5 and 0.33 μM) were also reported for the ODC enzyme from human fibroblasts [14].

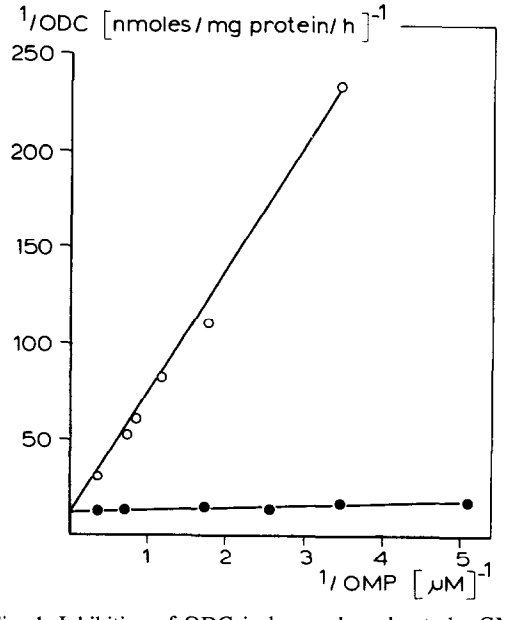

Fig. 1. Inhibition of ODC in human hemolysate by CMP. Control (●), 2.5 × 10^{-3} M CMP (○).

Inhibition of ODC activity by CMP was also observed for the enzyme from cow brain [2] and human liver [13] with K_i values of 0.14 mM and 40 μM, respectively. AMP and GMP were also effective inhibitors of cow brain ODC when present at 5 mM [2]. ODC from human hemolysate was not inhibited by IMP or GMP and only slightly by AMP at 5 μM OMP [3]. The tetramer form of ODC, purified from human hemolysate by Brown and O'Sullivan [5] was inhibited by less than 10 per cent by 5 μM AMP, ADP, ATP, GMP, TMP or CMP at 0.5 μM OMP. The results of inhibition studies on the OPRT/ODC enzyme complex from Ehrlich ascites cells [8] seem to be completely opposite to our data. AMP, ADP, IMP, TMP and TTP were found to have little effect on the OPRT activity while being inhibitors of the ODC activity. The conditions used in these experiments, however, were markedly different from the ones we applied. ODC activity was measured at 0.1 μM OMP which is much lower than the K_m value (0.8 μM) of the enzyme preparation used [15] while OPRT activity was assayed at a saturating concentration of PRPP. Inhibition of OPRT from human hemolysate is, however, competitive with PRPP for all inhibitors except TTP as was stated before [7] and confirmed in the present investigation. Inhibition of ODC activity cannot be the cause of the measured decrease in $^{14}CO_2$ production from [carboxyl–^{14}C]-orotic acid in the presence of purine and pyrimidine nucleotides, as suggested by Traut and Jones [8].

The K_i values with respect to ODC activity, measured for the naturally occuring necleotides CMP, AMP and dAMP are high both in comparison to their physiological concentration and in comparison to the K_m value for OMP. Thus, the inhibition of ODC by these nucleotides does not seem to be of physiological significance. The intracellular concentration of PRPP is well below the K_m value of OPRT for this compound [16]. Inhibition of OPRT which is competitive with respect to PRPP may be important for the *in vivo* regulation of pyrimidine biosynthesis and deserves further investigation.

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REFERENCES

1. W. A. Creasey and R. E. Handschumacher, *J. biol. Chem.* **236**, 2058 (1961).
2. S. H. Appel, *J. biol. Chem* **243**, 3924 (1968).
3. W. N. Kelley and T. D. Beardmore, *Science, N. Y.* **169**, 388 (1970).
4. R. E. Handschumacher, *J. biol. Chem.* **235**, 2917 (1960).
5. G. K. Brown and W. J. O'Sullivan, *Biochem. Pharmac.* **26**, 1947 (1977).
6. J. A. Fyfe, R. L. Miller and T. A. Krenitsky, *J. biol. Chem* **248**, 3801 (1973).
7. W. J. M. Tax, J. H. Veerkamp, J. M. F. Trijbels and E. D. A. M. Schretlen, *Biochem. Pharmac.* **25**, 2025 (1976).
8. T. W. Traut and M. E. Jones, *Biochem. Pharmac.* **26**, 2291 (1977).
9. K. Ishii and H. Green, *J. Cell Sci.* **13**, 429 (1973).
10. E. R. Giblett, J. E. Anderson, F. Cohen, B. Pollara and H. J. Meuwissen, *Lancet* II, 1067 (1972).
11. E. R. Giblett, A. J. Amman, D. W. Wara, R. Sandman and L. K. Diamond, *Lancet* I, 1010 (1975).
12. G. K. Brown, R. M. Fox and W. J. O'Sullivan, *J. biol. Chem,* **250**, 7352 (1975).
13. M. T. Campbell, N. D. Gallagher and W. J. O'Sullivan, *Biochem. Med.* **17**, 128 (1977).
14. T. E. Worthy, W. Grobner and W. N. Kelley, *Proc. natn. Acad. Sci U.S.A.* **71**, 3031 (1974).
15. P. R. Kavipurapu and M. E. Jones, *J. biol. Chem.* **251**, 5589 (1976).
16. I. H. Fox and W. N. Kelley, *Ann. intern. Med.* **74**, 424 (1971).