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Inhibition of HIV-1 TAR RNA–Tat peptide complexation using poly(acrylic acid)

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Abstract

HIV-1 is regulated at the transcriptional level by the interaction of Tat protein with the transactivation responsive region (TAR) RNA, a 59-base stem–loop structure located at the 5′-end of all nascent HIV-1 transcripts. Here, by targeting the Tat peptide, we found that negatively charged poly(acrylic acid) (PAA) had high affinity with Tat peptide and could inhibit the interaction of TAR with Tat. Therefore, PAA could block HIV replication by binding to Tat not to TAR RNA, providing a new thinking for the design of novel anti-HIV drugs.

Keywords: Inhibition; Poly(acrylic acid); Tat peptide; TAR RNA; Quartz crystal microbalance

RNA–protein interactions are vital for many regulatory processes such as translation, RNA splicing, and transcription. One representative example of such interactions is the mechanism of transactivation in the human immunodeficiency virus type 1 (HIV-1). It has been reported that HIV-1 is regulated at the transcriptional level by the interaction of Tat protein with the transactivation responsive region (TAR) RNA, a 59-base stem–loop structure located at the 5′-end of all nascent HIV-1 transcripts [1]. Tat protein is a potent transactivator and is essential for viral replication [2,3]. Tat can be divided into five structural domains, which are called N-terminal, cysteine-rich, core, basic (arginine-rich), and C-terminal domains [4]. The basic region is specific for binding to the TAR RNA; other amino acid residues outside this region are in favor of the overall binding affinity and kinetic stability of the TAR–Tat complex [5]. TAR RNA contains a six-nucleotide loop and a three-nucleotide pyrimidine bulge which separates two helical stem regions (Fig. 1) [6,7]. The trinucleotide bulge is essential for high affinity and specific binding of the Tat protein [8,9]. The loop region is required for in vivo transactivation but is not involved in Tat binding [10,11]. NMR studies indicate that the Tat basic domain forms a stable α-helix [12]. Upon Tat binding to TAR RNA, both of them undergo conformational change [13].

The functional importance of the Tat–TAR interaction to the viral life cycle makes it an attractive target for intervention with antiviral agents. Drugs that inhibit the Tat–TAR complex formation may be promising inhibitors of HIV replication [14–16]. A number of studies reported designed substances prevented formation of the Tat–TAR complex by binding to the viral TAR element, thus blocking HIV replication through Tat–TAR [17–22]. Indeed, in AIDS pathology, HIV-infected cells can secrete Tat. Extracellular Tat can enter the cell and nucleus to stimulate the transcriptional activity of HIV-LTR. Especially extracellular Tat induces several biological effects on uninfected target cells [23]. Therefore, compounds that can block HIV replication by binding to Tat not to TAR RNA may provide a new thinking for the design of novel anti-HIV drugs. It has been reported that the electrostatic interaction is required for Tat–TAR recognition [24], and a class of negatively charged compounds could inhibit Tat/TAR complexation by binding to Tat [25]. In addition, Presta and co-workers [26,27] have reported that extracellular Tat has high affinity with

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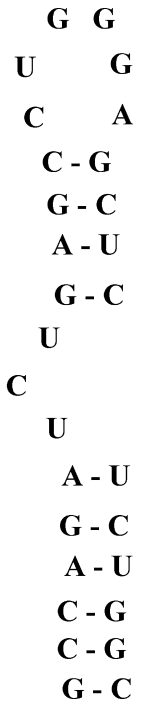

Fig. 1. Secondary structure of HIV-1 TAR RNA.

polyanions, e.g., pentosan polysulfate, heparin, and suramin, thus, these polyanions inhibit the uptake of extracellular Tat and its HIV-LTR transactivating activity. At physiological conditions (pH 7.4), poly(acrylic acid) (PAA) is a kind of polyanion, which has a variety of uses in such diverse fields as mining, textiles, superabsorbents, hydrogels, drug delivery coatings, and cosmetics. Therefore, poly(acrylic acid) was presumed to bind with Tat resulting in blocking the interaction of Tat with TAR.

