Polyamidoamine dendrimers inhibit binding of Tat peptide to TAR RNA

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Received 16 January 2004; revised 20 February 2004; accepted 10 March 2004

First published online 18 March 2004

Edited by Hans-Dieter Klenk

Abstract The binding of polyamidoamine (PAMAM) dendrimer or Tat peptide to trans-acting responsive element (TAR) RNA has been studied using microgravimetric quartz crystal microbalance (QCM). Experimental results showed that PAMAM dendrimer could form complexes with TAR RNA. Especially, PAMAM dendrimer could disrupt the interaction of Tat peptide with TAR RNA, which is essential for HIV-1 virus replication, suggesting that QCM is a powerful tool for studying the binding processes of Tat peptide–TAR RNA and drug–TAR RNA and has great significance for the design of new drugs. An equation to measure the binding ability between TAR RNA and other species has been proposed.

Key words: Trans-acting responsive element RNA; Tat peptide; Quartz crystal microbalance; Polyamidoamine dendrimer

1. Introduction

Arya et al. [1] reported that the human immunodeficiency virus type 1 (HIV-1) Tat protein is essential for HIV replication, which viewpoint was further proved by Dayton et al. [2]. Tat is introduced to the transcription complex following binding to an RNA stem-loop structure, the trans-acting responsive element (TAR), located at the 5′ end of the viral mRNA [3–6]. TAR RNA has a stable hairpin structure [7]. Recent experiments have demonstrated that Tat binds to TAR RNA in the region of a three base bulge and those adjacent Watson–Crick base pairs and the positions of surrounding phosphate groups [8–10]. Tat protein is 86–101 amino acids long, contains the N-terminal activation domain and the C-terminal RNA binding domain. The binding affinity and characteristics of Tat peptides, consisting of a highly basic domain encompassing amino acid residues 47–58, are remarkably similar to those of the full-length protein [11,12].

The interaction of Tat with TAR is crucial for the production of full-length viral transcripts and the proliferation of the virus. Therefore, finding drugs that bind TAR RNA, and block binding by Tat, would provide a strategy for inhibiting HIV replication. A number of recent studies have shown that aminoglycoside antibiotics [13,14], TAR RNA decoys [15,16], TAR ribozyme [17] and Tat peptide analogs [18,19] could block Tat–TAR binding. In addition, our previous studies [20] have also shown that poly(allylamine hydrochloride) (PAH) could disrupt the Tat–TAR interaction. As we know, PAH belongs to the traditional linear polymers. Compared with traditional linear polymers, polyamidoamine (PAMAM) dendrimers are a relatively new and exciting class of nanoscopic, spherical, mono-dispersed, highly branched three-dimensional polymers. More importantly, PAMAM dendrimers have been determined to be non-immunogenic and exhibit low mammalian toxicity [21]. These characteristics, along with water solubility, are some of the features that make them attractive for biological and drug-delivery applications [22]. In addition, PAMAM dendrimers could form complexes with DNA through electrostatic interactions between negatively charged phosphate groups of the nucleic acid and protonated (positively charged) primary amino groups on the dendrimer surface [23]. Therefore PAMAM dendrimers are promising inhibitors, through binding strongly with the phosphate groups on TAR RNA preventing Tat binding to TAR RNA.

Gel shift and filter binding assays are common approaches for the study of protein–nucleic acid interaction [6,24]. The microgravimetric quartz crystal microbalances (QCMs) are suitable transducers for chemical and biochemical sensing in general. They have been used to monitor DNA–protein formation in real time [25]. In the present paper, we report the application of QCM to the study of TAR RNA–protein and TAR RNA–PAMAM dendrimer interaction at interfaces.

2. Materials and methods

2.1. Materials

Neutravidin was obtained from Pierce (Rockford, IL, USA). Third-generation (G3) PAMAM dendrimers (Fig. 1) were purchased from Aldrich. They were used without further purification. Tris–HCl buffer (10 mM Tris–HCl, 70 mM NaCl, 0.2 mM EDTA, pH 7.4) was used to prepare solutions. They and all other chemicals were analytical reagent grade.

