Purine Nucleoside Phosphorylase, a multiple-faced enzyme

Catalytic activity

Purine Nucleoside Phosphorylase (PNP) is involved in salvage pathway of the purine metabolism. PNP catalyzes the cleavage of the glycosidic bond of ribo- or deoxyribonucleosides, in the presence of inorganic phosphate as a second substrate, to generate the purine base and ribose- or deoxyribose-1-phosphate. The reaction is reversible for natural substrates:

\[
\text{Purine nucleoside} + \text{Pi} \rightleftharpoons \text{Purine base} + (\text{deoxy})ribose-1\text{P}
\]

Therapeutic potential of PNP inhibitors

PNP deficiency leads to T-lymphocytopenia, usually with no apparent effects on B-lymphocyte function. These symptoms suggest possible chemotherapeutic applications of potent inhibitors of PNP, as selective immunosuppressive agents, to treat T-cell leukemias or T-cell-mediated autoimmune diseases, such as lupus erythematosus and rheumatoid arthritis. The decrease in plasma and urine levels of urate is an additional symptom of PNP deficiency. PNP inhibitors may contribute to treat hyperuricemic states, such as secondary or xanthine gout.

Some PNP inhibitors have undergone clinical trials for the treatment of cutaneous T-cell lymphoma, acute lymphoblastic leukemia (ALL), HIV infections, and psoriasis.

Peldesine (BCX-34) was granted orphan drug status for the treatment of T-cell lymphoma and reached phase III as an immunomodulator for inflammatory autoimmune diseases. It has recently entered clinical trial for HIV infections.

Forodesine (BCX-1777) has US orphan drug status for the treatment of T-cell non-Hodgkin's lymphoma, including cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukemia (CLL) and related leukemias, including T-cell prolymphocytic leukemia (PLL), adult T-cell leukemia and hairy cell leukemia, and for the treatment of acute lymphocytic leukemia (ALL). Forodesine was also designed Orphan drug in Europe for ALL in December 2006, and for CTCL in February 2007.

PNP inhibitors are also investigated to prevent the cleavage, and the resulting deactivation of Nucleoside Analogues by PNP.

Note: Protozoan parasites are auxotrophic for purine and have their own PNPs which have specific activities and properties that differ from the human PNP. Protozoan parasites PNPs are considered to be reasonable target against infection (e.g. Plasmodium falciparum).

PNP, a threat for therapeutic efficacy of Nucleoside Analogues

In vivo, phosphorylation is highly favoured over purine nucleoside synthesis and is coupled with two additional enzymatic reactions: oxidation of the liberated purine base by xanthine oxidase (XO) and its phosphorolysis by hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Thus, PNP plays a key role in the salvage pathway of the purine metabolism, enabling the cell to utilize purine bases recovered from metabolized purine ribo- and deoxyribonucleosides to synthesize purine nucleotides.

This phosphorylation reaction of purine nucleosides by PNP has a direct impact on the therapeutic efficacy of Nucleoside Analogues. Antitumour or antiviral nucleoside analogues are likely to be cleaved by PNP before being phosphorylated by the cell nucleoside kinases and converted to the active nucleotide form. For instance, 2’,3’-dideoxyguanosine (ddG), 9-β-D-arabinofuranosyl guanine (araG) as well as one of its produg, Nelarabine (Arranon®, GSK®), which is intracellularly converted to AraG by Adenosine deaminase (ADA), are PNP resistant nucleoside analogues, whereas 2’,3’-dideoxynosine (ddI) is easily cleaved in vivo by PNP.

Since acyclonucleoside analogues are particularly resistant to cleavage by PNP though phosphorylated by viral thymidine kinases (TK), they are generally considered as excellent candidates as antiviral agents (e.g. aciclovir, ganciclovir). Note that Ganciclovir is not only PNP resistant, but is also a PNP inhibitor.

PNP, a tool for enzymatic synthesis of Nucleoside Analogues

The reversible reaction catalyzed by PNP can be favorably exploited to synthesize nucleoside analogues, especially when chemical synthesis is difficult to prepare and / or gives low yields.

(coming soon, “Transribosylation by PNP”)

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8. L. C. Gravatt et al. (1993): Efficacy and toxicity of 9-β-D-arabinofuranosylguanine (araG) as an agent to purge malignant T-cells from murine bone marrow: application to an in vivo T-cell leukemia model. Leukemia 7(8), 1261-1267
PNP - Purine Nucleoside Phosphorylase

*Human, recombinant expressed in E. coli*

E.C. 2.4.2.1

**Description**

*Metabolic function*

Purine Nucleoside Phosphorylase (PNP) is involved in salvage pathway of the purine metabolism.