Conventional methods for the determination of protein–nucleic acid interaction are gel shift and filter binding assays [28,29]. The microgravimetric quartz crystal microbalance (QCM) is a suitable transducer for chemical and biochemical sensing in general, where a decrease of the resonant frequency is correlated to the mass accumulated on its surface. They have been used to monitor DNA–protein formation in real time [30,31]. According to the mass change at QCM after TAR–Tat or PAA–Tat complexation, the apparent coefficients of them could be obtained, and the quantitative investigations of the TAR–Tat or PAA–Tat interactions at the interfaces become possible. In addition, to our knowledge, there are no reports on the utility of transmission electron microscope (TEM) to study the interaction of TAR–Tat or drug–Tat. Thus, in the present study we use QCM cooperated with TEM to study the TAR–Tat or PAA–Tat interaction.

Materials and methods

Cysteamine (CA) was obtained from Sigma. Poly(acrylic acid) (PAA), sodium salt (MW 1200) was purchased from Aldrich. Glutaraldehyde (GA) was obtained from Beijing Chemical Reagents (Beijing, China). They were used without further purification. Tris–HCl buffer (10 mM Tris–HCl, 70 mM NaCl, and 0.2 mM EDTA, pH 7.4) was used to prepare solutions. They and all other chemicals were of analytical reagent grade.

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg

Fig. 2. Primary structure of HIV-1 Tat peptide.

TAR RNA (5′-GCCAGAUCUGAGCCUGGGAGCUCUCUG GC-3′) was purchased from TaKaRa Biotechnology (Dalian, China). Tat-peptide (Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg) (Fig. 2) was received from Shanghai Sangon Biological Engineering Technology and Service (Shanghai, China). They were used as received.

Gravimetric measurements with QCM. AT-cut quartz crystals with a fundamental frequency of 9 MHz were purchased from Seiko EG&G (Tokyo, Japan). These crystals were coated with thin gold layers on both sides (effective surface area, 0.196 cm^2). Before use, the Au surface of the quartz resonator was cleaned with piranha solution (H_2SO_4: 30% H_2O_2 = 3:1) for 2 min. They were then thoroughly washed with double-distilled water and used immediately afterwards. Au electrodes were modified with a cysteamine monolayer by incubation of the electrodes in an aqueous 20 mM cysteamine solution for 2 h. The resulting electrodes were rinsed with double-distilled water and placed in 2.5% glutaraldehyde solution for 1 h. After washing with Tris–HCl buffer and double-distilled water, they were immersed in 2.0×10^{-6} M Tat peptide solution for 2 h and rinsed with buffer and water similarly. Then they were immersed in a TAR RNA or PAA solution for 60 min. After rinsing, they were immersed in PAA or TAR RNA solution for 60 min. All experiments were carried out at room temperature (22 ± 1 °C).

Transmission electron microscope analysis. The morphologies of Tat, Tat–TAR, and Tat–PAA complex were examined by JEOL JEM-2010 transmission electron microscope (Japan). Samples were placed onto carbon-coated parlodion film supported by a copper grid, washed three times in bi-distillated water, and negatively stained with 1% uranyl acetate. They were dried at room temperature and then examined using a TEM.

Results and discussion

The binding affinity and characteristics of Tat peptides, consisting of a highly basic domain encompassing amino acid residues 48–57 such as Tat_{10} used in this study, are remarkably similar to the full-length protein [32,33].

The interface sensing processes were prepared as schematically outlined in Fig. 3. To obtain an amine group on the QCM gold electrode surface, a cysteamine monolayer was assembled. Then the glutaraldehyde was added as a functional group modifier to alter amino group to aldehyde on the gold electrode surface. After that, Tat peptide, with the amine group in the peptide amino acid, was coupled with aldehyde group in GA. The resulting Tat-functionalized interfaces were treated with TAR RNA or PAA to yield the active sensing interface. The assembly of the layered sensing interface was characterized by following the crystal frequency changes after each modification step. The frequency shift, ΔF, was related to the mass accumulated, Δm, on the quartz crystal electrode surface according to the Sauerbrey equation [34], given as follows:

