Inhibitory Effects of 7-Methylguanine and Its Metabolite 8-Hydroxy-7-Methylguanine on Human Poly(ADP-Ribose) Polymerase 1

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Abstract—Previously, we have found that a nucleic acid metabolite, 7-methylguanine (7mGua), produced in the body can have an inhibitory effect on the poly(ADP-ribose) polymerase 1 (PARP1) enzyme, an important pharmacological target in anticancer therapy. In this work, using an original method of analysis of PARP1 activity based on monitoring fluorescence anisotropy, we studied inhibitory properties of 7mGua and its metabolite, 8-hydroxy-7-methylguanine (8h7mGua). Both compounds inhibited PARP1 enzymatic activity in a dose-dependent manner, however, 8h7mGua was shown to be a stronger inhibitor. The IC₅₀ values for 8h7mGua at different concentrations of the NAD⁺ substrate were found to be 4 times lower, on average, than those for 7mGua. The more efficient binding of 8h7mGua in the PARP1 active site is explained by the presence of an additional hydrogen bond with the Glu988 catalytic residue. Experimental and computational studies did not reveal the effect of 7mGua and 8h7mGua on the activity of other DNA repair enzymes, indicating selectivity of their inhibitory action.

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INTRODUCTION

7-Methylguanine (7mGua) and 8-hydroxy-7-methylguanine (8h7mGua, Fig. 1) are metabolites of nucleic acids detected in small amounts in human urine [1-6]. In the messenger RNA guanosine is methylated at the 5'-terminal cap structure. This RNA modification is important for regulation of gene expression and is carried out enzymatically [7-9]. In the DNA deoxyguanosine is modified by various exogenous and endogenous methylating

Abbreviations: 7mGua, 7-methylguanine; 8h7mGua, 8-hydroxy-7-methylguanine; AP site, apurinic/apyrimidinic site; APE1, apurinic/apyrimidinic endonuclease 1; PAR, poly(ADP-ribose); PARP1, poly(ADP-ribose) polymerase 1; Polβ, DNA polymerase β; TDP1, tyrosyl-DNA phosphodiesterase 1; TGT, tRNA-guanine transglycosylase.

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Fig. 1. Chemical structures of natural nitrogenous bases 7mGua and 8h7mGua.

agents, and amounts of such adducts are increasing with aging [10-12]. It should be noted that the 7mGua free base is not involved in the synthesis of nucleotides and is not incorporated into DNA [13-15]. A certain amount of 7mGua is converted into 8h7mGua by xanthine oxidase or is demethylated [3, 16, 17]. Thus, after intravenous administration of 5 mg of the ¹⁵N-labeled 7mGua to a healthy volunteer the content of [¹⁵N]7mGua and [¹⁵N]8h7mGua in the daily urine output was 48% and 16% of the injected substance amount, respectively [16].

Virtual screening of the poly(ADP-ribose) polymerase 1 (PARP1) inhibitors conducted by our group revealed potential ability of the 7mGua to bind to the enzyme and interact with the key residues of the active site, Gly863 and Tyr907 [18]. A preliminary study confirmed the inhibitory effect of 7mGua against recombinant human PARP1 [19]. PARP1 is activated in the cell under various stress factors and synthesizes poly(ADP-ribose) (PAR) using NAD⁺ as a substrate [20-22]. Cancer chemotherapy often involves the use of DNA-damaging agents. Activation of the PARP1 enzyme by the drug-induced DNA breaks and formation of the signal PAR polymer recruit DNA repair proteins to the damaged site, which results in the decreased efficiency of the drugs [23-26]. In the cases of pathologies of the cardiovascular, nervous, immune, and respiratory systems, PARP1 activation also occurs leading to the depletion of NAD⁺ pool and triggering mechanisms of cell death [27-29].

The development of PARP1 inhibitors is a current trend in medicinal chemistry and pharmacology. Recently, the synthetic inhibitors olaparib, rucaparib, and niraparib have been approved for cancer treatment [30-32]. Unfortunately, widespread use of these compounds is hindered due to the serious side effects they cause (in particular, development of myelodysplastic syndrome in some patients) [33-35]. Natural compounds such as 7mGua and its derivatives may have a more favorable toxicity profile compared to the synthetic PARP1 inhibitors, as evidenced by QSAR modeling of 7mGua properties [18]. It should be noted that 7mGua is also known as an inhibitor of tRNA-guanine transglycosylase (TGT), which catalyzes replacement of guanine with queuine in tRNA [36, 37]. It has recently been shown that TGT deficiency signifi-

cantly suppresses proliferation and migration of cancer cells [38]. The inhibitory effect of 7mGua on several molecular targets, PARP1 and TGT, may be of interest from the point of view of polypharmacology [39, 40].