*Catalytic activity*

PNP catalyzes the cleavage of the glycosidic bond of ribo- or deoxyribonucleosides, in the presence of inorganic phosphate as a second substrate, to generate the purine base and ribose- or deoxyribose-1-phosphate. The reaction is reversible for natural substrates:

\[
\text{Purine nucleoside} + \text{Pi} \rightleftharpoons \text{Purine base} + \text{(deoxy)ribose-1P}
\]

NOVO CIB’s PNP is a human recombinant Purine Nucleoside Phosphorylase expressed in *E. coli*. It has an apparent molecular weight of 32.12 kDa.

**Interests**

**PNP inhibition**

Several PNP inhibitors have been developed to treat cancer, viral infection and auto-immune diseases. (see sheet # IVS-Nov2, "PNP Inhibition - In vitro Screening Assay" for further details)

**PNP, a threat for therapeutic efficacy of Nucleoside Analouges**

PNP’s activity in vivo can be responsible for the cleavage and the subsequent deactivation of Nucleoside Analouges, thus unable to be phosphorylated by nucleoside kinases. The resistance to cleavage by PNP is worth being investigated to increase the therapeutic efficacy of Nucleoside Analouges. (see sheet # NCR-Nov2, "PNP Cleavage activity - Nucleoside Resistance Assay" for further details)

**PNP, a tool for enzymatic synthesis of Nucleoside Analouges**

PNP can be exploited for the reversible reaction that it catalyzes to synthesize nucleoside analogues, for instance with potential antiviral and antineoplastic activities, especially when chemical synthesis is difficult to prepare and / or gives low yields. (coming soon, "Transribosylation by PNP" for further details)

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**NOVO CIB** has cloned and purified a human recombinant Purine Nucleoside Phosphorylase (PNP) and has developed a range of related PRECICE® services.

**PNP Services**

- **PNP Inhibition - In Vitro Screening Assay**
- **PNP Cleavage activity - Nucleoside Resistance Assay**
- (coming soon) Transribosylation by PNP
**PNP Inhibition - In vitro Screening Assay**

**Aim:** To screen compounds for their abilities to inhibit human PNP in vitro.

**Therapeutic potential of PNP inhibitors**

PNP deficiency leads to T-lymphocytopenia, usually with no apparent effects on B-lymphocyte function. These symptoms suggest possible chemotherapeutic applications of potent inhibitors of PNP, as selective immunosuppressive agents, to treat T-cell leukemias or T-cell-mediated autoimmune diseases, such as lupus erythematosus and rheumatoid arthritis. The decrease in plasma and urine levels of urate is an additional symptom of PNP deficiency. PNP inhibitors may contribute to treat hyperuricemic states, such as secondary or xanthine gout.

Some PNP inhibitors have undergone clinical trials for the treatment of cutaneous T-cell lymphoma, acute lymphoblastic leukemia (ALL), HIV infections, and psoriasis.

Peldesine (BCX-34) was granted orphan drug status for the treatment of T-cell lymphoma and reached phase III as an immunomodulator for inflammatory autoimmune diseases. Forodesine (BCX-1777) has US orphan drug status for the treatment of non-Hodgkin's lymphoma, cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukaemia (CLL) and related leukemias, including prolymphocytic leukaemia (PLL), adult T-cell leukaemia, hairy cell leukaemia and acute lymphocytic leukaemia (ALL). In December 2006, Forodesine was designed Orphan drug in Europe for ALL.

PNP inhibitors are also investigated to prevent the cleavage, and the resulting deactivation of Nucleoside Analogues by PNP. (see sheet # NCR-Nov2, "PNP Cleavage activity - Nucleoside Resistance Assay" for further details)

**Note:** Protozoan parasites are auxotrophic for purine and have their own PNPs which have specific activities and properties that differ from the human PNP. Protozoan parasites PNPs are considered to be reasonable target against infection (e.g. Plasmodium falciparum).