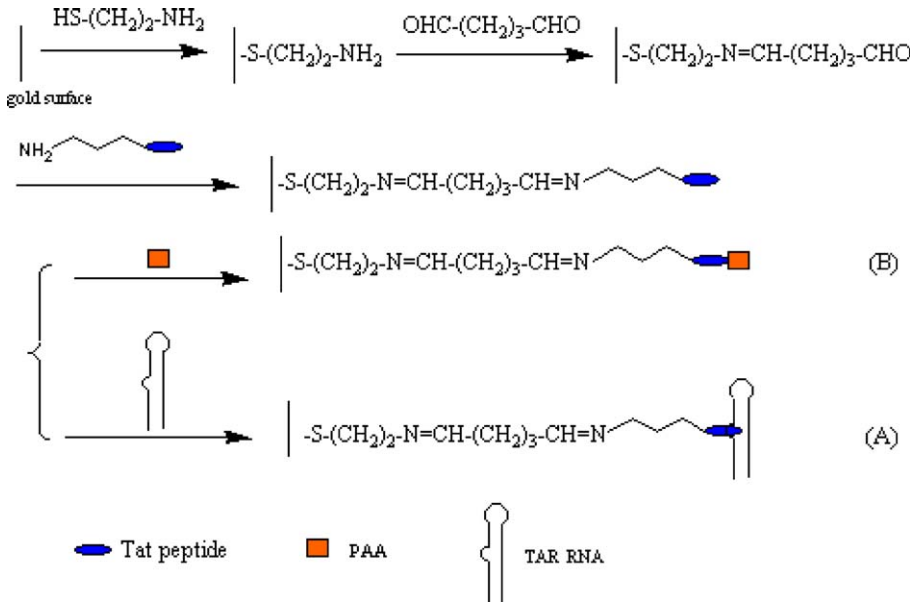

Fig. 3. Schematic illustration of the recognition processes.

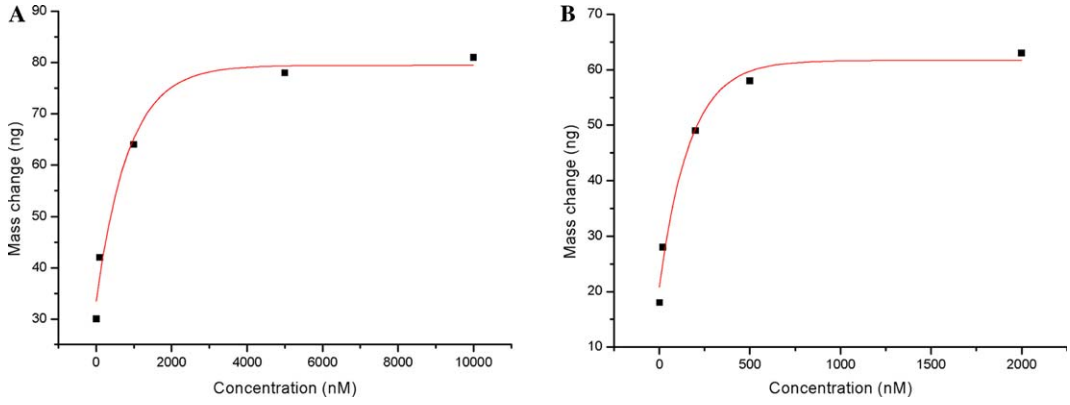

Fig. 4. Mass–concentration plots for the binding of TAR (A) or PAA (B) to Tat.

$$\Delta F = -2.26 \times 10^{-6} F^2 \Delta m / A, \quad (1)$$

where ΔF is the resonant frequency shift; F is the basic resonant frequency of the crystal; Δm is the mass accumulated on the crystal surface; and A is the area of the electrode surface (cm^2).

Fig. 4 shows the mass change on the gold electrodes upon interaction of the electrode with different concentrations of TAR RNA or PAA. In both systems, as the concentration of TAR RNA or PAA increases, the mass increases, implying the association of TAR RNA or PAA to the sensing interface. The TEM images of Tat, Tat–TAR, and Tat–PAA are shown in Figs. 5A–C, respectively, indicating the difference of complex formation of Tat–TAR and Tat–PAA. Furthermore, the interaction between fixed Tat and the species in solution could be depicted by an equation similar to Langmuir adsorption isotherm. That is

$$\Gamma = \Gamma_\infty \frac{C}{(K_D + C)}, \quad (2)$$

where Γ is the binding amount (mol) of TAR or PAA to Tat (capacity for TAR–Tat or PAA–Tat binding), Γ_∞ is the maximum amount of TAR or PAA binding (mol) to Tat, which should be equal to the amount of Tat if they are 1:1 combination, K_D is the dissociation constant of TAR–Tat or PAA–Tat complex, and C is the concentration of TAR or PAA in solution. Calculating results show that the combination coefficient (K_D^{-1}) of TAR–Tat is $7.5 \times 10^7\ M^{-1}$, which compared well with the value reported in the literature [33], although the physical background is different. While the combination coefficient of PAA–Tat is $4.2 \times 10^8\ M^{-1}$, suggesting that PAA has much stronger affinity with Tat than TAR does.

