Colorimetric Detection of HIV-1 Ribonuclease H Activity by Gold Nanoparticles

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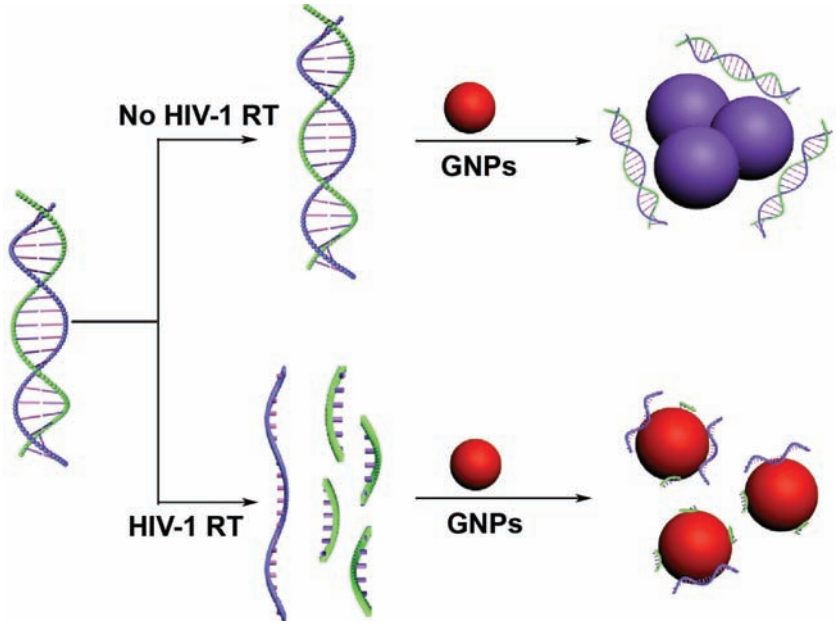
Acquired immune deficiency syndrome (AIDS) is generally characterized by infection of human immunodeficiency virus type 1 (HIV-1). Despite enormous efforts in developing antiretroviral drugs, coping with the drug resistance of HIV-1 remains a daunting task.[1] The ribonuclease H (RNase H) activity of HIV-1 reverse transcriptase (RT) plays a crucial role in the viral replication cycle and degrades the RNA strand of an RNA–DNA hybrid.[2] However, to date, very few inhibitors of the RNase H with potential therapeutic values have been identified.[1,3] A major barrier to the discovery of an RNase H inhibitor is attributed to the lack of suitable assay systems.[1,4] Commonly used methods that rely on gel electrophoresis and high-performance liquid chromatography are generally cumbersome, time-consuming, and unsuitable for real-time measurements.[3b,5] In contrast, fluorescence resonance energy transfer (FRET) methods allow high-throughput homogenous screening for inhibitors of the RNase H with high detection sensitivity, but they suffer from several drawbacks that include DNA/RNA premodification with organic fluorophores and quenchers as well as the requirement of specific oligonucleotide sequences.[1,4,6]



Scheme 1. Illustration of colorimetric detection of HIV-1 RNase H activity using unmodified gold nanoparticles.

Recently, nanoparticles have shown great promise for potential applications in biological detection and imaging.[7] In particular, gold nanoparticle-based colorimetric assays have led to low-cost, low-volume, and rapid detection of an extraordinary array of analytes owing to the unique optical and surface properties of the gold nanoparticles.[8] There are two major classes of gold nanoparticle-based colorimetric assays. The first class is based on a three-component sandwich assay format that includes a target oligonucleotide strand and two sets of oligonucleotide-modified nanoparticles.[9,10] The second class of the colorimetric assays relies on different binding affinities of a single-stranded DNA (ssDNA) and a double-stranded (dsDNA) toward unmodified gold nanoparticles.[8c] An important feature of the second class is that negatively charged ssDNA sequences can effectively stabilize nanoparticles against salt-induced aggregation, providing a convenient route for colorimetric assays without surface biomodification of the nanoparticles.[8c,11] On the basis of previous studies, we show that it is plausible to develop a rapid method for the colorimetric detection of HIV-1 RNase H activity by using unmodified gold nanoparticles.

