Single-walled carbon nanotubes binding to human telomeric i-motif DNA: significant acceleration of S1 nuclease cleavage rate†

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Single-walled carbon nanotubes (SWNTs) binding to human telomeric i-motif DNA can significantly accelerate S1 nuclease cleavage rate by increasing the enzyme turnover number.

Single-walled carbon nanotubes (SWNTs) exhibit unique electronic, mechanical and structural properties,[1,2] and have been considered as the leading candidate for nanodevice applications.[3–9] Molecular recognition between carbon nanotubes and DNA molecules has great potential for developing nanodevices, biosensors, field-effect transistors, and gene delivery.[5,6,10–16] By screening a library of DNA oligomers, Zheng *et al.* reported that a particular sequence of single-stranded DNA self-assembles into a helical structure around individual carbon nanotubes.[17,18] A novel optical detection system based on DNA B–Z transition on SWNTs that can serve as a sensor in living mammalian cells has been reported.[19] We have reported[20,21] that SWNTs bind to double-stranded DNA in the major groove and result in a sequence-dependent B–A transition,[20] and our recent studies show that SWNTs can selectively stabilize human telomeric C-rich DNA[21] and induce i-motif DNA formation under physiological conditions or even at pH 8.0, and compete with DNA duplex association, showing the intriguing potential to modulate human telomeric DNA structures *in vivo* which is of great interest for drug design and cancer therapy.[21] Fluorescence results and S1 nuclease cleavage patterns show that SWNTs bind to i-motif DNA in the 5′-end major groove.[21] S1 nuclease is widely used as an analytical tool for the determination of nucleic-acid structure. It binds to an exposed single strand, such as in a hairpin loop, and attacks on the O–3′-P bond.[22–26] Therefore, it is important for carbon nanotube application in biocatalysis and biomaterials to study how SWNTs can affect S1 nuclease kinetics. The sequence of human telomeric i-motif DNA used here is d$(CCCTAA)_3CCCT$. The four cytidine stretches form an i-motif which includes six intercalated $C{\cdot}C^+$ pairs and terminates with the cytidines at the 5′ extremity of each stretch at slightly acidic pH. The second TAA loops across one of the narrow grooves while at the bottom the first and third TAA loop across the wide grooves (shown in Fig. 1).[21,27] Therefore, there are three TAA loops in i-motif DNA, which give the chance for S1 nuclease cleavage.[21,28] Our previous studies show that SWNTs bind to the i-motif in the 5′-end major groove by interacting with $C{\cdot}C^+$ base pairs and the TAA loop.[21] In this report, we studied the effect of SWNTs on the kinetics of S1 cleavage and found that SWNTs can significantly accelerate S1 enzymatic cleavage rate by increasing the turnover number, k_{cat}, more than 22-fold, which was analysed by the Lineweaver–Burk method, high performance liquid chromatography (HPLC) and gel electrophoresis. To our knowledge, there is no report to show that SWNTs can accelerate the enzyme catalytic rate and significantly increase the turnover number.

The sequence we studied was labeled by fluorescein at the 5′-terminal of natural sequence[21,28] d[$(CCCTAA)_3CCCT$] (i-motif DNA), and the modified group does not change the i-motif structure and its enzyme cleavage.[21,28–30] From the electrophoresis, digestion of i-motif DNA alone and i-motif DNA/SWNTs complex by S1 nuclease resulted in three major cleavages,[21,28] labeled by bands 2, 3 and 4 (shown in Fig. 1A).

Semi-quantitative analysis was performed by the LabWorks 4.5 program and band intensities were compared (Fig. 1(B)). Band 1 was the excess i-motif DNA which was not cleaved by S1 nuclease. The other three bands appeared to be due to cleavage within the three TAA loops,[21] consistent with previous studies on c-myc promoter cleavage.[28,30] The first S1 cleavage (band 4) occurred near the 5′-end in the major groove with the shortest length. The second cleavage (band 3) occurred in the narrow groove with intermediate length, and the third cleavage (band 2) near the 3′-end in the major groove with the longest length, consistent with a previous report.[21] In the presence of SWNTs a higher amount of S1 nuclease cleavage was observed near the 5′-end in the major groove. At the same time, in the presence of SWNTs cleavage in the narrow groove was decreased, and a modest decrease in S1 cleavage was also observed for the site near the 3′-end in the major groove. This cleavage pattern[21] suggests that SWNTs bind to the i-motif in the 5′-end major groove by interacting with $C{\cdot}C^+$ base pairs and the TAA loop, thereby increasing the accessibility of this loop to the solvent and being more sensitive to S1 nuclease.[21,28]

