Designing Processive Catalytic Systems. Threading Polymers through a Flexible Macrocycle Ring

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Supporting Information

ABSTRACT: The translocation of polymers through pores is widely observed in nature and studying their mechanism may help understand the fundamental features of these processes. We describe here the mechanism of threading of a series of polymers through a flexible macracyclic ring. Detailed kinetic studies show that the translocation speed is slower than the translocation speed through previously described more rigid macrocycles, most likely as a result of the wrapping of the macrocycle around the polymer chain. Temperature-dependent studies reveal that the threading rate increases on decreasing the temperature, resulting in a negative activation enthalpy of threading. The latter is related to the opening of the cavity of the macrocycle at lower temperatures, which facilitates binding. The translocation process along the polymer chain, on the other hand, is enthalpically unfavorable, which can be ascribed to the release of the tight binding of the macrocycle to the chain upon translocation. The combined kinetic and thermodynamic data are analyzed with our previously proposed consecutive-hopping model of threading. Our findings provide valuable insight into the translocation mechanism of macrocycles on polymers, which is of interest for the development of processive catalysts, i.e., catalysts that thread onto polymers and move along it while performing a catalytic action.

INTRODUCTION

DNA polymerase and λ-exonuclease are examples of naturally occurring rotaxane-like catalytic systems that bind to DNA and move along it, while performing a catalytic action, e.g., duplication or cleavage of the DNA strand. Catalysts of this type are called processive and are different from conventional catalytic systems, which convert substrate molecules in a distributive way, i.e., molecule by molecule in a random fashion without continuous attachment to a substrate.1,2 Rotaxane-like architectures have also been shown to be essential for the transport of proteins across membranes and for the packaging and release of RNA and DNA through holes or openings in viruses.3,4 In previous papers we reported that relatively simple molecules, comprising a glycoluril cage compound provided with a porphyrin roof (Figure 1a, compound 1) can behave in a similar way as the above-mentioned processive enzyme systems. As a virtue of its toroidal shape, it can bind to linear polymer chains, which thread through its cavity.5−7 The manganese(III) derivative of this porphyrin cage was found to epoxidize the alkene double bonds of a polybutadiene thread while gliding along it.8 An important question to be answered was whether the catalytic oxidation of polybutadiene by the manganese porphyrin macrocycle can be assumed to follow a random hopping mode, i.e., the random processive mechanism.9

In order to obtain a synthetic catalytic system that is capable of performing catalysis in a more sequential processive fashion, the translocation rate and the rate of the catalytic reaction need to be similar. This requires a system that displays either a slower translocation process or a faster catalytic reaction. Since the rate of catalysis is difficult to adjust, we chose for the first option and designed a porphyrin macrocycle that has a larger affinity for the polymer chain, which should lead to a slower translocation rate. We herein describe a new porphyrin cage compound 2 (Figure 1a), that has additional oxyethylene spacers in the glycoluril moiety, which results in a larger and more flexible cage structure compared to the previously reported porphyrin cage 1. We present here the polymer threading studies on the flexible macrocycles H2 2 and Zn 2 and compare these with our previous findings on the porphyrin macrocycles H2 1 and Zn 1. Threading studies on macrocycles 2 reveal a slower translocation process compared to macrocycles 1 as well as remarkable differences in polymer length dependency and energy profile of threading, which suggests that the manganese derivative of compound H2 2 may be a promising candidate for achieving sequential processive catalysis.

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Figure 1. (a) Structures of porphyrin macrocycles. Left: rigid porphyrin 1 with four single oxyethylene spacers between the porphyrin and the glycoluril moiety. Right: porphyrin 2 with four bis(oxyethylene) spacers between the porphyrin and the glycoluril moiety. (b) Viologen-functionalized polytetrahydrofurans with different number of repeating units, which are used in the threading studies.

Figure 2. (a) Schematic representation of the (de)threading equilibrium; $k$ describes the rate constant for threading, whereas $k'$ is the rate constant for dethreading. (b) Energy diagram of the threading model with the rate constants ($k$ and $k'$) and energy levels of all the individual processes ($M$: macrocycle, $P$: polymer, $V$: viologen trap, $C$: local minimum).