Scheme 1 shows the principal design of our approach. A synthetic RNA–DNA duplex substrate is first incubated with HIV-1 RT. Under predefined buffer conditions, the HIV-1 RT should effectively cleave the RNA into fragments, resulting in the dissociation of ssDNA and ssRNA probes at room temperature (≈28 °C). Upon addition of gold nanoparticles to the solution, we reason that the dissociated probes can form

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DOI: 10.1002/smll.201002150

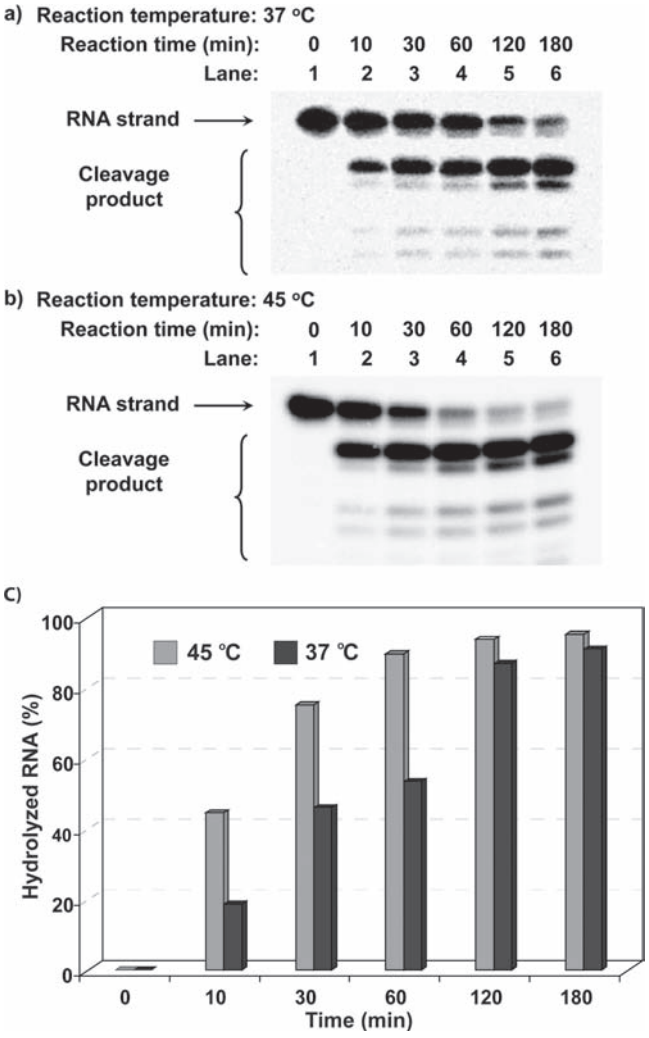

Figure 1. RNA cleavage assays by HIV-1 RNase H. a,b) Autoradiograms of polyacrylamide gel-separated RNA cleavage products obtained by addition of HIV-1 RNase H and incubation at 37 and 45 °C, respectively. Lane 1 (control): without HIV-1 RT. Lanes 2–6: with added HIV-1 RT. c) The amounts of hydrolyzed RNA obtained as a function of time after incubation at 37 and 45 °C, respectively.

a charged protecting layer on the surface of the nanoparticles and subsequently stabilize the nanoparticles at a precisely defined salt concentration. However, if there is no HIV-1 RT or if the enzyme is inactive, the RNA–DNA duplex should remain intact. Upon adding gold nanoparticles, salt-induced aggregation of the particles occurs with a concomitant change in color.

