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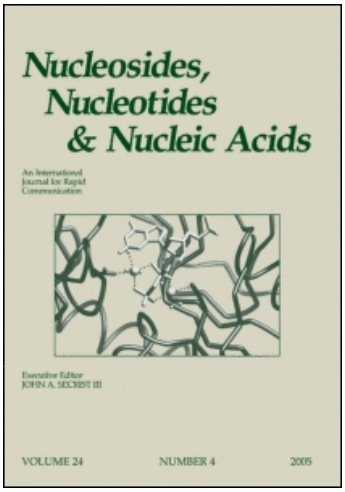

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Interactions of Trimeric Purine Nucleoside Phosphorylases with Ground State Analogues—Calorimetric and Fluorimetric Studies

Beata Wielgus-Kutrowska[a]; Joachim Frank[b]; Antonin Holý[c]; Gertraud Koellner[d]; Agnieszka Bzowska[ae]
[a] Department of Biophysics, University of Warsaw, Institute of Experimental Physics, Warsaw, Poland [b] Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin, Germany [c] Academy of Sciences of the Czech Republic, Institute of Organic Chemistry and Biochemistry, Czech Republic [d] Freie Universität Berlin, Institut für Chemie-Kristallographie, Berlin, Germany [e] Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland

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Interactions of Trimeric Purine Nucleoside Phosphorylases with Ground State Analogues—Calorimetric and Fluorimetric Studies

Beata Wielgus-Kutrowska,[1] Joachim Frank,[2] Antonin Holý,[3] Gertraud Koellner,[4] and Agnieszka Bzowska[1,*]

[1]Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland
[2]Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin, Germany
[3]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Czech Republic
[4]Institut für Chemie-Kristallographie, Freie Universität Berlin, Berlin, Germany

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ABSTRACT

Binding enthalpies, dissociation constants and stoichiometry of binding for interaction of trimeric calf spleen and *Cellulomonas* sp. purine nucleoside phosphorylases with their ground state analogues (substrates and inhibitors) were studied by calorimetric and spectrofluorimetric methods. Data for all ligands, with possible exception of hypoxanthine, are consistent with three identical non-interacting binding sites.

Key Words: Purine nucleoside phosphorylase; Isothermal titration calorimetry; Fluorescence; Mechanism; Stoichiometry; Dissociation constants.

*Correspondence: Agnieszka Bzowska, Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Zwirki i Wigury 93, 02 089 Warsaw, Poland; Fax: +48 22 554 0771; E-mail: abzowska@biogeo.uw.edu.pl.

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INTRODUCTION

Ubiquitous purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1.) catalyzes the reversible phosphorolysis of purine nucleosides, as follows: β-purine nucleoside + orthophosphate ⇔ purine base + α-D-pentose-1-phosphate. Two families of PNPs with different specificity were characterized: homotrimers and homohexamers.[1] PNP is of interest as a drug target because of its role in some immunological diseases and in the intracellular degradation of some antitumor and antiviral drugs. Recently antitumor gene therapy has profited from some PNP substrates specific for the *E. coli* enzyme.[1] Details of the catalytic mechanism of the reaction catalyzed by PNPs are not yet explored. PNPs show complex kinetic characteristic. Deviations form Michaelis-Menten kinetics is observed with some substrates, e.g., with phosphate as variable substrate, suggesting negative cooperativity or different non-interacting sites.[1] For hexameric *E. coli* enzyme X-ray and solution studies have shown that binding of phosphate and nucleoside is associated with negative cooperativity.[2] By contrast, for trimeric PNPs negative cooperativity was documented only for transition state events, e.g., for the calf spleen enzyme third-the-sites-binding of transition state inhibitors – immucillins was observed, and of hypoxanthine only in the absence of phosphate.[3,4]

RESULTS AND DISCUSSION

Here we describe interactions of several ground state analogue ligands with trimeric PNPs from calf spleen and *Cellulomonas* sp., studied by calorimetric and fluorimetric titrations. The studies were conducted for several substrates, guanine (Gua), hypoxanthine (Hx) and phosphate (P_i), as well as some inhibitors, namely acyclic nucleoside phosphonates, 2,6-diamino-(S)-9-[2-(phosphonomethoxy)propyl]-purine ((S)-PMPDAP), 2-amino-6-oxo-9-[2-(phosphonomethoxy)ethyl]-8-azapurine (PME-8-azaGua) and 2-amino-9-[2-(phosphonomethoxy)ethyl]-6-sulfanylpurine (PME-6-thioGua),[5] known to act as multisubstrate analogue inhibitors.[6]

The titrations conducted in the present study using both fluorimetric and calorimetric methods, although involve quite different enzyme concentrations (as monomer 0.5–5.0 μM and 90–300 μM, respectively), are sufficiently described by the one-binding site model[7] for all ligands studied. Stoichiometry of binding, n, was found in most cases (with possible exception of Hx, see below) to be consistent with three ligand molecules bound per enzyme trimer. For example stoichiometry for P_i, the ligand most often suggested to bind cooperatively with trimeric PNPs,[1] was found (by calorimetric titration) to be $n = 2.8 \pm 0.7$ for the calf spleen enzyme. Hence our data for the calf and *Cellulomonas* PNPs are consistent with three identical non-interacting binding sites, in contrast to previous preliminary fluorimetric studies for the calf enzyme for these and similar multisubstrate analogue inhibitors.[8]