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

2.2. 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. The resonator was immersed in a 1 mg/ml neutravidin solution for 30 min to obtain the neutravidin layer. It was rinsed with buffer and water. Then it was immersed in a 1.0×10^{-6} M biotin-TAR RNA for 60 min, and rinsed with buffer

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0014-5793/04/$30.00
doi:10.1016/S0014-5793(04)00284-4

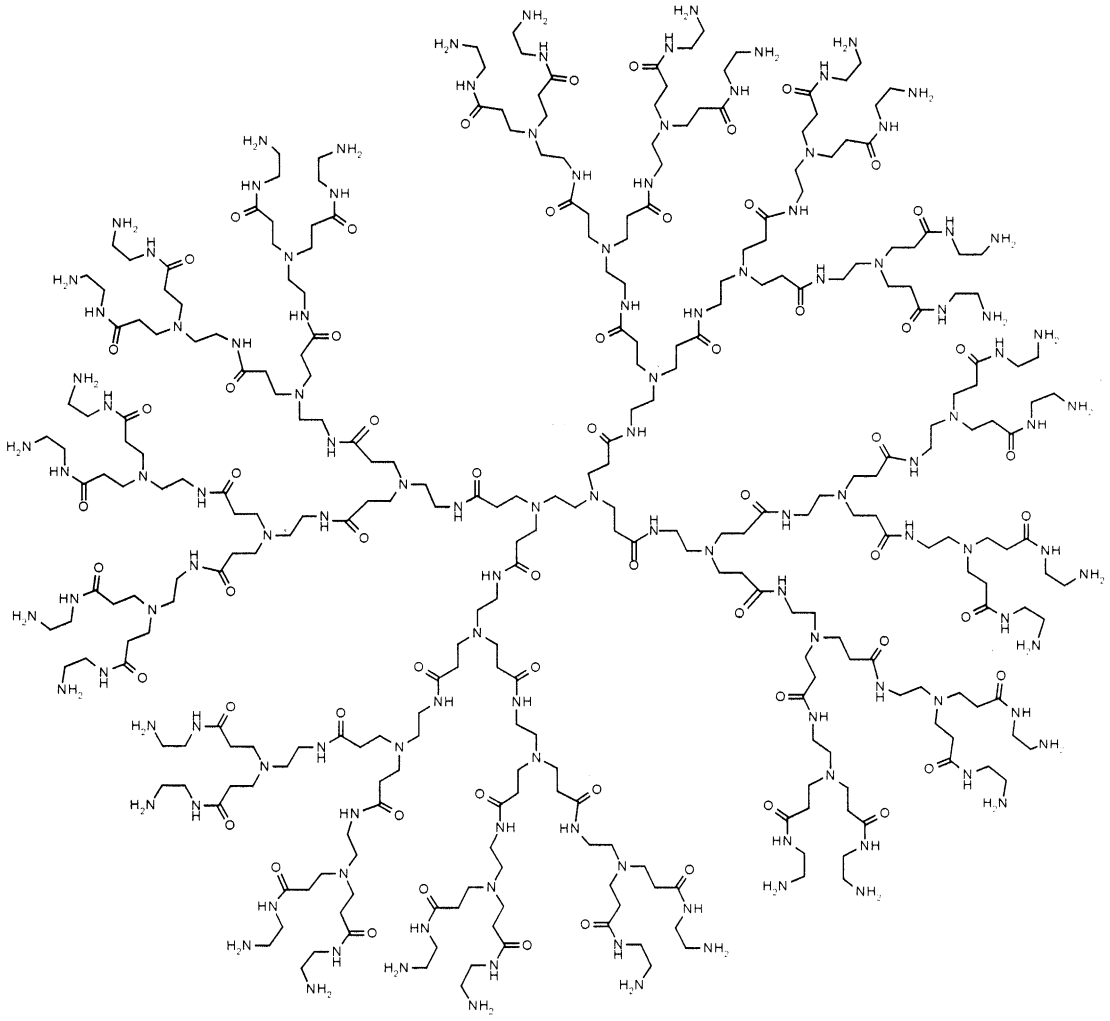

Fig. 1. Chemical structure of G3 PAMAM dendrimers.