We first used ^{32}P-end-labeled RNA to screen and optimize RNase H reaction conditions. In a typical experiment, the RNase H reaction was carried out in a buffer solution (50 mM Tris-HCl pH 8, 3 mM $MgCl_2$, 20 mM NaCl) containing 8.1 units of HIV-1 RT. The reaction was initiated by addition of HIV-1 RT to a preprepared RNA–DNA duplex (100 pmol) with 18 base pairs. After incubation for different time intervals (10, 30, 60, 120, and 180 min), the reaction was quenched by addition of denaturing loading buffer. To verify the cleavage products of the RNase H, we analyzed the reaction mixtures by denaturing polyacrylamide gel electrophoresis (PAGE, 20%).[12] The results of PAGE analysis showed that the RNase H could cleave the RNA in the RNA-DNA duplex into fragments (lower bands shown in **Figure 1**a,b). The amount of the RNA fragments increased with extended reaction time. Importantly, the rate of the cleavage reaction was strongly affected by the temperature (Figure 1c). At 45 °C, approximate 90% of the RNA was cleaved by RNase H after 60 min, in stark contrast to ≈50% of the RNA cleaved by RNase H at 37 °C.

In a further set of experiments, we examined the effect of active RNase H enzyme on the stability of colloidal solutions. It is well known that certain enzymes are able to stabilize unmodified gold nanoparticles, thereby causing interference with the received color readout signal.[13] As a control experiment, we found that the enzyme-mediated stabilization of gold nanoparticles can be largely suppressed through enzyme deactivation by incubating the reaction mixture at 37 °C overnight (Supporting Information (SI), Figure S1). To optimize the reaction conditions, we carried out enzyme deactivation studies at different temperatures.[14] As shown in **Figure 2**,

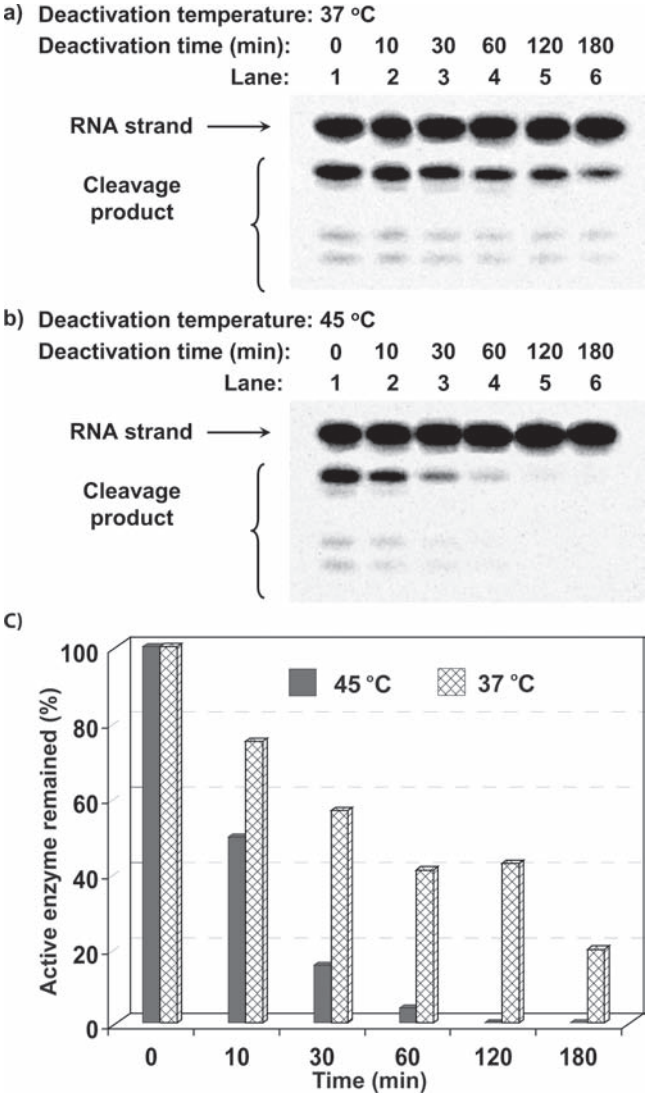

Figure 2. Thermal deactivation screening of RNase H. a,b) Comparative autoradiograms of polyacrylamide gel-separated RNA cleavage products obtained by addition of pre-deactivated HIV-1 RNase H at 37 and 45 °C for different times. c) The amount of remaining active enzyme obtained as a function of time after predeactivation at 37 and 45 °C, respectively.