We used HPLC to further study the effect of SWNTs on the S1 nuclease digestion. The cleavage was stopped by NaOH (pH 12) and formamide which served to stop the enzymatic reaction and to denature the DNA, so the peaks only represent the size of the digestion products. Peaks 1–4 in HPLC (Fig. 1(C)) correspond to bands 1–4 in the PAGE with size range from 22-mer, 17-mer, 11-mer and 5-mer (with an error of ± 1 base), respectively. The error may come from the cleavage of the TAA loop because S1 can cleave TAA at either the dT_pA or dA_pA site, the cleavage size difference being just one base. This is why peaks 1–4 are all shown as multiple peaks. Peak 5 is observed as a 'frayed end'. The difference between HPLC and PAGE is that the peak in HPLC showed the total amount of the same size, while the PAGE only indicated the fluorescent fragments with 5′ terminals. Peak 4 has

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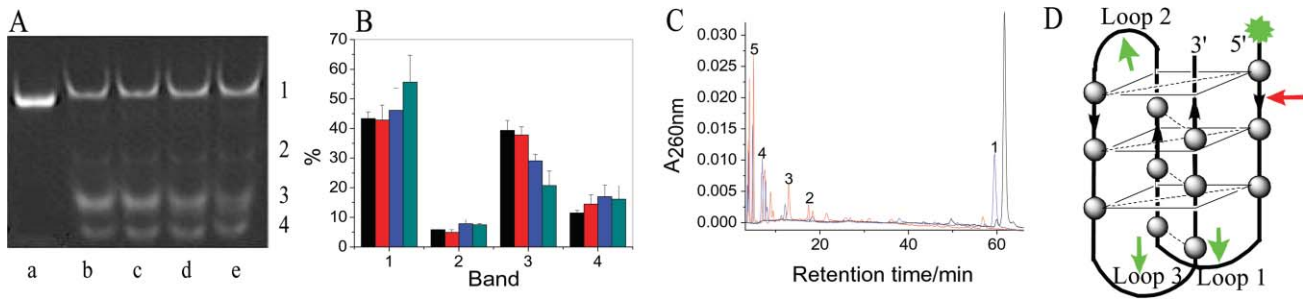

Fig. 1 (A) Image of native gel electrophoresis after S1 nuclease digestion. Untreated i-motif DNA (lane a); S1 treated complexes of i-motif DNA and SWNTs at pH 5.5 (0.75, 1.75, 5 μg, respectively) (lanes c–e). (B) Bar graph of the band intensity. The band intensity in (A) was analyzed by the software of LabWorks 4.5: black (lane b); red (lane c); blue (lane d); dark cyan (lane e). (C) HPLC analysis of the digestion products obtained by incubations of i-motif DNA and S1 nuclease in the absence (blue line) or presence (red line) of SWNTs. Separations were performed on a ZORBAX column. (D) Schematic illustration of i-motif conformation in slightly acidic pH. Cytosine bases are represented as circles. Fluorescent label is represented as a green starburst. Arrows show the possible S1 nuclease sensitive sites, bold green arrows indicate the major cleavage sites corresponding to bands 4 and 3 in Fig. 1(A); narrow green arrow represents the minor cleavage sites (band 2); red arrows represents the cleavage site of end fraying shown as peak 5 in HPLC.

the largest peak area and represents the shortest length, showing that the cleavage may occur at both 5′- and 3′-ends. Peak 2 with the longest length has the lowest peak area, which indicates that the 17-mer of the cleavage products is long enough to be cleaved a second time by S1 nuclease. In the presence of SWNTs, the area of peak 4 increased, while at the same time peaks 2 and 3 decreased, which was consistent with PAGE results. The schematic cleavage is shown in Fig. 1(D). The advantage of HPLC analysis is that it can present quantitative relations between the cleavage fragments. In combination with PAGE, it can provide detailed cleavage information which is helpful for the S1 kinetic studies in the presence of SWNTs.

The initial enzymatic reaction rate was measured as a function of time by tracking the increase in absorbance at 265 nm of the reaction mixture. Lineweaver–Burk graphs were constructed from the initial velocity data (Fig. 2). Plotting $1/V_0$ *vs.* 1/[S] yields a straight line which indicates that the S1 enzymic reaction followed the conventional Michaelis–Menten equation. Several important kinetic parameters such as V_{max} (maximum initial velocity), K_m (Michaelis–Menten constant), k_{cat} (turnover number) can be determined from the Lineweaver–Burk plot (Table 1).[31–36] The ratio of i-motif DNA : SWNTs is 9.2 : 11 (μg ml^{-1}) and the i-motif DNA concentration was varied in the linear range of the velocity. In the absence of SWNTs, V_{max} and K_m were 3.1 × 10^{-11} M s^{-1} and 3.5 × 10^{-7} M, respectively. In the presence of SWNTs, V_{max} and K_m were 6.9 × 10^{-10} M s^{-1} and 8.7 × 10^{-6} M. K_m is the character constant which is independent of the concentration of enzyme and substrate. For S1 nuclease K_m has been reported to vary from 5 × 10^{-3} to 2 × 10^{-8} M; such a significant difference in K_m is caused by different reaction conditions and the various substrates used by different authors.[32] In the presence of SWNTs, both K_m and V_{max} were increased. The catalytic efficiency (V_{max}/K_m) ranged from 9 × 10^{-5} s^{-1} for DNA alone to