### RESULTS AND DISCUSSION

**Previous Studies on Compound 1.** In previous papers\(^6\,\,7\) we described a method for studying the threading of porphyrin cage compounds onto polymers of different length. To this end the polymers were blocked on one side with a di-tert-butylphenyl group and provided with a trap, i.e., a viologen molecule, which has a high affinity for the macrocyclic compound. The trap is located close to the blocking group and can only be reached by the porphyrin macrocycle if it first threads onto the open end of the polymer and then fully traverses the polymer chain (Figure 2a). Detailed analysis performed by NMR spectroscopy and MALDI-TOF mass spectrometry revealed the formation of only a 1:1 complex between porphyrin macrocycle and polymers under the conditions of the experiments.\(^6\) The threading process can be followed by recording the fluorescence emission of the porphyrin, which is quenched when it reaches the viologen trap. This quenching only occurs when the viologen is bound inside the cavity. It was found that the threading process obeys second-order kinetics and the dethreading process follows first-order kinetics and that these processes are strongly dependent on the number of atoms of the thread, i.e., the length of the polymer chain. The threading kinetics were explained by using a consecutive-hopping mechanism (Figure 2b).\(^7\,\,8\)

In this model the threading is dependent on the initial binding event and the chance of arrival of the macrocycle at the viologen trap.\(^7\) The overall threading rate constant $k$ is described by $k = k_{\text{initial}}/(n + 1)$ and the overall dethreading rate constant $k'$ by $k' = k_{\text{off}}/(n + 1)$. The rate constant for initial binding ($k_{\text{initial}}$) is independent of the length of the polymer chain, i.e., the number of atoms $n$, whereas the chance of arrival of the macrocycle to the viologen trap ($1/n + 1$) is dependent on the polymer length. In the proposed consecutive-hopping mechanism the macrocycle finds the open end of the polymer and it "hops" from one local energy minimum to the other (Figure 2b).\(^7\) Threading rates are usually high, and the association constant of the complexes between macrocycle 1 and the viologen traps of the polymers is $K_a = 10^7 \text{M}^{-1}$. The translocation speed of the macrocycle along the polymer chain is therefore not explicitly expressed in the consecutive-hopping model; however, it is statistically present in $n + 1$. Temperature-dependent measurements showed that the activation enthalpy of threading ($\Delta H^\ddagger$) is positive and remains constant, whereas the activation entropy ($\Delta S^\ddagger$) is negative and becomes more negative as the number of the atoms per chain increases. Thus, the energy barrier that has to be overcome for threading to occur is entropic in origin and depends on the length of the polymer chain.

**Kinetics and Thermodynamics of Threading for Macrocycle 2.** The target compounds $\text{H}_2^2$ and Zn 2 were synthesized according to a published procedure.\(^9\) The synthesis, characterization, and binding properties of these compounds will be described elsewhere.\(^9\) Initial conformational analysis of macrocycle $\text{H}_2^2$ based on variable-temperature NMR, UV-vis spectroscopy, and computer modeling revealed that the flexible oxyethylene units are bent toward the inside of the cavity, which leads to a closed conformation at room temperature (Figure 3). At elevated temperatures, however, the oxyethylene units move away from the cavity leading to an open conformation, which will be discussed in the next sections.