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the RNase H enzyme retained ≈40% activity after incubation at 37 °C for 60 min. In contrast, less than 5% enzyme activity was observed after incubation at 45 °C for 60 min and essentially no enzyme activity was detected after 120 min. Taken together, our results show that the optimum temperature of 45 °C allows both efficient cleavage of RNA and rapid deactivation of RNase H enzyme.

To validate our hypothesis that the colorimetric detection of HIV-1 RNase H activity is feasible by using unmodified gold nanoparticles, we first added RNase H enzyme (8.1 units) to a reaction solution (20 μL) containing a preprepared RNA-DNA duplex (5 μM). The resulting reaction mixture was incubated at 45 °C for 120 min. It should be noted that no dithiothreitol (DTT) or bovine serum albumin (BSA) was added to avoid DTT-induced aggregation or BSA-assisted stabilization of gold nanoparticles.[7a] Upon addition of gold nanoparticles (≈14 nm) to the reaction mixture, no aggregation of nanoparticles was observed (**Figure 3**, inset). In stark contrast, we observed a clear red-to-blue colorimetric response for the reaction mixture without the RNase H enzyme, which was further confirmed by the presence of a broad 650 nm absorption peak. More importantly, the colorimetric response of the particle solution is highly sensitive to the concentration of the RNase H enzyme. With the aid of a UV–vis spectrometer, a low concentration of 27 units mL^{-1} can be readily detected (**Figure 4**).

In conclusion, we have demonstrated a rapid, convenient method to the colorimetric detection of HIV-1 RNase H activity through use of unmodified gold nanoparticles. In contrast to conventional methods, this approach offers significant advantages in terms of non-premodified RNA and fast assay response. We expect that this method, once refined, could be particularly useful for screening other types of enzymes and drug molecules.

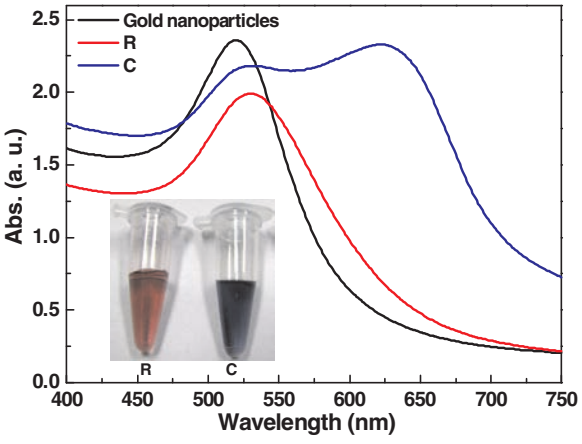

Figure 3. UV–vis spectra of gold nanoparticle solutions obtained upon addition of reaction mixtures in the presence or absence of HIV-1 RNase H (8.1 units). The inset shows the corresponding colorimetric responses of the resulting reaction mixtures. R refers to the reaction containing a RNA–DNA duplex and the RNase H enzyme. C refers to the control reaction without the RNase H enzyme.