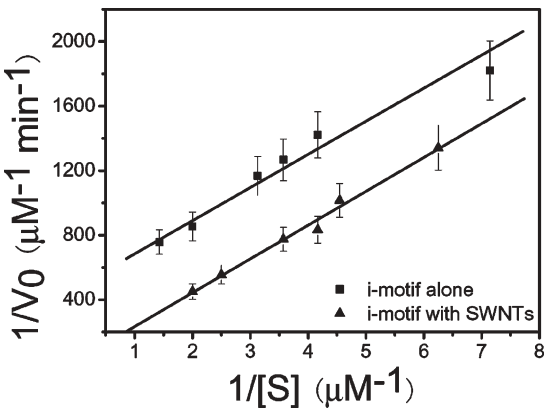

Fig. 2 Lineweaver–Burk plots of the cleavage reaction of i-motif alone (■) and i-motif with SWNTs (▲) by S1. [S1] = 10 U, the concentration of i-motif DNA was varied from 0.1 to 0.5 μM. The ratio of i-motif DNA and SWNTs was 9.2 : 11 (μg ml^{-1}). Absorbance measurements were carried out on a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. The temperature was maintained at 25.0 ± 0.2 °C.

8 × 10^{-5} s^{-1} for i-motif DNA/SWNTs. There is no obvious difference between the catalytic efficiency in our experiments. However, it has been reported that for soybean peroxidase (SBP) covalently bound with MWNTs, the catalytic efficiency (V_{max}/K_m) was nearly 40% of that for native SBP in aqueous solution.[35]

k_{cat}, the enzyme turnover number, is the catalytic rate constant, a measure of an enzyme's maximal catalytic activity, or the number of the substrate molecules converted to product per enzyme molecule per unit time. When SWNTs bind to i-motif DNA, the k_{cat} value is significantly enhanced. The k_{cat} value for i-motif DNA alone is 40 s^{-1}, but increases to 885 s^{-1} for the

Table 1 Summary of S1 nuclease kinetics parameters[a]

DNA	V_{max}[b]/mol L^{-1} s^{-1}	K_m[b]/mol L^{-1}	k_{cat}[c]/s^{-1}	(V_{max}/K_m)/s^{-1}
i-Motif alone	3.1 × 10^{-11}	3.5 × 10^{-7}	40	9 × 10^{-5}
i-Motif with SWNTs	6.9 × 10^{-10}	8.7 × 10^{-6}	885	8 × 10^{-5}

[a] $1/V_0 = 1/V_{max} + (K_m/V_{max})(1/[S])$. [b] Correlated coefficient: $R_1 = 0.98$ for i-motif alone, $R_2 = 0.99$ for i-motif with SWNTs. [c] $k_{cat} = V_{max}/[E]$, E = 10 U, 1U = 7.8 × 10^{-14} mol L^{-1}. Errors in the kinetic parameters were <12%.

i-motif DNA/SWNTs complex as the substrate. More than 22-fold increase of k_{cat} showed that the catalytic rate was increased and the number of i-motif molecules turned into product per minute was increased 22-fold when SWNTs bound to i-motif DNA. There are two possible reasons which are responsible for the increase of the turnover number. One may be the i-motif conformational change when SWNTs are bound. It should be noted that the i-motif has considerable conformational flexibility in the orientation of its phosphate groups. Our previous fluorescence and circular dichroism studies have shown that slight conformational change was observed and made the TAA loop more exposed to the solvent and more sensitive to S1 nuclease cleavage;[21] another reason may come from the stability of i-motif DNA when SWNTs are bound. NMR studies have shown that the i-motif structure is dynamic and its configuration is in fast exchange. Therefore, stabilization of the i-motif structure would make the collision between the enzyme molecule and i-motif DNA more efficient and accelerate the enzyme cleavage. We have reported that the thermal stability of the i-motif was greatly increased when SWNTs are bound.[21] The stabilized i-motif would favor S1 nuclease binding and attacking on the O–3′-P bond in the loop. Therefore, the more solvent-exposed loop structure and more stabilized i-motif DNA can make S1 nuclease cleavage much faster and significantly increase the enzyme turnover number.

In summary, SWNTs, as the leading nanodevice candidate, have potential application in biocatalysis and biomaterials. Our results show that SWNTs can significantly accelerate S1 nuclease cleavage rate and increase the turnover number 22-fold when bound to human telomeric i-motif DNA in the major groove.

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