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Interaction of aurintricarboxylic acid (ATA) with four nucleic acid binding proteins DNase I, RNase A, reverse transcriptase and Taq polymerase

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ABSTRACT

In the investigation of interaction of aurintricarboxylic acid (ATA) with four biologically important proteins we observed inhibition of enzymatic activity of DNase I, RNase A, M-MLV reverse transcriptase and Taq polymerase by ATA in vitro assay. As the telomerase reverse transcriptase (TERT) is the main catalytic subunit of telomerase holoenzyme, we also monitored effect of ATA on telomerase activity in vivo and observed dose-dependent inhibition of telomerase activity in Chinese hamster V79 cells treated with ATA. Direct association of ATA with DNase I (K_d = 9.019 μM)), RNase A (K_d = 2.33 μM) reverse transcriptase (K_d = 0.255 μM) and Taq polymerase (K_d = 81.97 μM) was further shown by tryptophan fluorescence quenching studies. Such association altered the three-dimensional conformation of DNase I, RNase A and Taq polymerase as detected by circular dichroism. We propose ATA inhibits enzymatic activity of the four proteins through interfering with DNA or RNA binding to the respective proteins either competitively or allosterically, i.e. by perturbing three-dimensional structure of enzymes.

1. Introduction

The triphenylmethane dye aurintricarboxylic acid (ATA) has been reported to interact with a large number of protein molecules. The ATA molecule carries three carboxylic acid moieties that take negative charge at physiological pH. The structure of ATA was shown in Fig. 1. These charged groups are responsible for extensive ionic binding with positively charged groups on cellular proteins [1]. ATA was initially shown to inhibit template binding to QB replicase, *E. coli* RNA polymerase, T7 RNA polymerase and to prevent the binding of RNA to ribosome [2,3]. It was postulated that ATA would inhibit association of the nucleic acid binding proteins with nucleic acid. Subsequently, ATA's general inhibitory effect to nucleases was reported [4]. Inhibition of apoptosis by ATA was widely documented in various cell lines under different stimulations [5–10]. Our previous study showed that aurintricarboxylic acid (ATA) inhibited apoptosis induced by two different inducer gamma radiation and benzamide [an inhibitor of poly(ADP)-ribose polymerase] [11]. ATA reduced significantly nuclear fragmentation and nucleosomal ladder formation without affecting upstream events like cytochrome c release or caspase-3 activation in Chinese hamster V79 treated with gamma radiation or benzamide. Further, we observed treatment with protein kinase C (PKC) inhibitors (staurosporin and H-7) induced apoptosis (unpublished) and ATA pre-treatment reduced DNA ladder formation by PKC inhibitors without changing caspase-3 activation (unpublished). This result implicated that ATA might be interacting with down-stream events of caspase-3 in the apoptosis pathway, most likely with DNases involved in apoptosis. A large number of DNases localizing in cytoplasm are involved in apoptosis and their activity is either Ca^{2+}/Mg^{2+}-dependent or cation independent. For example, DNase II and L-DNase II are cation independent [12]. DNases translocate into nucleus during apoptosis and cleave DNA into nucleosomal ladder. DNase I, DNase-γ, DNAS1L3, NUC70, NUC18 are all cation-dependent endonuclease and gets activated in the process of apoptosis and cleaves the DNA into large and smaller fragments [13–16]. It has been reported that ATA inhibits endonucleases and promotes long-term survival of cells [6,7].

In modeling studies by docking, it has been shown that ATA can bind with several viral proteins like RNA-dependent RNA polymerase (RdRp), S1, HIV integrase and likely to alter their functions. The same study also reported that ATA bind to the catalytic domain of RdRp in severe acute respiratory syndrome (SARS) coronavirus [17,18]. Anti-viral activity of ATA has been shown due to ATA's ability to interfere with the attachment of viral mRNA to host polyribosome complex in cell free system [17–19]. Anti-reverse transcriptase activity of ATA has been demonstrated by in

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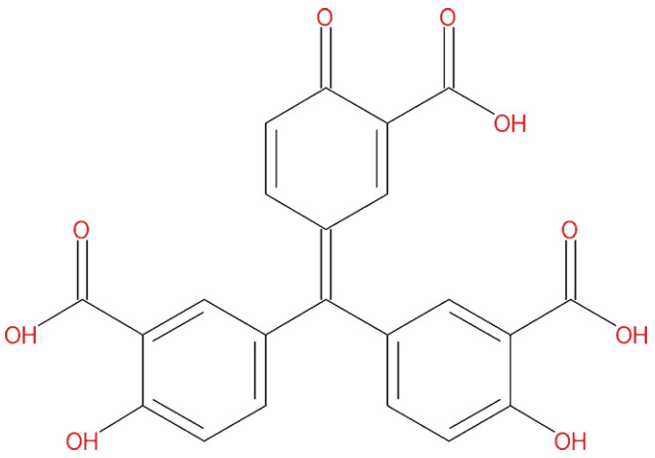

Fig. 1. Chemical structure of aurintricarboxylic acid (ATA).

vitro assay [20]. A potent inhibitor of RNA-dependent DNA polymerase (reverse transcriptase) is important for the discovery of therapeutic agents against retroviruses. ATA's anti-reverse transcriptase activity inspired us to investigate its role in regulation of telomerase which has telomerase reverse transcriptase (TERT), the catalytic subunit. Telomerase is a ribonucleo-protein complex having the main catalytic subunit – TERT [21–23], associated RNA template-telomerase RNA component (TERC or TR or TER) and other associated proteins like telomerase associated protein TEP1/TP1, HSP90, P23, etc. [21,24–26]. Besides, various other proteins have been identified that interact with the telomere. Role of these proteins and their interacting partners for maintenance of the telomere and regulation of telomerase activity is being discovered. The reverse transcriptase replicates the end of chromosomes for synthesizing the telomeric repeats with help of its own RNA molecule as the template [26]. Here we demonstrated inhibition of telomerase activity by ATA treatment in V79 cells.

