Thiostrepton binds to malarial plastid rRNA

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Abstract Binding of the thiazolyl peptide antibiotic thiostrepton to the GTPase domain of 23S rRNA involves a few crucial nucleotides, notably A1067 (*E. coli*). Small RNA transcripts were prepared corresponding to the GTPase domain of the plastid 23S rRNA and the two forms of cytosolic 28S rRNAs found in the human malaria parasite *Plasmodium falciparum*, as well as the plastid form of rRNA of the AIDS-related pathogen *Toxoplasma gondii*. Binding affinities of the wild type and mutated RNA sequences were as predicted; the malarial plastid sequence had by far the highest affinity, whereas that from toxoplasma did not bind thiostrepton.

Key words: Malaria; Toxoplasma; Thiostrepton; GTPase domain; rRNA

1. Introduction

Like plants and algae, apicomplexan parasites (malaria, toxoplasma etc.) carry two DNA-containing organelles, a mitochondrion (mt) and a plastid (pl) [1,2]. The plastid, enveloped by several membranes, is vestigial and probably of ancient endosymbiotic origin, but it is also demonstrably active and could be a new target for chemotherapeutic agents [3]. Nucleotide (nt) sequences are available for the GTPase domain of the two types of 28S rRNA specified by the nucleus of the human malaria pathogen *Plasmodium falciparum* (Pf) [4], as well as the pl 23S rRNA genes [6] and the 23S rRNA specified by the mt genome (only a partial sequence, see [5]). These data indicate that the high affinity binding site for the thiazolyl peptide antibiotic thiostrepton, A1067 in *E. coli* [7–9], is conserved in the GTPase domain encoded by the plastid DNA, but is modified to a G in both nuclear and mitochondrial genomes (Fig. 1). This substitution is predicted to reduce rRNA binding by the antibiotic [8]. We have tested thiostrepton to ascertain whether it inhibits Pf in cultures of human blood and have determined the levels of antibiotic binding to nucleus and plastid forms of Pf 28/23S rRNA.

2. Materials and methods

Blood cultures of the malaria parasite *P. falciparum* were maintained in vitro and the uptake of radioactive tracers in the presence and absence of thiostrepton was determined by methods described previously [10].

Short transcripts of wild type rRNA (corresponding to the GTPase domain of Pf 23S $rRNA_{pl}$, nt 987–1078) were transcribed in vitro from a PCR product that included a T7 promoter sequence in one of the primers. Mutated malarial $rRNA_{pl}$ sequences (*E. coli* numbers A1067U and A1067G) were obtained by PCR methodology and transcribed in the same way. Transcripts of both types of Pf 28S rRNA also were prepared from PCR products, in this case using either total DNA or RNA as template, a reverse transcription reaction with random primers being carried out first in the latter case. All wild type and modified transcript sequences were verified prior to thiostrepton binding assays. A positive control transcript was used based on the 23S rRNA sequence of *E. coli* with a mutation (U1061A) that increases stability and binding (kindly supplied by Dr P. Ryan, Johns Hopkins University, Baltimore, MD). Thiostrepton filter-binding assays were performed as previously described [8] except that transcripts were labelled with ^{32}P and the KCl buffer was replaced with NH_4Cl [11].

3. Results and discussion

Reproducible inhibition of uptake of both [^{3}H]hypoxanthine and [^{14}C]isoleucine was found with *P. falciparum* in blood cultures (50% inhibition at 3–5 μM thiostrepton ml^{-1}, respectively, see Fig. 2A). As shown in Fig. 2B, onset of inhibition of protein synthesis by thiostrepton was more rapid (5 h) than by tetracycline (8 h). Specificity was demonstrated by the lack of effect of viomycin (data not shown), an unrelated antibiotic that also can inhibit translocation [12].

Evidence that the highest affinity interaction of thiostrepton is with 23S $rRNA_{pl}$ was obtained from a thiostrepton filter-binding assay [8]. Fig. 3A shows that the mutation Pf_{pl} (*E. coli* number A1067U) markedly reduced thiostrepton binding (~14% of wild type). An intermediate level of binding (~35% of wild type) was obtained with the mutation Pf_{pl} (*E. coli* number A1067G). Thiostrepton binding to transcripts corresponding to the GTPase domain (nt 1334–1427) of both the sporozoite and erythrocytic forms of Pf 28S rRNA [4] were ~10% of that for Pf 23S $rRNA_{pl}$. These data show