In the present work, a detailed analysis of the inhibitory effect of 7mGua and its metabolite 8h7mGua on PARP1 was carried out using the previously developed method for assessing enzyme activity through changes in fluorescence anisotropy of the DNA substrate [41]. To prove selectivity of the effects of 7mGua and 8h7mGua on PARP1, their influence on the enzyme activity of other DNA repair proteins, such as apurinic/apyrimidinic endonuclease 1 (APE1), DNA polymerase β (Pol β), and tyrosyl-DNA phosphodiesterase 1 (TDP1) was also studied.

MATERIALS AND METHODS

7mGua was purchased from Sigma-Aldrich (USA, product No. 67073). Synthesis of 8h7mGua was carried out based on the previously published procedures [42, 43], according to the scheme shown in Fig. 2. A more detailed description is given in the Online Resource 1.

Baculovirus carrying human PARP1 cDNA was kindly provided by Dr. V. Schreiber (École Supérieure de Biotechnologie de Strasbourg, France). Recombinant PARP1 protein was expressed in a suspension culture of Sf9 insect cells and isolated according to the previously described procedure [44]. PARP1 was activated using a DNA duplex formed by oligonucleotides 5'-GGAAGACCCTGACGTTCCCAACTTTATCGCC-FAM-3' (containing FAM fluorophore at the 3'-end), 5'-GGCGATAAAGTTGGG-3', and 5'-p-AACGTCAG-GGTCTTCC-3' (containing 5'-terminal phosphate). This DNA duplex represents a model of damaged DNA.

PARP1 activity was assessed using a method based on monitoring fluorescence anisotropy of the DNA molecule [41]. A reaction mixture included a buffer (50 mM NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM DTT, 5 mM MgCl₂), 100 nM labeled DNA duplex, 200 nM PARP1, and tested compound at a certain concentration. Reaction was initiated by addition of NAD⁺. Fluorescence was measured



Fig. 2. Synthesis of 8h7mGua. Theobromine was used as an initial compound.

at 25°C with a microplate reader CLARIOstar (BMG Labtech, Germany) using kinetic analysis mode, fluorescence excitation wavelength was set at 482 nm, and fluorescence emission was measured at 530 nm. Duration and number of measurement cycles were determined individually depending on the number of scanned wells. In a typical experiment, duration of the fluorescence measurement was 30-40 min, and intervals between the two consecutive measurements were 30-40 s. Anisotropy was calculated using formula:

$$A = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp}), \tag{1}$$

where I_{\parallel} and I_{\perp} – intensities of fluorescent light polarized in two perpendicular planes.

Effects of 7mGua and 8h7mGua on activity of the DNA repair enzymes APE1, Pol β , and TDP1 were studied using the previously described protocols [45-47], the enzymes and their substrates were prepared at the Institute of Chemical Biology and Fundamental Medicine; a more detailed description is given in the Online Resource 1.

Docking of 7mGua and 8h7mGua molecules into the active site of the previously obtained PARP1 model [18] was performed using the Lead Finder 1708 program in an "extra precision" mode [48, 49]. The VMD 1.9 program [50] was used for visualization of the obtained structures. Biological activity spectra of 7mGua and 8h7mGua were predicted using the PASS 2020 program [51]. This software predicts mechanism of action of organic compounds by analyzing structure–activity relationships for a training set containing information on the structures and activities of more than 1.3 million biologically active compounds, with average accuracy of about 97%.

RESULTS AND DISCUSSION

To study the effect of 7mGua and 8h7mGua on PARP1 activity, we used the previously developed assay based on measuring fluorescence anisotropy of the labeled DNA duplex that binds to PARP1 [41]; some modifications were made to the methodology regarding the algorithm for calculating the initial reaction rate. The level of fluorescence anisotropy is related to the size of the complex containing the fluorophore. Anisotropy of the free DNA duplex is minimal, as it is a small, rapidly rotating molecule. Upon formation of the PARP1-DNA complex, a significant increase in the fluorescence anisotropy is observed due to restriction of the fluorophore mobility. Addition of NAD⁺ to the reaction mixture initiates reaction of PARP1 auto-modification. Growth of the negatively charged PAR polymer attached to PARP1 affects association of PARP1 with the DNA duplex and, consequently, leads to the gradual decrease in anisotropy (Fig. 3).

