Potent inhibition of specific diadenosine polyphosphate hydrolases by suramin

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Abstract The cytosolic enzymes asymmetrical diadenosine tetraphosphate hydrolase (EC 3.6.1.17, Ap3'Aase) and diadenosine triphosphate hydrolase (EC 3.6.1.29, Ap3'Aase) are inhibited competitively by suramin. Ap3'Aase and Ap3'Aase were assayed in cytosolic rat brain extracts using fluorogenic analogues of the respective substrates diadenosine tetraphosphate (Ap4'A) and diadenosine triphosphate (Ap3'A). Kі values for suramin as inhibitor of Ap3'Aase and Ap3'Aase were 5×10^{-6} M and 3×10^{-6} M, respectively. Results indicate that suramin or suramin-like derivatives may be useful tools to investigate diadenosine polyphosphate cleaving enzymes and that the intracellular diadenosine polyphosphate metabolism may be a pharmacological target of suramin with biological and clinical implications.

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Key words: Suramin; Diadenosine tetraphosphate; Diadenosine triphosphate (asymmetrical) hydrolase; Diadenosine triphosphate hydrolase; Rat brain

1. Introduction

Diadenosine polyphosphates (ApnA, n = 3–6) are a family of dinucleotides with increasing biochemical and pharmacological significance as intra- and extracellular signalling molecules [1,2]. Some recent reports suggest that some ApnA are involved in the intracellular regulation of ATP-gated K+ channels [3,4], in the signalling mechanisms associated with interferon actions [5] and in the mechanisms of differentiation and apoptosis in human cells [6]. These all contribute to an increasing interest in ApnA. Specific hydrolytic enzymes regulate the intracellular levels of these dinucleotides in mammalian cells: asymmetrical Ap3'Aase, a 20-kDa peptide, preferentially cleaves Ap3'A over Ap4'A and Ap2'A but not Ap1'A or Ap2'A, and Ap3'Aase, a 30-kDa peptide, hydrolyzes Ap3'A but not Ap1'A or Ap2'A [7,8]. It has recently been demonstrated that the protein encoded by the putative human tumor suppressor FHIT gene [9,10] displays enzymatic properties similar to those of the previously characterized Ap4'Aase.

Suramin is a symmetrical hexa sulfonated naphthyamine derivative of urea used therapeutically as antiparasitic, antiviral and anticancer drug but is severely limited by toxic side effects [11,12]. Suramin is also used in the pharmacology of purinergic transmission since its effects as antagonist of P2 purinoceptors and inhibitor of ecto-ATPases [13] and neural ecto-Ap3'Aase [14] have been reported. We now report inhibition of the specific Ap4'A and Ap3'A hydrolases present in rat brain cytosol by suramin, using fluorogenic derivatives of ApnA as substrates [15,16], and show that Ap4'Aase is a particularly sensitive enzyme.

2. Materials and methods

Young Wistar male rats were killed by decapitation, brains rapidly removed and homogenized in ice-cold 50 mM Tris-HCl pH 7.4 and centrifuged at 100 000 × g for 1 h. Supernatants were dialyzed against homogenization buffer and used as Ap3'Aase and Ap4'Aase source. The fluorogenic substrates ε-(ApnA), n = 2–5, were used to measure enzymatic activities by continuous fluorometric and chromatographic assays [8,14]. Continuous assays were performed in fluorimeter microcuvettes, containing Tris-HCl 50 mM, pH 7.5, 4 mM MgCl2 and appropriate protein, substrate and inhibitor concentrations (250 μM final volume) under stirring at 37°C. A Hitachi F-2000 spectrofluorimeter (λex 305 nm, λem 410 nm) was used to record the fluorescence increase associated with substrate hydrolysis. When necessary, results of reaction rates were corrected for the quenching effect of suramin on the fluorescence emission of etheno compounds: quenching was noted at suramin concentrations higher than 5 μM. HPLC analysis was performed in a Waters instrument with a 470 fluorescence detector (λex 305 nm, λem 410 nm). A Nova-Pak C18 column (Waters) was eluted with 10 mM KPO4, pH 2.5 acetonitrile, 2 mM tetrabutylammonium bromide (Sigma), pH 7.0 at 0.8 mL/min. Preparation of ε-(ApnA) was as previously described [8]. Acetonitrile and salts used for chromatographic analysis were HPLC grade from Merck and Scharlau. C. durissus phosphodiesterase was from Boehringer. Suramin was from RBI. All other products were reagent grade.

3. Results

3.1. Hydrolysis of ε-(ApnA) by rat brain extracts

The fluorogenic dinucleotides ε-(ApnA), ε-(ApnA) and ε-(ApnA) but not ε-(ApnA) were hydrolyzed by rat brain cytosolic extracts. Using both continuous fluorimetric and chromatographic assays it was found that hydrolytic activity on ε-(Ap3'A) and ε-(Ap3'A) was inhibited by Ap2', F−, Zn2+ and Ca2+; however, ε-(Ap4'A) hydrolysis was not affected by Ap2', F−, Zn2+ and Ca2+ but by Zn2+ (Fig. 1A,B). The lack of ε-(Ap4'A) cleavage indicates the absence of non-specific phosphodiesterase activity able to hydrolyze ApnA, n = 2–6 from brain cytosol according to known results [17]. These results

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closely similar inhibitory profile to that of O and slopes in the presence of: (2) Ca action mixtures for at least 5 min to determine control slopes (1) to soltic extracts. Linear fluorescence increases were recorded from re-

on the hydrolysis of (A) inhibited as percentages (means, n = 3) of slope in the presence of inhibitor relative to control slope. Hydrolysis of (A) and hydrolysis of (B) was not detectable (not shown).

indicate that only the specific hydrolases asymmetrical ApAase and ApAase contributed to the cleavage of (A) and (A), respectively. The (nucleotide moieties released from (A) and (A) by asymmetrical ApAase and ApAase, (ATP+AMP and ADP+AMP, respectively, were catabolized by nucleotidases and/or phosphatases up to adenose as the final product (Fig. 2A,B). Km and specific activity values obtained for ApAase and ApAase were respectively: 2.1 ± 0.4 and 11.2 ± 1.7 μM, and 10.5 ± 1.8 and 2.3 ± 0.6 nmol/min/mg (n = 4).