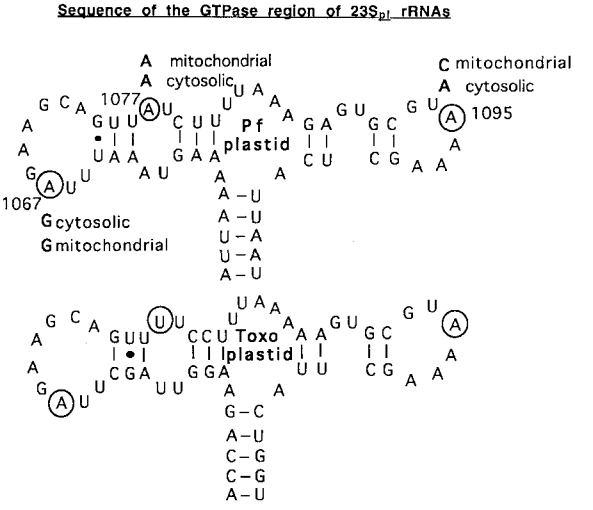

Fig. 1. Sequence of the GTPase region of the plastid 23S rRNAs of *Plasmodium falciparum* (Pf) and *Toxoplasma gondii* (Toxo) (numbers based on *E. coli*), showing substitution sites (circled) affecting the binding of thiostrepton [7]. The corresponding nucleotides in Pf cytosolic 28S rRNA and Pf mitochondrial 23S rRNA are indicated.

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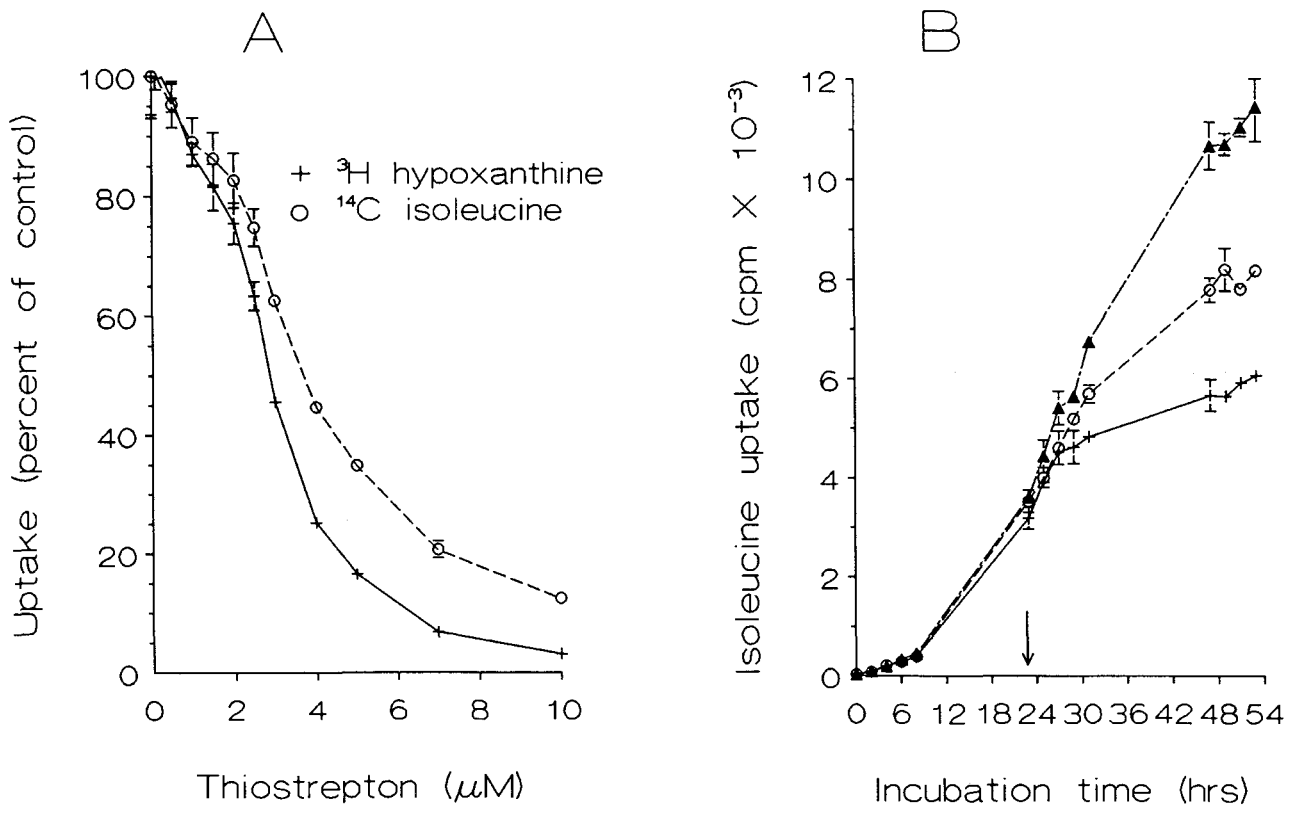

