The exceptional properties of Plasmodium deoxyguanylate pathways as a potential area for metabolic and drug discovery studies

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ABSTRACT

In Plasmodium falciparum, deoxyguanylate was found to be a substrate for several DNA metabolizing enzymes. Guanylate kinase utilizes dGMP with very low specificity, which is estimated to be the lowest among well-known prokaryotic and eukaryotic enzymes. Furthermore, thymidylate kinase, which is a pyrimidine specific enzyme, was found to phosphorylate dGMP with a surprisingly high specificity similar to that of the natural substrate. The above mentioned distinctions are specific for the Plasmodium protozoa and provide an interesting method for tracking dGMP metabolism during development and a starting point for drug development.

INTRODUCTION

Plasmodium falciparum is a virulent parasite that causes severe illness and high mortality. Lack of effective vaccination and the spread of drug resistance have necessitated the search for new drug targets. For developing antimalarial drugs, it may be preferable to select targets from pathways that are present in the parasite but absent in humans; nevertheless, even if the target is common to the parasite and its host, slight structural differences can be exploited in the development of new drugs.

Rational drug development starts with the identification of new molecular targets critical for parasite life. Enzymes of nucleic acid metabolism are intriguing candidates due to their indispensable biological role; nucleoside monophosphate kinases, which play an important role in the synthesis of DNA and RNA precursor nucleotides, are of particular interest. Guanylate kinase (GK, ATP: GMP phosphotransferase, guanosine monophosphate kinase, EC 2.7.4.8) belongs to the nucleoside monophosphate kinase superfamily and is critical for the synthesis of GTP/dGTP since it catalyzes the reversible phosphorylation of GMP/dGMP to its diphosphate form GDP/dGDP. Thymidylate kinase (TMK, ATP:TMP phosphotransferase, dTMP kinase, deoxythymidine monophosphate kinase, EC 2.7.4.9) belongs to the nucleoside monophosphate kinase superfamily and is critical for the synthesis of TTP. It catalyzes the reversible phosphorylation of dTMP to its diphosphate form, dTDP.

Viral thymidine kinases (TKs) have unusual substrate specificity and differ from the corresponding human enzyme; therefore, they are important targets for chemotherapy. Several viral thymidine kinases have the ability to bind purine nucleosides; thus, this is an attractive finding from the perspective of drug discovery. Similarly, we show in this paper the unusual catalytic properties of guanylate and thymidylate kinases from Plasmodium falciparum. These findings constitute a starting point for drug development as well as for further investigation of the metabolic pathways in parasitic protozoa.

RESULTS AND DISCUSSION

P. falciparum guanylate kinase efficiently uses GMP; however, the catalytic efficiency for dGMP is much lower than known guanylate kinases. Compared with the human enzyme, PfTMK has a broader spectrum of substrate specificity (Fig. 1). The enzyme not only phosphorylates dTMP and dUMP but can also tolerate the bulkier purines. Thus, PfTMK is not a dual function thymidylate/guanylate kinase enzyme; however, the differences in the catalytic properties for dGMP kinase activity provide an interesting contribution to the knowledge of nucleic acid metabolism in P. falciparum.

In human TKM, the cavity in which bases bind is very tight to accommodate purine bases, and this consequently reduces the specificity to pyrimidines. Thus, the parasite enzyme can be targeted by the development of highly selective purine-based inhibitors. The most striking and unique feature of PFTMK is its ability to utilize the purine nucleosides GMP, dGMP and dTMP. The specific activities for GMP and dTMP were as low as 0.12 and 2.2 U/mg, respectively. However, the enzyme can utilize dGMP with very high efficiency, quite similarly to dTMP.

The specific activity of PfGK was 750 U/mg for GMP and unexpectedly, it was as low as 30 U/mg for dGMP. The extremely low reactivity of PfGK toward dGMP is a unique feature among known GKS. Under the normal assay conditions of 200 ng PfGK used for GMP, the activity for dGMP was completely non-detectable, contrary to many GKS from other sources, which have similar or only slightly lower reaction rates.
The $K_m$ values were 22 and 74 $\mu$M for GMP and dGMP, respectively. Surprisingly, the catalytic efficiency for dGMP was not comparable to that for GMP. The $K_{cat}$ for dGMP was 22-fold lower than that for GMP. This value appears to be the lowest among known GKS (Table 1).

**Table 1** Summary of kinetic properties of wild-type PfGK. The substrate concentration of GMP was 0.002–1 mM, while that of dGMP was 0.01–5 mM.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{cat}$ (GMP) (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (GMP) (s$^{-1}$$\mu$M$^{-1}$)</th>
<th>$k_{cat}$ (dGMP) (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (dGMP) (s$^{-1}$$\mu$M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>946</td>
<td>42</td>
<td>43</td>
<td>0.58</td>
</tr>
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</table>

A decrease in kinase activity was observed in the presence of dGMP, a poor substrate for PfGK. The $K_i$ value of inhibition was calculated to be 0.148 mM. Inhibition data indicated that dGMP is a competitive inhibitor of GMP. Furthermore, dGMP was found to be a mixed-type inhibitor of ATP with a measured $K_i$ value of 0.4 mM.

GK was found to react with GMP and dGMP with nearly the same efficiency in calf thymus, human erythrocyte, hog brain and rat liver, although the reactivity only doubled in the case of bovine eye GK. In *Arabidopsis thaliana*, the $K_{cat}$ for GMP was ~3-fold higher than that for dGMP, while the yeast enzyme had a $K_{cat}$ that was ~5-fold higher.

*Mycobacterium* GK accepted dGMP as a substrate at a catalytic rate that was 8% of the rate for GMP and *E. coli* GK had a rate for dGMP that was 30% of the rate for GMP, while this ratio was 50% in the case of human GK. Accordingly, PfGK has an unusually poor ability to phosphorylate dGMP compared with GKS in mammals or lower organisms.

**CONCLUSION**

In summary, dGMP is a poor substrate for *Plasmodium* guanylate kinase and is a very good substrate for *Plasmodium* guanylate kinase. Both of these properties are unique to *Plasmodium* and in contrast with the enzymes in human and other parasites. In conclusion, the unique properties of *Plasmodium* deoxyguanylate pathways are an attractive area of investigation in future metabolic and drug discovery studies.

**REFERENCES**


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