When calculating the initial reaction rate, it was assumed that at least in the initial period of time the change in fluorescence anisotropy relates linearly to concentration of the catalytically active PARP1–DNA complex, and that the dissociated form of PARP1 is catalytically inactive. The kinetic data (anisotropy vs. time) were approximated by a bi-exponential decay equation:

$$A = A_{\infty} + a \cdot \mathrm{e}^{-b \cdot t} + c \cdot \mathrm{e}^{-d \cdot t}, \qquad (2)$$

where t - time; a, b, c, d - coefficients of the equation; $A_{\infty} - \text{calculated}$ value of anisotropy at $t \rightarrow \infty$. The use of a simpler exponential decay equation produced a poorer result (Table S1 in the Online Resourse 1).



Fig. 3. Analysis of the rate of the PARP1-catalyzed reaction by monitoring fluorescence anisotropy.



Fig. 4. Effect of 7mGua (a) and 8h7mGua (b) on the initial rate of the PARP1-catalyzed reaction at different concentrations of the NAD⁺ substrate.

The obtained coefficients *a*, *b*, *c*, and *d* were used to calculate the initial reaction rate according to the formula:

$$V = R \cdot (a \cdot b + c \cdot d) / (A_0 - A_\infty), \qquad (3)$$

where A_0 – calculated value of anisotropy at t = 0.

Since $A_0 = A_{\infty} + a + c$, the reaction rate equation becomes:

$$V = R \cdot (a \cdot b + c \cdot d) / (a + c), \tag{4}$$

where R – coefficient for obtaining the reaction rate values in M/min:

$$R = [\text{NAD}^+]_0 \text{ if } [\text{NAD}^+]_0 / [\text{E}] \le n, \tag{5}$$

$$R = n \cdot [E] \text{ if } [NAD^+]_0 / [E] > n, \qquad (6)$$

where $[NAD^+]_0$ – initial concentration of NAD⁺, [E] – PARP1 concentration, n – experimentally determined minimum value of the ratio $[NAD^+]_0/[E]$ at which $A_0 - A_\infty$ (or a + c) increases to a maximum value (i.e., at which complete dissociation of the PARP1–DNA complex is achieved). Analysis of the dependency of (a + c)on NAD⁺ concentration at constant PARP1 concentration revealed that the average value of n is 210. Apparently, this value corresponds to the critical number of

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ADP-ribose residues in the auto-modified PARP1 molecule, which leads to the complete loss of ability of the enzyme to bind to the DNA duplex.

To calculate IC_{50} (inhibitor concentration at which the initial reaction rate is reduced by 50%), the reaction rates were determined at different concentrations of the potential inhibitor, and then the obtained experimental data were approximated by two-parameter logistic equation:

$$V_{\rm i}/V = 1/(1 + ([{\rm I}]/{\rm IC}_{50})^h),$$
 (7)

where V_i – initial reaction rate in the presence of the test inhibitor, V – reaction rate in the absence of inhibitor; [I] – inhibitor concentration; h – cooperativity coefficient.

It was found that 8h7mGua, like its metabolic precursor 7mGua, exhibits a pronounced inhibitory effect on the PARP1 enzyme activity (Fig. 4). Both compounds suppress PARP1 activity in a dose-dependent manner at NAD⁺ concentrations of 10-100 μ M, but 8h7mGua is a more potent inhibitor (Fig. 4, and Figs. S1, S2 in the Online Resourse 1). This is evidenced by the lower IC₅₀ values at all considered NAD⁺ concentrations (table). The IC₅₀ value of 7mGua and 8h7mGua is directly proportional to the concentration of the NAD⁺ substrate, which indicates competitive inhibition. Concentration of NAD⁺ in the cell nucleus is about 100 μ M [52]. At this substrate concentration, the obtained IC_{50} values for 7mGua and 8h7mGua are 78 and 11 μ M, respectively.

To elucidate molecular mechanism of 7mGua and 8h7mGua binding, models of their complexes with PARP1 were constructed. In the case of 7mGua, the earlier described interactions with active site residues were observed: hydrogen bonds with Gly863 and Ser904, hydrophobic contact with Ala898, and π -stacking with Tyr907 (Fig. 5a)

Calculated IC_{50} values for the inhibitory effect of 7mGua and 8h7mGua on PARP1 at different concentrations of NAD⁺

$[NAD^+]_0, \mu M$	IC ₅₀ , μM	
	7mGua	8h7mGua
10	8.0 ± 0.3*	3.6 ± 0.3
30	23.1 ± 3.1	7.3 ± 0.6
50	29.4 ± 4.2	7.6 ± 0.5
100	78.1 ± 14.1	11.0 ± 1.1

* Data are presented as mean \pm standard error. The values were obtained by fitting the experimental reaction rates at seven concentrations of the inhibitor with equation (7).