**Description of the In vitro screening assay**

**PNP enzyme** used in the assay is a human recombinant PNP, cloned by NOVOCIB from human cells, expressed in E. coli, and produced and purified in NOVOCIB's laboratory. (see sheet # E-Nov 2 for further information) PNP purification is controlled before every assay by SDS-PAGE. Protein concentration is measured by Bradford method. PNP specific activity is then determined - 1 unit of PNP catalyzes the cleavage of 1 μmole of inosine per minute at pH 8.0 at 25°C.

**Procedure**

NOVOCIB has developed a spectrophotometric procedure to directly follow the phosphorolytic reaction on inosine (IR). The assays are performed at 25°C or 37°C in 200μl o f reaction buffer on 96-well microplate. Pipetting is done by a Multiprobe II Robotic Liquid Handling System (Packard BioScience). Ganciclovir is used as positive control for PNP inhibition. Replicate assays: One point is defined as a well per compound and per concentration. Both negative and positive controls are done in duplicate. Triplicates are available upon request.

Every assay, from one to 30 points, is done with one negative control, i.e. without inhibitor, and two positive controls containing Ganciclovir as a PNP inhibitor. Controls are done in duplicate. If an additional microplate is needed, it includes the three controls (in duplicate, i.e. 6 wells).

**Confirmation by HPLC:**

For every positive assay, an HPLC (Agilent 1100 series) control of PNP inhibition is performed by measuring inosine (IR) and Hypoxanthine (Hx) concentrations in the assay and in comparison with negative and positive controls.

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**PNP Cleaving activity - Nucleoside Resistance Assay**

**Aim:** To evaluate the resistance of Nucleoside Analogues to cleavage by human PNP.

**Nucleoside Analogues deactivation by PNP**

In vivo, phosphorylisis is highly favoured over purine nucleoside synthesis and is coupled with two additional enzymatic reactions: oxidation of the liberated purine base by xanthine oxidase (XO) and its phosphoribosylation by hypoxanthine-guanine phosphoribosyltransferase (HGPRT)\(^1\). Thus, PNP plays a key role in the salvage pathway of the purine metabolism, enabling the cell to utilize purine bases recovered from metabolized purine ribo- and deoxyribonucleotides to synthesize purine nucleotides.

This phosphorylisis reaction of purine nucleosides by PNP has a direct impact on the therapeutic efficacy of Nucleoside Analogues. Antitumour or antiviral nucleoside analogues are likely to be cleaved by PNP before being phosphorylated by the cell nucleoside kinases and converted to the active nucleotide form. For instance, 2',3'-dideoxyguanosine (ddG)\(^2\), 9-β-D-arabinofuranosyl guanine (AraG)\(^3\) as well as one of its produg, Nelarabine (Arranon\(^4\), GSK)\(^4\), which is intracellularly converted to AraG by Adenosine deaminase (ADA), are PNP resistant nucleoside analogues, whereas 2',3'-dideoxynosine (ddI)\(^5\) is easily cleaved in vivo by PNP.

Since acyclonucleoside analogues are particularly resistant to cleavage by PNP though phosphorylated by viral thymidine kinases (TK), they are generally considered as excellent candidates as antiviral agents (e.g. aciclovir, ganciclovir)\(^6\).

Note that Ganciclovir is not only PNP resistant, but is also a PNP inhibitor.

**NOVOCIB’s has developed a PNP enzymatic assay which consists in evaluating the PNP phosphorylisis activity on Nucleosides Analogues in comparison with natural purine nucleoside substrates.**

**Description of the Nucleoside Resistance assay**

The assays are performed at 25°C or 37°C in 200µl of reaction buffer (lower volumes are available if compound saving is a constraint) on 96-well microplate. Pipetting is done by a Multiprobe\(^®\) II Robotic Liquid Handling System (Packard BioScience).

Inosine is used as a positive control and adenosine as a negative control.

Note that if inosine is a natural substrate of PNP and is consequently actively cleaved by a wide range of PNPs, adenosine resists to Human PNP phosphorolytic activity but is easily cleaved by other PNPs, such as E.coli PNP for instance. This is why we consider as a decisive advantage to evaluate the cleavage resistance of Nucleoside Analogue using Human PNP.

Identification and quantification of the purine nucleosides and the related purine bases produced by Human PNP cleaving activity are performed by HPLC.

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3. L. C. Gravatt et al. (1993): Efficacy and toxicity of 9-β-D-arabinofuranosylguanine (araG) as an agent to purge malignant T-cells from murine bone marrow: application to an in vivo T-cell leukemia model, Leukemia 7(8), 1261-1267