Fig. 2. Thiostrepton inhibition of *Plasmodium falciparum* in blood cultures. A: Dose-response curve. B: Inhibition of incorporation of [^{14}C]isoleucine in cultures following addition (arrow) of 10 μM thiostrepton ml^{-1} (+), or 100 μM tetracycline ml^{-1} (○) at 23 h. Untreated control (▲).

that the nts crucial for thiostrepton binding to Pf 23S rRNA are as predicted from *E. coli*, and that the plastid form has the highest binding affinity. It is not yet possible to reconstruct the complete sequence for the GTPase domain of the 23S $rRNA_{mt}$ [5], however, the fragments of sequence available do not have the critical consensus nts required for thiostrepton binding at positions A1067 and A1095 (*E. coli* numbers) (see Fig. 1).

In a similar way, we tested a transcript corresponding to the GTPase domain of the 23S $rRNA_{pl}$ of *Toxoplasma gondii* (Tg), a related apicomplexan that is an important opportunistic pathogen in patients with AIDS. In this case, the wild type sequence has a substitution at a different site (*E. coli* number A1077U) (see Fig. 1), that inhibits binding by thiostrepton in *E. coli* [8]. This was found also to be the case with a transcript derived from a PCR product covering the GTPase domain of Tg_{pl} 23S rRNA (nt 926–1024) (Fig. 3B). Corrective mutation of the Tg_{pl} transcript (*E. coli* number U1077A) conferred a significant increase (×5) in thiostrepton binding (Fig. 3B).

These thiostrepton binding studies constitute the first direct evidence that components of the malarial plastid organelle could be preferentially targeted by drugs. The results complement earlier studies [13,14] which inferred that toxoplasma's 23S $rRNA_{pl}$ might be the target of the macrolide antibiotic, clindamycin, acting at a different effector site.

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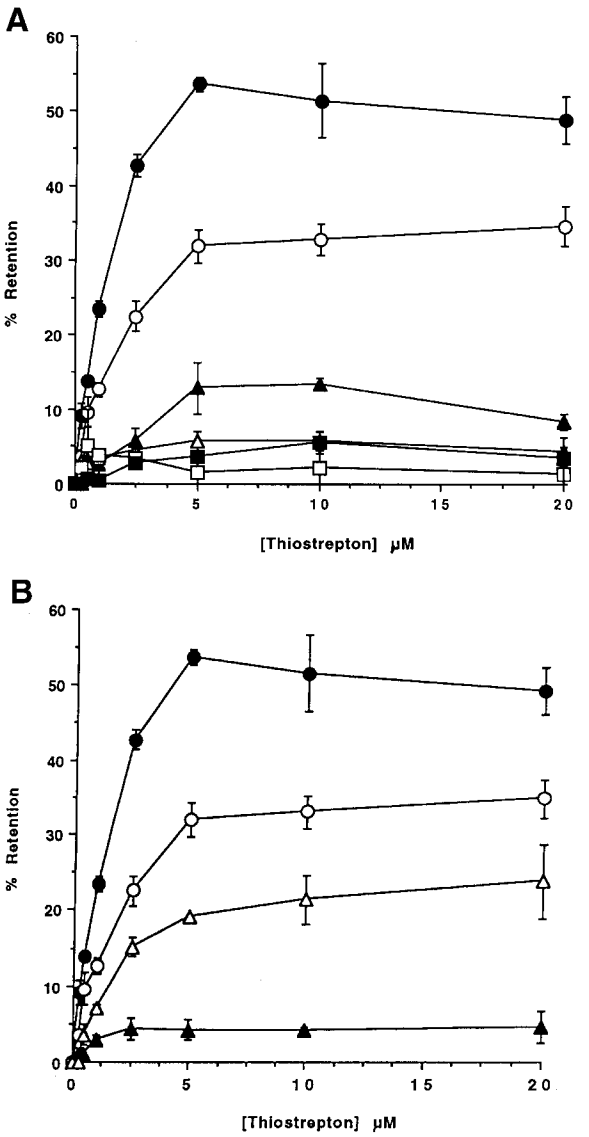

Fig. 3. Thiostrepton binding curves [8] (mean of duplicates, bar = range). A: Short 23S transcripts of *P. falciparum* (Pf) wild type $rRNA_{pl}$ (○, A1067) and mutated forms (□, A1067U and ▲, A1067G), as well as Pf 28S rRNA sporozoite-type transcripts (△) and erythrocytic-type (■), compared with an optimized *E. coli* control transcript (●). All nucleotide numbers correspond to *E. coli* for convenience. B: *T. gondii* wild type $rRNA_{pl}$ transcript (▲) and mutated transcript (△) compared with control transcripts from *P. falciparum* $rRNA_{pl}$ (○) and *E. coli* (●).