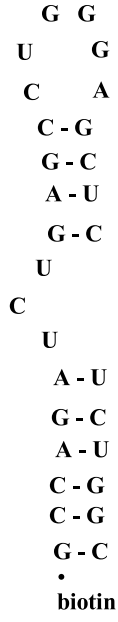

Fig. 2. Secondary structure of biotinylated HIV-1 TAR RNA.

and water similarly. Then it was immersed in a PAMAM dendrimer (Fig. 4A) or Tat peptide (Fig. 4B) solution for 60 min. After rinsing, it was immersed in a PAMAM or Tat peptide solution for 60 min. All experiments were carried out at room temperature (22 ± 1°C).

2.3. Atomic force microscope (AFM) measurements

The surface morphology of the thin film was studied by tapping mode AFM (Nanoscope III, Digital Instruments, USA).

3. Results

3.1. AFM analysis

Mica was chosen as substrate because of its atomically flat surface over a large area. A drop of PAMAM dendrimer solution spreads rapidly across the mica surface in a thin layer because of the high hydrophilicity of the mica surface when it is deposited on a freshly cleaved mica surface. Cationic PAMAM dendrimers can be easily adsorbed on the negatively charged mica surface by electrostatic attractive force. It can be seen from Fig. 5A that PAMAM dendrimers are uniformly adsorbed on mica. Comparing Fig. 5B to Fig. 5A, it can be

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg

Fig. 3. Primary structure of HIV-1 Tat peptide.

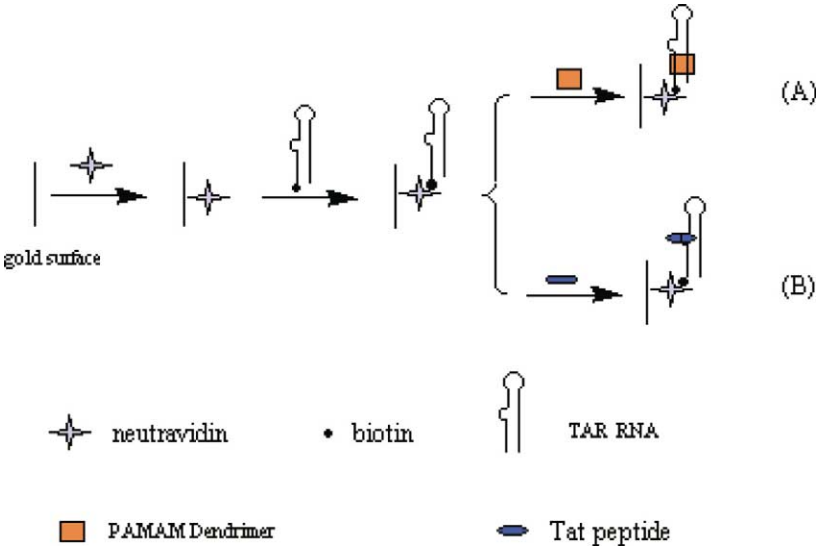

Fig. 4. Schematic illustration of the recognition processes.

easily seen that TAR RNA can form complexes with PAMAM dendrimers.

3.2. QCM analysis

Using the highly specific and extremely strong non-covalent binding of biotin to the deglycoprotein neutravidin ($K_D = 10^{-15}$ M) [26], biotin–TAR RNA was immobilized onto the neutravidin-modified QCM surfaces.

As shown in Fig. 6A, an increase in response is observed when the concentration of PAMAM dendrimer is increased. No obvious mass change was observed when only neutravidin was present on the sensor surface (Fig. 7B), suggesting that PAMAM dendrimers interact specifically with TAR RNA.

Fig. 6B shows that an increase of Tat peptide concentration resulted in increased mass change, subsequent leveling off is achieved as the surface saturates. Fig. 7D shows that Tat peptide could not be attached to the neutravidin layer, indicating that Tat peptide interacted specifically with TAR RNA.