Polymers P1–P7 were synthesized as described previously.\(^7\) The threading of $\text{H}_2^2$ onto polymers P1–P7 was studied by fluorescence spectroscopy. Typically, to a known volume of $\text{H}_2^2$ 2 ([$\text{H}_2^2$] = 1 $\mu$M) in CHCl$_3$/CH$_3$CN, 1:1, v/v), 2.5 mol equiv
of polymer solution was added at 296 K, and the fluorescence emission intensity of H$_2$ 2 was measured as a function of time. The fluorescence intensity decreased over time, indicating that the macrocycle finds the open end of the polymer and threads onto the polymer chain, eventually reaching the viologen trap, after which the fluorescence of the porphyrin is quenched (Figure 4a). A similar degree of fluorescence quenching was observed for each polymer at equilibrium, independent of the polymer length, which suggests that H$_2$ 2 binds to polymers P1–P7 with similar association constants (vide infra, Table 1). While the trend in fluorescence quenching rate of H$_2$ 2 upon the addition of viologen-functionalized polymer is similar to that of H$_2$ 1 (i.e., the rate of quenching decreases as the polymer length increases), the degree of quenching is much lower in the case of H$_2$ 2. The fraction of macrocycle—viologen complex in the mixture can be quantified by the decrease in fluorescence intensity. Addition of 2.5 equiv of polymer to H$_2$ 2 gives rise to only 25% decrease in fluorescence emission, while addition of 1 equiv of guest to H$_2$ 1 under identical conditions results in 75% fluorescence decrease (see the Supporting Information Figure S1 for P2). The association constants of the polymers and H$_2$ 2, which were determined by applying second-order 1:1 kinetic binding isotherms ($K_a = 10^4$ M$^{-1}$, Table 1) (see the Supporting Information, part 3), revealed that H$_2$ 2 displays significantly lower affinities ($K_a \sim 10^3$ M$^{-1}$) for viologen derivatives P1–P7 than H$_2$ 1 ($K_a = 10^5$ M$^{-1}$). The threading experiments with H$_2$ 2 were further analyzed, and the rate constants of threading ($k$) and subsequently the free energy of activation for threading ($\Delta G^\ddagger$) were determined. The values of $k$ and $\Delta G^\ddagger$ are depicted in Figure 4b,c as a function of number of atoms per polymer chain. The trend in the polymer length dependency of the $k$ of H$_2$ 2 is different from that of the $k$ of H$_2$ 1, which in both cases shows a gradual shift from higher $k$ to lower $k$ (Figure 4b and Table 1). However, as the number of atoms in the polymer chain doubles (P2–P5) the rate of H$_2$ 2 decreases by a factor of 4, which is more than the expected factor of 2 predicted by the consecutive-hopping model for H$_2$ 1. Presumably, the energy barrier associated with traversing the polymer chain upon increasing the polymer length increases more for H$_2$ 2 than it does for H$_2$ 1. The initial threading event of H$_2$ 2 is apparently the rate-determining step for the short chain (P1–P3) threading process. However, when the chains are longer (P4–P7), the translocation step becomes the rate-determining step. We propose that the measured lower threading rates exhibited for the longer polymer chains are the result of a stronger affinity of the macrocycle for the polymer chain. Consequently, this leads to a slower translocation process, which becomes more pronounced for longer chains. Therefore, translocation becomes the rate-determining step in the overall threading process for longer chains, and this leads to deviations in the length dependency of threading of H$_2$ 2 as compared to H$_2$ 1.

The experimental data depicted in Figure 4b,c could not be explained by using the conventional consecutive-hopping model. As mentioned above, the translocation process was not included in our previous model. In order to find out if the deviation is in fact the result of the translocation process and to fit the experimental data in Figure 4b,c better, we extended our model by adding a new parameter, $\Delta G^\text{translocation}$.

The free energy of activation of the threading ($\Delta G^\ddagger$), which is calculated from the measured rate constants, can be divided into three parts:

$$\Delta G^\ddagger = \Delta G^\ddagger_{\text{initial}} + \Delta G^\ddagger_{\text{ca}} + \Delta G^\ddagger_{\text{translocation}}$$

where $\Delta G^\ddagger_{\text{initial}}$ is the free energy of activation of initial binding, $\Delta G^\ddagger_{\text{ca}}$ is the additional free energy of activation corresponding to the length-dependent chance of arrival (ca) at the trap, and $\Delta G^\ddagger_{\text{translocation}}$ is the observed extra free energy of activation of the translocation process.

