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Accelerating chemical reactions by molecular sledding†

Lei Zhang,^{‡,ab} Lifei Zheng,^{‡,*a} Zhuojun Meng,^a Konstantin Balinin,^a Mark Loznik^a and Andreas Herrmann^{id} ^{*a}Cite this: *Chem. Commun.*, 2017, 53, 6331Received 5th April 2017,
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The speed-up of covalent bond formation was achieved between a sulfhydryl group and a 2-bromopropionic acid derivative by utilizing sliding peptide-modified substrates. Moreover, a new type of DNA cleaving reagent was developed, consisting of pVlc covalently coupled to verteporfin. This peptide–porphyrin conjugate allowed targeting of DNA and resulted in increased photodegradation of double-stranded nucleic acids.

Proteins' sliding, hopping, and jumping between DNA segments are believed to be essential to the remarkable efficiency of the search for specific binding sites in large amounts of nonspecific DNA sequences.¹ The wide-spread occurrence of one-dimensional (1D) diffusion in cellular environments facilitates many fundamental biochemical processes such as controlling transcription, unwinding, supercoiling or cleaving DNA and repairing damaged polynucleotides.² In addition to those occurring in cells, another example of 1D diffusion was uncovered in adenovirus.³ During viral maturation, the viral protease is responsible for the proteolytic processing of viral proteins to render the virus infective. Recently, single-molecule experiments have shown that this protease employs a short 11-amino acid pVlc peptide (GVQSLKRRRCF)⁴ to slide along DNA inside the virus particle and thus effectively processes its protein substrates by reducing the dimensionality of search from three to one.^{3b} Moreover, the pVlc peptide has been proven to be a universal 'molecular sled', capable of carrying heterologous cargos along DNA.⁵ Thanks to this feature, we demonstrated the speed up of the non-covalent association of a pair of biomolecules by coupling each of them to a sliding peptide and adding DNA as a catalyst to induce 1D diffusion of both substrates.⁶

Besides biology, performing chemical reactions in one dimension can be equally important in chemistry because decreasing the dimensionality of 3D diffusion of reactants may drastically influence the reactivity, and thus may exhibit large potential in synthesis and catalysis. To date, studies on 1D chemistry are very rare. The few successful examples were based on topologically interlocked rotaxane-like architectures.⁷ However, the complex fabrication procedures of these supramolecular assemblies greatly limit their practicality and widespread application.

Here, we demonstrate that the ability of a peptide sliding along DNA can be used to perform chemistry in one dimension. First, we selected a nucleophilic substitution reaction between a thiol and 2-bromopropionamide as a model and show that this conversion is accelerated 18 times when employing pVlc conjugated substrates in the presence of DNA. Second, we present a new strategy to improve the photocleavage of DNA by conjugating pVlc to a clinically approved photosensitizer, namely verteporfin.⁸ Upon irradiation, the efficiency of the DNA photocleavage by the verteporfin–pVlc (vp–pVlc) conjugate was significantly enhanced compared to that of the unmodified drug.

Scheme 1 shows the components to enable covalent bond formation mediated by pVlc peptide sliding along DNA. As the first reactant, pVlc was conjugated with 2-bromopropionamide (either (*R*)- or (*S*)-enantiomer) at its N-terminus, while the thiol group was protected by a *S*-acetaminomethyl group. For simplicity, we refer to these conjugates as (*S*)- or (*R*)-Br–pVlc. The other reactant was Cy3-labelled pVlc (Cy3–pVlc) with a free thiol group acting as a nucleophile. The cyanine dye Cy3 was introduced to improve the absorbance signal of the as-formed product and thus gave rise to the opportunity of tracking the reaction kinetics by High Pressure Liquid Chromatography (HPLC). Fig. S1 (ESI†) shows a typical set of HPLC traces obtained from the reaction mixtures between 16 μ M (*R*)-Br–pVlc and 20 μ M Cy3–pVlc. Over time, the product peak at a retention time of 31.7 min increased. The formation of the product was additionally confirmed by MALDI-TOF mass spectrometry (Fig. S2, ESI†). Concomitantly, the intensity of the starting material peak of Cy3–pVlc at a retention time of 33.9 min decreased.

^a Zernike Institute for Advanced Materials, Nijenborgh 4, 9747 AG Groningen, The Netherlands. E-mail: zhenglifei0926@gmail.com, a.herrmann@rug.nl

^b Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering,

Qingdao University of Science and Technology, Qingdao, 266042, P. R. China

† Electronic supplementary information (ESI) available: Experimental procedures, synthesis procedure and Fig. S1–S11. See DOI: 10.1039/c7cc02500a

‡ These authors contributed equally.

Scheme 1 Schematic representation of the reaction between the sulfhydryl group in the dye containing the Cy3-pVlc conjugate and 2-bromopropionamide (either (*S*) or (*R*)-enantiomer) attached to the N-terminus of another pVlc molecule.