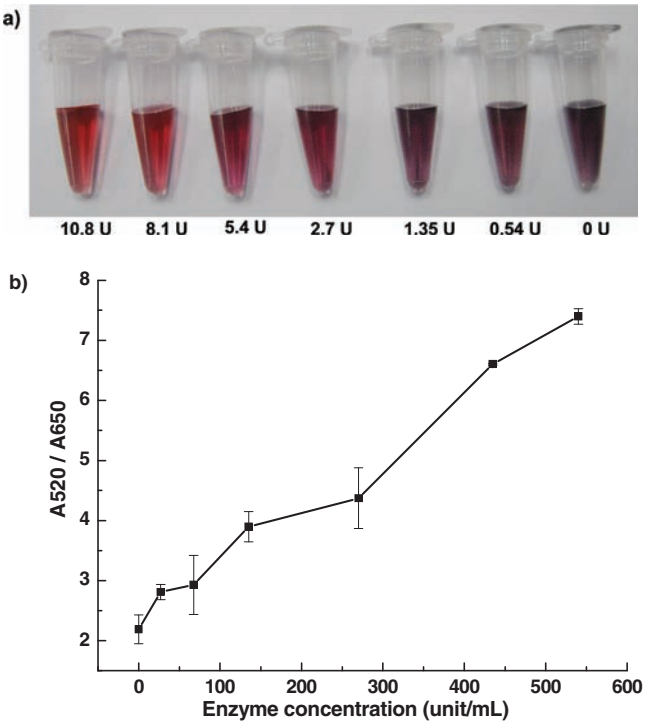

Figure 4. Colorimetric assays of RNase H enzyme at different concentrations. a) Photographic images showing colorimetric responses of the reaction mixtures on addition of different amount of RNase H enzyme. b) The corresponding absorption ratio of the resulting solution at 520 and 650 nm as a function of the enzyme concentration.

Experimental Section

HIV-1 RNase H Activity Detection: In a typical experiment, HIV-1 RNase H activity was measured in a 20 μL solution containing 50 mM Tris-HCl pH 8, 3 mM $MgCl_2$, 20 mM NaCl, 5 μM DNA and 5 μM RNA (DNA: 5'-AGC TCC ACG GCT CAG ATC-3'; RNA: 5'-GAU CUG AGC CGU GGA GCU-3'). The solution was first heated to 70 °C and then slowly cooled to room temperature. The RNA cleavage reaction was subsequently initiated by addition of HIV-RT (Worthington Biomedical Corporation) to the RNA-DNA duplex. The resulting reaction mixture was incubated at 45 °C for 2 h, at which time a 100 μL solution of unmodified gold nanoparticles was added. The colorimetric response of the solution was recorded after 5 min.

Polyacrylamide Gel Electrophoretic Analysis: The RNA cleavage process was monitored by denaturing PAGE with autoradiography. In a typical experiment, a RNA strand (200 pmol) was first labeled with [γ-^{32}P] ATP at the 5' end in the presence of T4 polynucleotide kinase, followed by PAGE purification (20%). Subsequently, the slice of band containing the [^{32}P]-labeled RNA strand was separated and eluted by soaking in Tris-HCl (10 mM, pH 7) for 3 h, followed by purification by gel filtration chromatography (NAP-25, GE Healthcare) eluting with ultrapure water. The purified [^{32}P]-labeled RNA strand (ca. 0.16 pmol) was then added to the solution containing an equal amount (100 pmol) of RNA and DNA strands. The mixed strands were annealed at 70 °C for 1 h, at which time RNase H enzyme (8.1 units) was added. The reaction was stopped by addition of denaturing loading buffer (10 μL, 95% (v/v) formamide, 18 mM EDTA, 0.025% SDS, 10% (w/w) xylene FF and bromophenol blue). The reaction products were analyzed by electrophoresis in a

20% polyacrylamide gel. Autoradiographic images were analyzed and quantified using the ImageQuant software.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work is supported by the National University of Singapore (R-143-000-427), the Ministry of Education (R-143-000-453), the Singapore-MIT Alliance, and the Agency for Science, Technology, and Research (R-143-000-366). This Communication is part of the Special Issue on Nanotechnology with Soft Matter.

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Received: December 1, 2010
Revised: January 24, 2011
Published online: March 24, 2011