As seen from Fig. 7A, no obvious mass shift is observed when Tat peptide binds to TAR–PAMAM complex, implying that the interaction of Tat with TAR is blocked by PAMAM dendrimer. On the other hand, significant mass change is obtained when PAMAM dendrimer binds to Tat–TAR complex (Fig. 7C), indicating that a ternary complex of TAR–Tat–PAMAM dendrimer is formed, in agreement with [27].

4. Discussion

The interactions between Tat and TAR are critical for virus replication. Furthermore, RNA is the genetic material of HIV, indicating that targeting of specific RNA structure will provide numerous opportunities for the design of novel anti-viral drugs. Indeed, various types of cationic molecules, including aminoglycoside antibiotics [13,14], heterocyclic molecules [28,29], polyamine-acridine-based compounds [30] and PAH [20], have been employed as HIV inhibitors. These diverse materials have in common the ability to bind TAR RNA via charge-based interactions.

PAMAM dendrimers are an interesting new class of highly defined, spherical, cationic polymer [31]. The unique structural features and properties of PAMAM dendrimers make them ideally suited both for a wide range of biomedical applications and as platforms for biomimetic chemistry. Because of the high number of positive charges on their surfaces, PAMAM dendrimers form a stable electrostatic complex with negatively charged nucleic acids in buffer at physiological pH. Our studies indicated that PAMAM dendrimers could form a complex with TAR RNA and effectively inhibit the binding of Tat to TAR RNA, suggesting that binding of PAMAM dendrimers to TAR RNA changes the molecular structure of TAR RNA. And the structural transition induced by PAMAM dendrimers

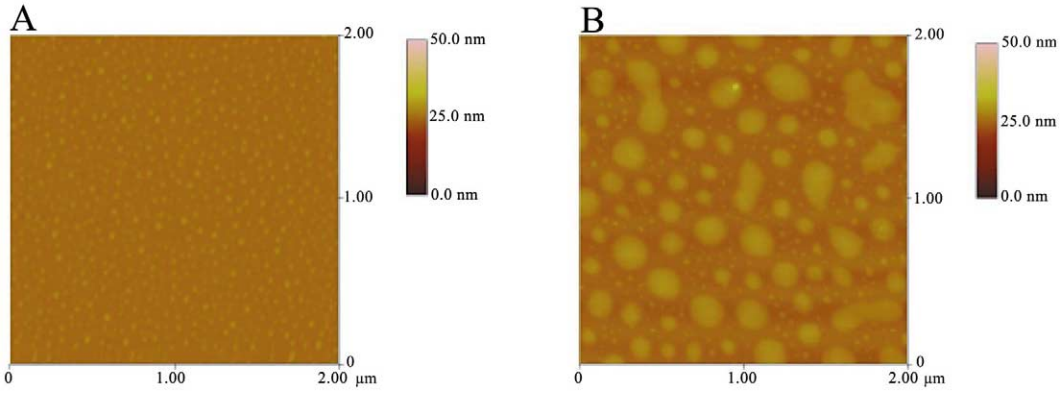

Fig. 5. AFM images of G3 PAMAM dendrimer (A) and G3 PAMAM dendrimer–TAR RNA complex (B) on mica.

is apparently incompatible with the binding of Tat. We have not studied specific binding. We think it will be a good subject for our future studies. Just as reported by Du et al. [33], compounds capable of locking TAR into a stable conformation, regardless of similarity to the Tat binding conformation, should prove useful in inhibiting HIV-1 viral replication. Therefore PAMAM dendrimers are potent inhibitors for HIV-1 viral replication. Furthermore, the interaction between fixed TAR RNA and the species in solution could be depicted by an equation similar to the Langmuir adsorption isotherm. That is