In the resulting kinetic curves, the concentrations of the formed product were plotted against time. As shown in Fig. 1, the substitution on both substrates ((*S*)- and (*R*)-Br-pVlc) proceeded significantly faster in the presence of 100-mer double-stranded DNA (100 bp DNA) compared to the background reactions performed in the absence of DNA. This particular DNA length was chosen to ensure that the primary contributor to the rate acceleration is a 1D sliding mechanism.⁶ To further elucidate the catalytic

Fig. 1 Kinetics of product formation between Cy3-pVlc and (a) (*R*)-bromide-pVlc and (b) (*S*)-bromide-pVlc in the presence and absence of 100 bp double-stranded DNA.

activity, the initial rates (V_{init}) of the substitution reactions, in the presence and absence of DNA, were calculated from the initial increase of product formation (Fig. S3, ESI†). With the help of the DNA track, the reaction between Cy3-pVlc and (*R*)-Br-pVlc was accelerated by 14.5-fold, while that applying (*S*)-Br-pVlc as a substrate reacted with a higher rate acceleration of 17.9-fold. This slight preference for the (*S*)-enantiomer indicates that the chirality of DNA was transferred to the product formation to a certain extent. Although high enantioselectivities were achieved by employing DNA as a second coordination sphere in a series of chemical transformations,⁹ we wish to emphasize that our results show the potential ability of chiral induction within an 1D synthetic reaction system, in which the distance between the reaction center and the chiral environment provided by the DNA double helix is probably further apart. More importantly, our results clearly demonstrate that the reduction of dimensionality of diffusion of the reactants from three to one makes the starting materials find each other faster and, thus, results in the overall rate acceleration of the reaction.

Encouraged by these findings, we attempted to broaden the scope of 1D chemistry based on the current system including a sliding peptide and a DNA track. As another example, we decided to conjugate pVlc onto a photosensitizer, which can produce reactive oxygen species (ROS) under light irradiation.¹⁰ Due to the sliding ability of pVlc along DNA, we expect that the generated ROS could be concentrated locally in close proximity to DNA and thus enhance the efficiency of DNA photocleavage. Since photodynamic therapy (PDT) has been limited by the short half-life ($< 0.04 \mu\text{s}$) and limited action radius ($< 40 \text{ nm}$) of ROS in biological systems,¹¹ we also anticipated that this new strategy would offer an opportunity to possibly target DNA and improve PDT efficiency by overcoming some of these longstanding limitations.

Following our design strategy, verteporfin (vp) was chosen as a photosensitizer because of two reasons: first, it exhibits an absorption wavelength at $\sim 690 \text{ nm}$, which assists tissue penetration with less photosensitivity;¹² second, it exhibits a single carboxy group that allows the convenient conjugation to a peptide by amide bond formation. Verteporfin consists of two isomers which differ by the position of the ester group (either at ring C or D; Fig. S5, ESI†). These two isomers are often present in equal proportions and they were proven to be equally active photosensitizers both *in vitro* and *in vivo*.¹³ Scheme S1 (ESI†) shows the synthetic route for the verteporfin-pVlc conjugate (vp-pVlc). After activation with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide, a maleimide was introduced at the carboxy site of vp. Subsequently, the resulting verteporfin-maleimide (vp-maleimide) was reacted with the thiol of pVlc to form the desired conjugate. Following the same coupling strategy, we synthesized another control verteporfin-peptide conjugate (vp-s) using a scrambled peptide (s-peptide, SFRRCGLRQVK), which contains the same residues as pVlc but in a random order. Presumably, such alterations will affect the sliding behavior of the peptide yet preserving the net charge. The final products were purified by HPLC and characterized by UV-Vis spectroscopy. As shown in Fig. S6 (ESI†), both conjugates show a characteristic absorption peak of verteporfin at 683 nm. The successful conjugation was further confirmed by ESI-MS

spectrometry, which showed good agreement between the obtained and expected molecular weights (Fig. S7, ESI†).

Next, the photocleavage activity of the conjugates was evaluated by using supercoiled pUC19 plasmid DNA as a model substrate (Fig. S10, ESI†). It is worth noting that in the following experiments 35% (v/v) DMF was present as a co-solvent in the buffer under physiological pH because of the poor solubility of verteporfin in an aqueous medium. We first tested the DNA cleavage efficiency of the photosensitizers in the dark, because an ideal photosensitizer should not be harmful to the target until the treatment beam is applied. For that purpose, pUC19 DNA was incubated with verteporfin conjugates overnight in the dark. The resulting samples were analyzed by agarose gel electrophoresis. As shown in Fig. S11 (ESI†), without irradiation, no detectable cleavage of DNA was observed with all photosensitizers.