The only exception in the present study is Hx for which stoichiometry slightly lower than 3 was determined for both calf (calorimetric studies) and *Cellulomonas* (fluorimetric studies) enzymes. In the absence of P_i $n = 2.0 \pm 0.3$ and 2.2 ± 0.6 for calf and *Cellulomonas* PNPs, respectively, and for calf PNP and P_i in the range

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0.37–10 mM, $n = 1.9 \pm 0.3$. Although, in contrast to Kline & Schramm,[4] third-the-sites-binding of Hx in the absence of P_i was not observed, Hx was found to be an unusual ligand not only because of stoichiometry close to 2 molecules per enzyme trimer. In addition enthalpy associated with its binding to calf PNP is about $\Delta H = 60$ kJ/mol, as compared with $\Delta H = 10$, 25 and 21 kJ/mol for binding of P_i, (S)-PMPDAP and PME-8-azaGua, respectively.

Dissociation constants K_d for both enzymes and all ligands studied, except that for P_i, were in the range of about 0.5 μM to 5 μM. For P_i (calf spleen enzyme, calorimetric titrations) less potent binding was observed, $K_d = 71 \pm 26$ μM, in fairly good agreement with previous fluorimetric titrations.[9] Binding of Gua and Hx to both enzymes was less potent in the presence of P_i. For example, $K_d = 0.46 \pm 0.08$ μM and 1.7 ± 0.2 μM for Gua and *Cellulomonas* PNP were obtained in fluorimetric titrations, and $K_d = 0.49 \pm 0.36$ μM and 2.0 ± 0.7 μM for Hx and calf PNP were determined in calorimetric experiments, in the absence and in the presence of P_i, respectively.

CONCLUSIONS

Ligands studied (Gua, P_i, acyclic nucleoside phosphonates) bind uniformly to all three sites of the calf and *Cellulomonas* PNPs, in contrast to the transition state inhibitors – immucillins, and possibly Hx. These results support hypothesis that non-Michaelis kinetics of trimeric PNPs is not caused by cooperative interactions between enzyme subunits or by presence of different non-interacting sites but rather by complex kinetic mechanism of the phosphorolytic reaction involving random substrate binding, unusually slow and hence strongly rate limiting dissociation of some products (Gua, Hx), and dual function of P_i acting as a substrate and as a modifier[9] (see also two related papers in this volume).

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REFERENCES

1. Bzowska, A.; Kulikowska, E.; Shugar, D. Purine nucleoside phosphorylases: properties, functions, and clinical aspects. Pharmacol. & Therapeut. **2000**, *88*, 349–425.
2. Koellner, G.; Bzowska, A.; Wielgus-Kutrowska, B.; Luić, M.; Steiner, T.; Saenger, W.; Stępiński, J. Open and closed conformation of the E. coli purine nucleoside phosphorylase active center and implications for the catalytic mechanism. J. Mol. Biol. **2002**, *315*, 351–371.

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3. Wang, F.; Miles, R.W.; Kicska, G.; Nieves, E.; Schramm, V.L.; Angeletti, R.H. Immucillin-H binding to purine nucleoside phosphorylase reduces dynamic solvent exchange. Protein Sci. **2000**, *9*, 1660–1668.
4. Kline, P.C.; Schramm, V.L. Purine nucleoside phosphorylase. Inosine hydrolysis, tight binding of the hypoxanthine intermediate, and third-the-sites reactivity. Biochemistry **1992**, *31*, 5964–5973.
5. Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Structure-antiviral activity relationship in the series of pyrimidine and purine N-[2-(2-phosphonomethoxy)ethyl] nucleotide analogues. 1. Derivatives substituted at the carbon atoms of the base. J. Med. Chem. **1999**, *42*, 2064–2086.
6. Kulikowska, E.; Bzowska, A.; Holý, A.; Magnowska, L.; Shugar, D. Antiviral acyclic nucleoside phosphonate analogues as inhibitors of purine nucleoside phosphorylase. Adv. Exp. Med. Biol. **1998**, *431*, 747–752.
7. Eftink, M.R. Fluorescence methods for studying equilibrium macromolecule-ligand interactions. Methods Enzymol. **1997**, *278*, 221–257.
8. Wierzchowski, J.; Kulikowska, E.; Bzowska, A.; Holý, A.; Magnowska, L.; Shugar, D. Interactions of calf spleen purine nucleoside phosphorylase with antiviral acyclic nucleoside phosphonate inhibitors: kinetics and emission studies. Nucleosides and Nucleotides **1999**, *18*, 875–876.
9. Bzowska, A. Calf spleen purine nucleoside phosphorylase: complex kinetic mechanism, hydrolysis of 7-methylguanosine, and oligomeric state in solution. Biochim. Biophys. Acta **2002**, *1596*, 293–317.

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