3.2. Inhibition of asymmetrical ApAase and ApAase by suramin

Addition of suramin (0.1–30 μM) to the reaction mixtures in the fluorimeter cuvette produced immediate and sustained decreases of the slope of fluorescence traces associated with substrate hydrolysis. The slope changes induced by suramin were dose dependent and were not modified after preincubation of suramin in the reaction mixture. HPLC assays clearly demonstrated that the depressor effect of suramin on fluorescence trace slopes was associated with a decrease in the substrate cleavage efficiency (Fig. 2C,D).

Fig. 3 shows comparatively the effects of suramin on ApAase and ApAase. ApAase was found to be the most sensitive enzyme showing a complete inhibition at suramin concentrations higher than 6 μM. Increased substrate concentration in the reaction mixture attenuated suramin inhibition, suggesting competition between substrate and inhibitor at the binding site. Double reciprocal plots obtained for ApAase and ApAase were compatible with a competitive inhibition pattern by suramin (Fig. 4). Ki values determined from these plots were 5.03 ± 0.91 μM and 0.31 ± 0.04 μM for ApAase and ApAase, respectively, with Ki/Ki quotients of 0.4 and 36. A Ki/Ki quotient of 36 indicates that ApAase exhibits much greater affinity for suramin than for its own substrate ApA, although this is not the case for ApAase.

4. Discussion

Rat brain cytosolic activities hydrolyzing the fluorogenic substrates of ApA and ApA display the typical characteristics of asymmetrical ApAase and ApAase from bovine adrenomedullary tissue and various other mammalian sources [7,8]. Both Km and specific activities determined for these specific hydrolases compare well with those determined for enzymes from various rat organs, including the brain using ApA and ApA as substrates [8].

These Ki values for suramin as inhibitor of specific ApAases are within the range of lower Ki values reported
for most suramin-sensitive enzymes [11,14,18,19]. Competitive inhibition for Ap₄Aase and Ap₃Aase suggests that suramin, a polyanionic molecule, interacts primarily by electrostatic interactions with positively charged amino acid residues involved in the binding of the negatively charged substrates Ap₄A and Ap₃A. Hydrophobic interactions due to suramin polyaromatic rings and hydrogen bonding probably also contribute to suramin binding. Other enzymes using nucleotide substrates, e.g. ATP, like human folylpolyglutamate synthetase [18] or a Xenopus oocyte ecto-ATPase [20], have been reported to be competitively inhibited by suramin. We have tentatively proposed that lysine is not involved in substrate binding in bovine adrenomedullary Ap₄Aase [8]; consequently histidine and/or arginine could be involved in the binding of Ap₄A to Ap₄Aase. The complete inactivation of both asymmetrical Ap₄Aase and Ap₃Aase observed after preincubation of brain extracts with diethyl pyrocarbonate (unpublished observations) strongly suggests the presence of catalytically active histidine residues in both enzymes.

The similarity between the catalytic properties of FHIT protein [9] and Ap₄Aase (EC 3.6.1.29), an enzyme known for years [7], suggests that both could be the same protein. If this is so, it may then be predicted that suramin will be a FHIT protein-binding drug inhibiting its associated Ap₄Aase activity, which opens the question of the biological activity displayed by putative FHIT-suramin complexes.

Inhibition of Ap₄Aase and Ap₃Aase by suramin may have clinical and biological relevance. When used therapeutically, suramin serum concentrations in treated patients may be higher than 200 μM with a measured half-life of about 50 days and it has been reported that the drug may inhibit in vivo cytosolic, lysosomal, and nuclear enzymes [11,12,21,22]. Thus, suramin inhibition of Ap₄A hydrolases is a feasible event that could contribute partly to the great diversity of toxic effects described for suramin by increasing cellular Ap₄A levels.

Raised intracellular Ap₄A levels caused by suramin inhibition of both Ap₄A hydrolases could interfere with cellular energy metabolism through the very strong inhibition of adenosine and adenylyl kinases by Ap₄A and Ap₃A [23,24] or with the normal regulation of ATP-gated K⁺ channels, which are also Ap₄A-binding proteins [3,4]. It is worth noting that increased Ap₄A levels have recently been correlated with cellular differentiation in responsive human cells treated by interferons [5,6] and that suramin can induce cell differentiation [25]. This raises the question whether suramin induced differentiation could be mediated by increased Ap₄A levels or changes in the Ap₄A/Ap₃A ratio, a recently proposed sensitive indicator of cell status [6].

This study adds asymmetrical Ap₄Aase and Ap₃Aase to the list of enzymes inhibited by suramin. Since there are no suitable inhibitors of these enzymes, our results suggest that suramin or suramin-derived compounds more specifically inhibiting these Ap₄A hydrolases could be tools to further investigate molecular aspects of Ap₄A-cleaving enzymes including FHIT protein, and also the intracellular roles of Ap₄A, still a puzzling question far from being clearly understood.

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References