Subsequently, the efficiency of DNA cleavage by verteporfin derivatives was investigated in the presence of light. The samples containing DNA and different photosensitizers at various concentrations ranging from 1 to 5 μM were irradiated by light through a 680 nm band-pass filter (excitation power: 0.5 mW cm^{-2}). Each sample was irradiated for 30 minutes and then subjected to gel analysis (Fig. 2). Larger photodamage was achieved in the presence of the vp-s conjugate, which can be attributed to the electrostatic interactions between the positively charged S-peptide and the negatively charged DNA backbone. The most efficient photosensitizer was verteporfin conjugated to the pVlc sliding peptide. Notably, in the presence of 4 μM of each verteporfin compound, almost no pUC19 DNA was detected for vp-pVlc, while there was still 45% and 94% DNA substrate left for vp-s and pure verteporfin, respectively. It is noteworthy that in our experiments, vp-pVlc conjugates cut supercoiled DNA into

Fig. 3 (a) DNA cleavage as a function of irradiation time at a photosensitizer concentration of 2 μM in 35% DMF (v/v) solution at pH = 7.4 employing a 680 nm band-pass filter (excitation power: 0.5 mW cm^{-2}). (b) Gel electrophoretic analysis showing the cleavage of supercoiled pUC19 DNA: lanes 1–6, DNA control, verteporfin 5 min, 10 min, 15 min, 20 min, and 30 min respectively; lanes 7–12 DNA control, vp-s 5 min, 10 min, 15 min, 20 min, and 30 min respectively; lanes 13–18, DNA control, vp-pVlc 5 min, 10 min, 15 min, 20 min, and 30 min respectively.

small pieces and linear or nicked DNA strands were not obtained,¹⁴ as evidenced by the disappearance of these DNA bands in the agarose gel. These results indicate that the sliding ability of pVlc might play an important role in the way of cleaving DNA, namely, pVlc shuttles verteporfin along DNA which results in multiple damage sites as well as high efficiency of cleavage.

We further tested the effect of irradiation time on DNA cleavage using 2 μM of each verteporfin derivative. As shown in Fig. 3, with only 5 min irradiation time, the majority damage of DNA was achieved. For real clinical treatment, short time exposure to light is an appealing feature because it will decrease the side effects of PDT.

A question that still remains is whether vp-pVlc also cuts conventional double-stranded DNA equally well as the supercoiled one. To give an answer, the disappearance of nicked DNA, which is the first product of the photodamage of the corresponding supercoiled DNA substrate, was followed (see the ESI† for further information, Fig. S12). Again, this analysis revealed that verteporfin attached to the functional peptide sled degraded this DNA substrate more efficiently than the two controls, *i.e.* pristine verteporfin and vp-s.

In previous work, the speed up of the kinetics of supramolecular interactions with the help of pVlc sliding on DNA was demonstrated for the acceleration of the binding between streptavidin and biotin as well as for the search of primers during the annealing phase of PCR.⁶ In this contribution, the sledding of pVlc peptide on DNA was for the first time exploited in the context of speeding up covalent bond formation and photocleavage. The degree of acceleration of the nucleophilic substitution in the current work is comparable to the one observed

Fig. 2 (a) Photodamage of supercoiled pUC19 DNA with different verteporfin derivatives in a 35% DMF (v/v) solution (pH = 7.4) after 30 min of irradiation employing a 680 nm band-pass filter (excitation power: 0.5 mW cm^{-2}). (b) Gel electrophoretic analysis of DNA cleavage: lanes 1–6, DNA control, verteporfin 1 μM , 2 μM , 3 μM , 4 μM , and 5 μM , respectively; lanes 7–12, DNA control, vp-s 1 μM , 2 μM , 3 μM , 4 μM , and 5 μM , respectively; lanes 13–18, DNA control, vp-pVlc 1 μM , 2 μM , 3 μM , 4 μM , and 5 μM , respectively.

for streptavidin–biotin binding reported previously.⁶ On the other hand, an artificial catalyst for DNA cleavage in a processive fashion has been described by Nolte *et al.* before.¹⁵ This elegant hybrid catalyst system contains a large clamp protein (*ca.* 680 amino acids) acting as a processivity mediator and a synthetic ligand and a metal center. However, the biological part needs to be produced by laborious recombinant expression followed by difficult conjugation and purification steps. In this respect, we have significantly reduced the size of the sliding scaffold to only 11 amino acids, which renders our catalytic porphyrin–peptide hybrid appealing from a synthetic point of view.

In summary, we successfully demonstrated the use of sliding peptide-modified entities to accelerate covalent bond formation and to efficiently induce photocleavage of DNA. The speed-up of the chemical conversions was achieved by reducing the dimensionality of diffusion of substrates from three to one. As a proof-of-concept, the reactivity of a bimolecular nucleophilic substitution reaction was significantly enhanced by utilizing sliding peptide-modified substrates. The rate acceleration was accompanied by a small preference for one enantiomeric substrate, which indicates the potential of chiral induction within a 1D reaction system. Moreover, we have developed a new type of DNA cleaving reagent consisting of pVlc covalently coupled to the clinically approved photosensitizer verteporfin. This gives the opportunity to overcome the life-span and active radius limitation of reactive oxygen species by bringing them in close proximity to DNA targets. Upon irradiation, vp–pVlc exhibits the highest DNA cleavage activity, and more interestingly, it cleaves DNA into short fragments which might be attributed to the diffusional behaviour of pVlc sliding along the DNA substrate. Considering the fact that a series of artificial molecular sleds were successfully developed recently,¹⁶ we envision that this work will pave the way towards more types of 1D chemistry.

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