The individual free energy terms are given by eqs 2–4.
224% of the number of atoms in the polymer chains and the 225temperature dependent threading experiments also the enthalpic (ΔHf) 226and entropic (ΔSf) contributions to the free energy of 227activation of threading (ΔGf) for H2 2 for each polymer 228were obtained by constructing Eyring plots (see the Supporting 229Information, Figure S2). Although slight changes in concen- 230trations of the samples resulted in significant deviations, some 231clear trends could be observed (Table 1 and Figure 5a). The 232activation enthalpy (ΔHf) is negative for each sample (except 233for P6) 11 and becomes more positive as the number of atoms 234per polymer chain increases. On the other hand, the TΔSf 235values are negative and reveal a slight trend to become less 236negative upon increasing the number of atoms per polymer 237chain. Figure 5a shows the activation parameters as a function 238of the number of atoms in the polymer chains and the 239corresponding fits obtained by eq 1. The experimental and 240theoretical data are in good agreement for ΔGf (black line) but 241scatter quite a bit for the individual activation parameters ΔHf.
281 increases upon lowering the temperature (279 for 278 atoms per polymer chain at three di
274 parameters displayed in Figure 5a, the energy of activation In order to better comprehend the transition-state
272 hopping model.7
269 (from 268 Furthermore, the value of 267 as the number of atoms per polymer chain increases. 265 under identical conditions. The activation enthalpy of threading
263 macrocycle 2 and polymers P1–P7 are remarkably different
262 from those observed for the threading of H2 1 onto P1–P7
260 under identical conditions. The activation enthalpy of threading
259 for H2 1 is positive, 258 DH = +20 kJ mol−1, and remains constant
257 as the number of atoms per polymer chain increases. 256 Furthermore, the value of TΔS becomes more negative
255 (from −15 to −29 kJ mol−1) as the chain length increases (i.e., 254 threading becomes more entropically unfavorable as the chain
253 length increases), which is in agreement with the consecutive-
252 hopping model.7

In order to better comprehend the transition-state 253 parameters displayed in Figure 5a, the energy of activation 252 for threading, 251ΔG°, was analyzed as a function of number of 250 atoms per polymer chain at three different temperatures 249 (Figure 5b). By using the fits obtained in Figure 5b the values
248 for k initial, 247ΔG° atom, and subsequently the activation parameters,
247ΔH° initial, 246ΔS° initial, 245ΔH° atom, and 244ΔS° atom were derived (Figure 5c). For macrocycle H2 2 and polymer P1 the value of k initial
242 increases upon lowering the temperature (k initial = 1.4 × 108
241 M−1 s−1 at 296 K, k initial = 3.5 × 107 M−1 s−1 at 274 K),
indicating that the initial binding of H2 2 to the open end of P1
239 is faster at lower temperatures. This consequently results in a
238 negative activation enthalpy (ΔH° initial = −31 ± 10 kJ mol−1) and
237 a negative activation entropy (ΔS° initial = −63 ± 10 kJ mol−1), suggesting that the initial binding is enthalpically
235 favorable and entropically highly unfavorable. Furthermore, the
234 values derived for ΔG° atom at different temperatures revealed
232 that ΔG° atom increases upon decreasing temperature. (ΔG° atom
230 = 9.4 J mol−1 at 296 K, ΔG° atom = 13.6 kJ mol−1 at 276 K). 229 Apparently, the energy barrier for translocation is higher at 228 lower temperatures, leading to a slower translocation process at 226 lower temperatures. From the values of ΔG° atom at different 224 temperatures the values for ΔH° atom (66 ± 20 J mol−1) and
223 TΔS° atom (57 ± 20 J mol−1) were calculated. The obtained 221 values indicate that for H2 2 the translocation process is 219 entropically driven and enthalpically unfavorable.