Fig. 5. Positions of 7mGua (a) and 8h7mGua (b) inhibitors in the PARP1 active site obtained by molecular docking. The hydroxyl group of 8h7mGua is oriented towards the side chain of Glu988, which enables formation of the additional hydrogen bond.

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Fig. 6. Effects of 7mGua and 8h7mGua on activity of several DNA repair enzymes. a) Electropherogram demonstrating activity of APE1; *1*) DNA substrate (31 nt), *2*) DNA+APE1, *3*-6) DNA+APE1+7mGua (33, 75, 150, 300 μM), *7-10*) DNA+APE1+8h7mGua (33, 75, 150, 300 μM). b) Electropherogram demonstrating activity of Polβ; *1*) DNA substrate (11 nt), *2*: DNA+Polβ, *3*-6) DNA+Polβ+7mGua (33, 75, 150, 300 μM), *7-10*) DNA+Polβ+8h7mGua (33, 75, 150, 300 μM). c) TDP1 activity at different concentrations of test compounds.

[18, 19]. In the case of 8h7mGua, the above interactions were observed, as well as additional contact with the Glu988 catalytic residue. The hydroxyl group of 8h7mGua is oriented towards the side chain of Glu988 and able to form a hydrogen bond (Fig. 5b), which could explain more efficient inhibition compared to 7mGua.

The ability of 7mGua and 8h7mGua to inhibit other DNA repair proteins was investigated for APE1, Pol β (base excision repair enzymes), and TDP1 (an enzyme removing 3' adducts from DNA). APE1 cleaves the sugar-phosphate backbone of the apurinic/apyrimidinic (AP) site [53, 54]. A radiolabeled 31-bp DNA duplex containing an AP site at position 12 was used as a substrate for APE1. Cleavage of this substrate results in formation of the 11-nt product that can be detected on the electropherogram (Fig. 6a). Polß catalyzes removal of the 5'-terminal dRP fragment, formed as a result of the cleavage of the AP site by the APE1 enzyme, and further insertion of the complementary residue to fill the gap [55, 56]. The DNA duplex with cleaved AP site was used as a substrate for Pol β , and the reaction product was a 12-nt oligonucleotide (Fig. 6b). TDP1 removes 3' adducts of various origins, thus restoring the DNA structure [57, 58]. An oligonucleotide containing a fluorophore at the 5'-end and a fluorescence quencher at the 3'-end was used as a TDP1 substrate [47]. Cleavage of the quencher by TDP1 leads to the increase in fluorescence, which can be recorded in real time (Fig. 6c). No inhibitory effect of 7mGua and 8h7mGua on the activity of APE1, Polß, and TDP1 was observed in a wide range of concentrations.

According to the obtained results, 7mGua and 8h7mGua inhibit PARP1 and have no effect on some other DNA repair proteins, which indicates selectivity of their action. To support this conclusion, we have predicted possible activities of 7mGua and 8h7mGua against a

wider set of molecular targets using the PASS software. APE1, Pol β , TDP1, and other DNA repair proteins were not found in the list of activities for which the Pa probability exceeded 0.6. At the same time, activity against TGT (an additional target of 7mGua discussed in more detail in the Introduction; Pa = 0.685), as well as some enzymes of bacterial metabolism was predicted.

Thus, the use of technique based on monitoring fluorescence anisotropy made it possible to characterize and compare the inhibitory action of 7mGua and its metabolite 8h7mGua on the recombinant human PARP1. It was shown that both compounds inhibit PARP1 activity, but 8h7mGua has a more pronounced inhibitory effect. It is likely due to the additional interaction of 8h7mGua with the Glu988 catalytic residue. Furthermore, no influence of 7mGua and 8h7mGua was seen on the activity of other DNA repair enzymes (APE1, Pol
, TDP1), which indicates selectivity of their action. Since PARP1 is a recognized therapeutic target for the treatment of various diseases, suppression of its activity using natural compounds 7mGua and 8h7mGua may be of considerable interest. In further in vitro and in vivo experiments it should be taken into account that the 7mGua molecule has the properties of a prodrug, which is converted in the body into a more active metabolite, 8h7mGua.

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Ethics declarations. D. K. Nilov and V. K. Švedas are the authors of the patent on using 7mGua to suppress PARP1 enzyme activity. D. K. Nilov, S. I. Shram, T. A. Shcherbakova, and V. K. Švedas are the authors of the patent application on using 8h7mGua to suppress PARP1 enzyme activity. This article does not contain description of studies involving animals or human participants performed by any of the authors.

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