$$\Gamma = \Gamma_{\infty}\frac{C}{(K_D + C)} \quad (1)$$

where Γ is the binding amount (mol) of Tat or PAMAM to TAR (capacity for TAR–Tat or TAR–PAMAM binding), Γ_{∞} is the maximum amount of Tat or PAMAM binding (mol) to TAR, which should be equal to the amount of TAR if they are in a 1:1 combination, K_D is the dissociation constant of the PAMAM–TAR or Tat–TAR complex, and C is the concentration of PAMAM or Tat in solution. Calculating results shows that the combination coefficient (K_D^{-1}) of Tat–TAR is 1.1×10^5 M^{-1}, which compares well with the value reported in the literature [32], although the physical background and calculated equation are different (TAR in our case is fixed on the solid substrate), whereas the combination coefficient of PAMAM–TAR is 1.8×10^7 M^{-1}, 100 times higher than that of Tat–TAR, suggesting that PAMAM dendrimer has much stronger affinity for TAR than Tat does.

Comparing the present results with those of our previous studies [20], we found that the concentration of inhibitor decreased by one order. That is to say, the binding of Tat peptide to TAR RNA could be blocked by 3.0×10^{-7} M PAMAM dendrimer or 2.0×10^{-6} M PAH [20], which may be due to spherical PAMAM dendrimers having a lower steric effect than linear PAH when they bind to TAR RNA.

In conclusion, this paper demonstrates that PAMAM dendrimers could form a stable complex with TAR RNA. PAMAM dendrimer has a stronger affinity for TAR RNA than Tat does. PAMAM dendrimer could effectively disrupt Tat–TAR RNA interaction. The proposed methods and the unique structural features and properties of PAMAM dendrimers have great significance for the design of new drugs.

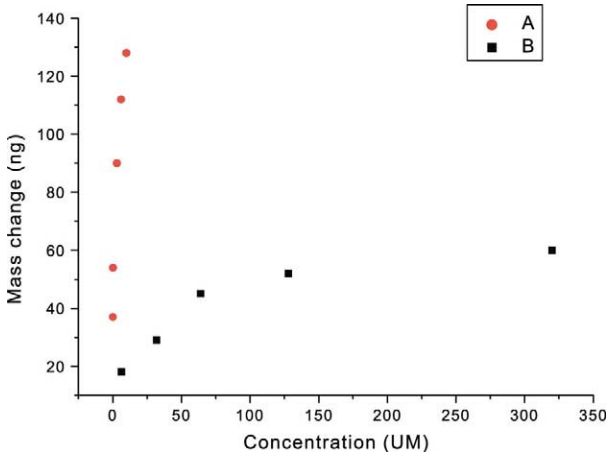

Fig. 6. Mass–concentration plots for the binding of PAMAM dendrimer (A) and Tat (B) to TAR RNA.

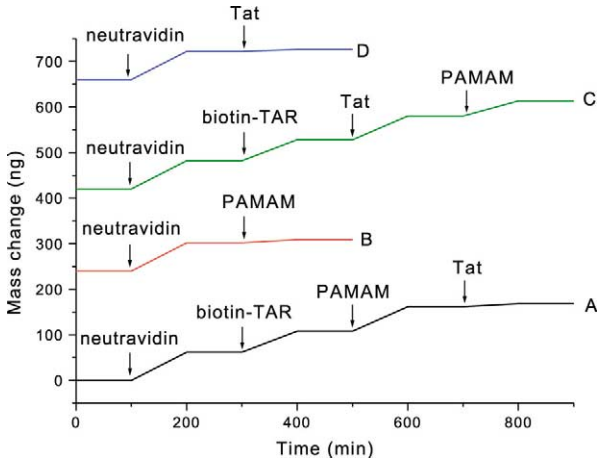

Fig. 7. Mass change for sequential introduction of analytes. A: Neutravidin (1.0 mg/ml), biotin–TAR (1.0×10^{-6} M), PAMAM (3.0×10^{-7} M) and Tat peptide (1.3×10^{-4} M). B: Neutravidin and PAMAM. C: Neutravidin, biotin–TAR, Tat peptide and PAMAM. D: Neutravidin and Tat peptide. Analytes of B, C and D have the same concentrations as those of A.

Acknowledgements: We thank the Chinese Academy of Sciences and National Natural Science Foundation of China (90206035) for their financial support.

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