In a separate set of experiments we performed temperature- 217 dependent threading measurements in order to determine the
215 enthalpic (ΔH°) and entropic (ΔS°) contributions to the free energy of binding (ΔG°). Association constants for complexes 213 between H2 2 and P1–P7 at equilibrium were determined at 211 different temperatures and thermodynamic parameters were 208 obtained by using Van’t Hoff plots (see Supporting Information 206 Figure S3). As depicted in Table 1, the enthalpy of binding for 204 complex formation between H2 2 and P1–P7 has a value 202 ranging from ΔH° = −23 to −31 kJ mol−1 while the entropic
200 contribution ranges from TΔS° = 7 to −1 kJ mol−1. This
indicates that the free energy of binding $\Delta G^\circ (-30 \text{ kJ mol}^{-1}$ for P4) is mainly the result of a favorable binding enthalpy. Thermodynamic parameters observed for complexes of $\text{H}_2$ 2 with P1–P7 complexes deviate significantly from the ones obtained for complexes of $\text{H}_1$ 1 with P1–P7. Typical thermodynamic parameters for complexes with $\text{H}_2$ 2 amounted to $\Delta H^\circ = -19 \text{ kJ mol}^{-1}$ and $\Delta S^\circ = 21 \text{ kJ mol}^{-1}$, which yields a free energy of binding $\Delta G^\circ = -40 \text{ kJ mol}^{-1}$. In this case, enthalpic and entropic parameters contribute almost equally to $\Delta G^\circ$.

**Threading of Zn 2.** The threading of Zn 2 over polymers P1–P7 was studied in a similar way as for $\text{H}_1$ 2. Analysis of the data revealed that the $k$ values for threading of Zn 2 are on average 2.9 times higher than those of $\text{H}_2$ 2 (compare Table 1 with Table 3). This observed threading behavior is in contrast to the behavior observed for $\text{H}_1$ 1 and Zn 1 in which threading of $\text{H}_1$ 1 is faster than that of Zn 1. This difference could be the result of the coordination of the zinc center of Zn 2 to the oxygen atoms in the oxyethylene moieties of the host (see the Supporting Information, Figure S6). The polymer length-dependency of the threading process of Zn 2, however, is similar to that observed for $\text{H}_2$ 2 and also the calculated activation parameters revealed similar values and length dependencies. Zn 2 showed a higher threading rate with decreasing temperature similar to $\text{H}_2$ 2. Furthermore, a negative and increasing (more positive) value for $\Delta H^\circ$ and a negative and increasing (less negative) value of $\Delta S^\circ$ as a function of the number of atoms in the polymer chain were observed (Table 3).

**Mechanism of Threading.** The mechanism proposed for the threading process of $\text{H}_1$ 2 is presented in Figure 6. The initial binding of the macrocycle to the open end of the polymer chain is entropically highly unfavorable ($T\Delta S^\circ_{\text{initial}} = -63 \pm 10 \text{ kJ mol}^{-1}$), which can be attributed to the loss of conformational freedom of both the flexible cavity and the open end of the polymer chain upon binding (Figure 6a). Once threaded, $\text{H}_2$ 2 moves along the polymer chain randomly, “hopping” from one local energy minimum to the other (Figure 6b,c). The derived values for $\Delta H^\circ_{\text{atom}} (66 \pm 20 \text{ J mol}^{-1})$ and $\Delta S^\circ_{\text{atom}} (T\Delta S^\circ_{\text{atom}} = 57 \pm 20 \text{ J mol}^{-1})$ indicate that the translocation process is entropically favorable and enthalpically unfavorable. This is unprecedented because one would expect that the movement along the polymer requires the stretching and ordering of the polymer chain, which is entropically unfavorable. The experimental observations, on the other hand, suggest that $\text{H}_2$ 2 has a relatively strong affinity for the chain, which leads to the conclusion that the translocation process becomes a more rate-determining factor than the rearrangement of the polymer chain. In order to translocate along the chain, $\text{H}_2$ 2 first has to adopt a more relaxed conformation in which it releases the tight binding geometry with the chain (Figure 6c,e), which is an enthalpically unfavorable but entropically favorable process. This results in a more positive $\Delta H^\circ$ and a less negative $\Delta S^\circ$ upon increasing polymer lengths, and because of this the translocation process becomes significantly more apparent in the threading process upon increasing chain length (as observed in the rate of quenching in fluorescence emission). Finally, the macrocycle $\text{H}_2$ 2 reaches the viologen trap and the system relaxes to find its energy minimum (Figure 6d,e). The chance of reaching the viologen is proportional to the polymer chain length. For $\text{H}_1$ 1 and Zn 1, where the translocation process is not expressed in the threading curves (as observed in the rate of quenching of the fluorescence emission) only the chance of arrival determines the observed length dependency, and this is expressed in extra activation entropy upon increasing chain lengths. This entropic effect is not observed for threading of polymers through $\text{H}_2$ 2 because it is compensated for by the entropically favorable translocation process.