When TAR RNA binds to PAA–Tat complex, no obvious mass shift is observed (Fig. 6A), implying that

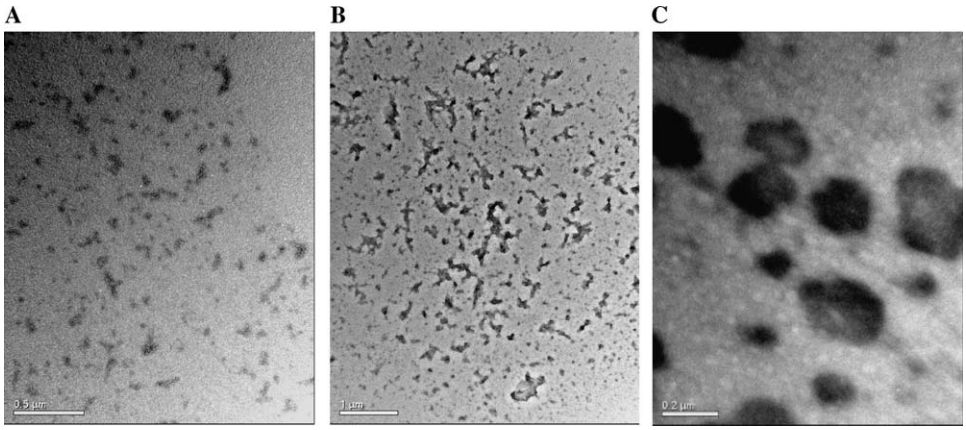

Fig. 5. Transmission electron microscope images of Tat peptide (A), Tat–TAR (B), and Tat–PAA (C).

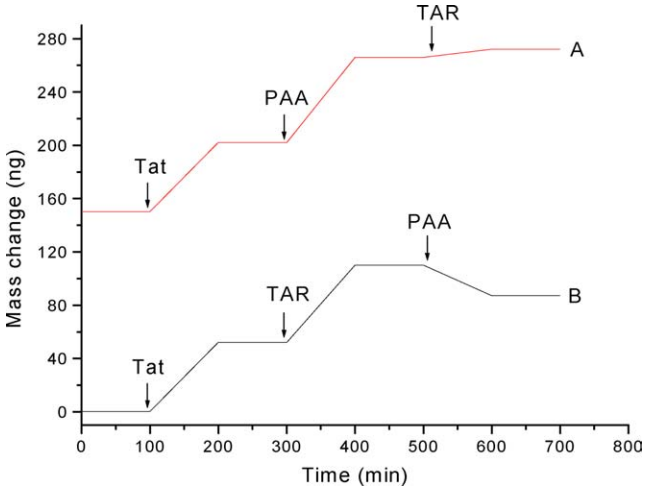

Fig. 6. Mass change for sequential introduction of analytes on CA/GA modified electrodes. (A) Tat peptide (2.0×10^{-6} M), PAA (1.0×10^{-6} M), and TAR (5.0×10^{-7} M). (B) Tat peptide (2.0×10^{-6} M), TAR (5.0×10^{-7} M), and PAA (1.0×10^{-6} M).

the interaction of Tat with TAR is blocked by PAA. This result may come from the change in the net positive charge of Tat peptide after interacting with negatively charged PAA, as the electrostatic interaction is critical for complex formation with TAR RNA [24]. By contrast, a negative mass shift is obtained when PAA binds to Tat–TAR complex (Fig. 6B), indicating that the binding of PAA to Tat–TAR complex induces dissociation of TAR from the ternary complex, since the molecular weight of PAA is lower than that of TAR.

In conclusion, by targeting the Tat peptide, we found that poly(acrylic acid) (PAA) had high affinity with Tat peptide and could inhibit the interaction of TAR with Tat. Therefore, PAA could block HIV replication by binding to Tat not to TAR RNA, providing a new thinking for the design of novel anti-HIV drugs.

Acknowledgments

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