Several in vitro studies reveal that ATA can inhibit activities of different DNA or RNA binding proteins such as restriction enzymes, DNA polymerase, RNA polymerase. It has been observed that restriction enzymes like Sal I, Bam H1, Pst I, Sma I are inhibited in vitro by ATA treatment [4]. In vitro DNA synthesis activity by DNA polymerase was also inhibited by ATA [27]. The activities of the purified human DNA polymerases alpha, beta, gamma, and DNA primase as well as those of DNA polymerase I and RNA polymerase from *Escherichia coli* and Rauscher leukemia virus are also inhibited by ATA [28]. ATA also inhibits amino acyl ^{t}RNA synthetase [29] and ribonucleotide reductase [30]. In protein synthesis ATA inhibits the interaction of mRNA with the ribosome complex has been modulated by ATA inhibiting the initiation and elongation of protein synthesis [31]. ATA also interfere DNA-NF-κB binding as detected by mobility shift assay [32].

Apart from its inhibitory effect on nucleic acid binding proteins to associate with nucleic acid, it plays significant role in phosphorylation. Apoptosis induced by TNF-alpha alone is suppressed by ATA and this event is paralleled by phosphorylation and nuclear translocation of Jak2, Stat2, Stat4 and NF-kB, along with inhibition of caspase activation in human B cells leading to promotion of survival [33,34]. ATA can also promote bcl2 phosphorylation at evolutionary conserved residue ser-70 whose phosphorylation is required for full anti-apoptotic function [35]. Increase in the phosphorylation of serine and tyrosine residues of proteins have been observed in cells treated with ATA [36–38].

In spite of large number of reports about the inhibitory effect of ATA with several nucleic acid binding proteins by biochemical assay, as well as modeling studies, no information is available about the direct interactions of ATA with the proteins. In this manuscript, we are presenting direct physical interaction of ATA with four biologically important nucleic acid binding proteins such as DNase I, RNase A, reverse transcriptase and Taq polymerase as detected by biochemical enzymatic assay and tryptophan fluorescence quenching study. Three dimension conformational analysis of DNase I, RNase A and Taq polymerase was also demonstrated using circular dichroism spectroscopy. In this connection we are also presenting inhibition of telomerase activity in V79 cells treated with ATA.

2. Materials and methods

2.1. Chemicals

Aurintricarboxylic acid (ATA), RNase A and DNase I were purchased from Sigma chemicals (USA). Medium MEM was procured from Hi-media, India. Goat blood was procured locally and processed in our laboratory for serum preparation as described [39]. M-MLV reverse transcriptase, first strand buffer and oligo dT were from Life Technologies (USA), gene specific oligonucleotides for PCR amplification were from MWG Biotech (Germany). Taq polymerase was purchased from Clontech.

2.2. Enzyme assay and cell culture

2.2.1. Gel electrophoresis

We have used 1.5% and 2% agarose gels in 0.5× TBE buffer for resolving PCR products and RNA, respectively. The gels were stained with ethidium bromide to visualize the DNA or RNA and photographed in ImageMaster VDS (Pharmacia Biotech). To check the purity of the enzymes we resolved in 12.5% SDS-PAGE. After completion of electrophoresis the protein bands were stained with coomasie blue and photographed (data not shown).

2.2.2. DNA digestion

PCR product of 315 bp DNA (obtained as described in the following PCR reaction section) was used as substrate for DNase I in a digestion buffer (40 mM Tris–Cl pH 8.0, 10 mM $MgSO_4$, 1 mM $CaCl_2$) in presence and absence of ATA at 37 °C for 15 min. The digested product was resolved in 1.5% agarose gel, stained with ethidium bromide, photographed under transluminator (data not shown).

2.2.3. RNA digestion

Total RNA was extracted from exponentially growing Chinese hamster V79 cells following the standard protocol and estimated spectrophotometrically. Five micrograms of RNA was digested with RNase A (4.5 μg/ml) in presence and absence of ATA (1, 10, 50 100, 150, 200 μM) in a digestion buffer (150 mM NaCl, 5 mM EDTA, 10 mM Tris–Cl pH 7.5) for 10 min at 37 °C. The product was resolved in 2% agarose gel and stained with ethidium bromide and photographed under transluminator (data not shown).

2.2.4. cDNA synthesis

We have used M-MLV reverse transcriptase for the preparation of cDNA from RNA isolated from V79 cells. We have followed the same method for cDNA preparation (RT product) as the manufacturer described. First, the reverse transcriptase taken in first strand buffer was incubated with different concentration of ATA for 15 min at room temperature. Five micrograms of RNA and 100 ng oligo dT was taken, heated at 70 °C for 10 min followed by chill on ice for 1 min. Then the reverse transcriptase with different concentrations of ATA was added to RNA solution along with DTT and dNTP (containing P^{32}α-dCTP). Then it was incubated at 37 °C for 1 h, heated at 90 °C for 5 min and chilled on ice for 10 min. Similarly RT product was produced using reverse transcriptase without ATA. The product was resolved in 10% non-denaturing acrylamide gel and