The measured negative enthalpy of activation for the threading (e.g., $\Delta H^\circ = -19 \text{ kJ mol}^{-1}$ for the combination $\text{H}_1$ 2 and P1, Table 1) and for the initial binding event (for $\text{H}_2$ 2 in $\Delta H^\circ_{\text{initial}} = -31 \pm 10 \text{ kJ mol}^{-1}$, see above) is highly uncommon in supramolecular systems.12,13 The rate of complexation is reported for various comparable synthetic supramolecular systems decreases upon lowering the temperature, and these processes all have positive activation enthalpies.14–17 In some protein systems, however, negative activation enthalpies for binding processes have been reported,18–20 and these are mostly associated with protein folding. Furthermore, a number of reactions including Diels–Alder reactions,21 radical reactions,22 and proton-23 and electron-transfer reactions24 display negative activation enthalpies.25

For $\text{H}_2$ 2, the observed negative $\Delta H^\circ$ can be explained by its specific conformational behavior. $\text{H}_2$ 2 adopts a geometry in which the oxyethylene spacers fill the space in between the glycoluril moiety and the porphyrin. Variable-temperature11 NMR experiments showed a strong downfield shift of the oxyethylene spacer protons, suggesting that these protons move away from the proximity of the porphyrin ring upon lowering the temperature, which leads to a wider cavity (see the Supporting Information, Figure S5). Therefore, the binding of the open end of the polymer chain to the cavity becomes easier at lower temperatures. The observed increase in initial threading rate at lower temperatures and the negative $\Delta H^\circ$ may be a result of the conformational change in the molecule. The initial binding therefore has an entropic penalty; however, it is enthalpically driven.

**Theoretical Evaluation.** As mentioned above we used the consecutive-hopping model to explain the mechanism of threading for regular porphyrins7 and we extended this model in order to describe the threading mechanism of macrocycles. $\text{H}_1$ 2 and Zn 2. This model also allows us to simulate threading curves for polymers with different chain-lengths and macrocycles with a larger affinity for the polymer chain. By using these simulations, we may describe the deviations in the threading kinetics of compounds 2 compared to compounds 1 and verify the presence of a slow translocation process in the threading of the first mentioned compounds.

We previously proposed that the observed chain-length dependency of the threading process originates from the rate of initial binding of the first few atoms of the polymer chain into the cavity of the macrocycle (called entron) and the chance of arrival of the macrocycle at the viologen trap.7 The relative rates of initial binding and the movement along the chain can be calculated, but the absolute magnitudes of $k_{\text{hop}}$ (the rate constant of “hopping” of the macrocycle from one energy minimum to another) and $k_{\text{entron-off}}$ (the rate constant of the macrocycle leaving the chain) are unknown, and it is therefore not possible to derive the value of the movement rate along the chain. In order to estimate the relative magnitudes of $k_{\text{hop}}$ and the number of steady-state intermediates $n$ (local energy minima), we extended our model. The two events in the threading process, i.e., the initial binding to the open end of the chain and the movement along the chain, will be treated separately, and expressed in terms of half-life times ($t_{1/2}$).
Table 4. Half-life Times for the Initial Binding Event ($t_{1/2\text{-}entron}$) and the Translocation Process ($t_{1/2\text{-}translocation}$) As a Function of the Number of Local Energy Minima ($n$) and the Rate Constant of the Hopping Steps ($k_{hop}$)
The authors declare no competing financial interest.