POLYPEPTIDE TRANSLOCATION THROUGH A HOLE. A MONTE CARLO STUDY*

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Dedicated to Professor Andrzej Fuliński on the occasion of his 70th birthday

In this work we studied a simple model of a copolymer (polypeptide) chain in a confined space. The model chain was restricted to a flexible [310] lattice. It was represented as a sequence of united atoms located at the positions of alpha carbons. The force field introduced into the model consisted of the long-range contact potential between amino acid residues and a local helical potential. The chain was built of hydrophilic and hydrophobic segments. The properties of such chains were determined by means of the Monte Carlo simulations using a Metropolis-like algorithm. During the simulations we observed and tracked the translocation of the chain during its passage through a hole in an impenetrable wall. The influence of the length of the chain and the structure of the polymer film on the translocation process were investigated. The dynamic properties of the system such as the translocation time were also studied and discussed.

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1. Introduction

Many experimental and theoretical efforts were undertaken to determine the structure and the properties of polypeptide chains at interfaces [1]. One of them is the process of the threading of chain molecules through a pore. This process is also an important problem in biological systems: it takes place in the translocation of proteins through an endoplasmic reticulum or into mitochondria as well as in the formation of signaling protein in a cellular membrane [2]. The problem of chain molecule threading through a membrane is a complicated phenomenon. One has to remember that passing

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through a pore is governed by the presence of an entropic barrier because of the limited number of chain conformations inside and near the pore. Polymer translocation through a hole was recently the subject of numerous theoretical studies [3–7] and computer simulations [8–11]. Most of these works used one-dimensional polymer diffusion equations and were mainly focused on the mechanism of translocation. Recently it was shown that simplified models of polypeptide chains were useful to provide an insight into the behavior of real biomolecules. A simple lattice model of a polypeptide was introduced by Kolinski and Madziar a few years ago [12]. In their work the influence of the sequence of amino acid residues in the chain on its secondary structures, α-helices and β-strands, was studied. This model was extended by Romiszowski and Sikorski by introducing a local preference of helical states which made the helical content at the correct level and improved the characteristics of the folding transition [13]. After combining the classical Metropolis-type simulation method with the Histogram Method the thermodynamic properties of the system could be investigated [14]. In this work we employed the above mentioned model of polypeptide chains in order to study the threading of polypeptide chains through a hole in a impenetrable surface. The hole was in a form of a square having the edge d (in lattice units) long. This system can be treated as a crude model of a chain translocating through a pore or an idealized membrane.

2. Model and simulation algorithm

We assumed that our model chains were built of structural elements that could be treated as an approximation of amino acid residues [12, 13].

The chain was represented as follows: each amino acid residue was represented by a single united atom located at the correct alpha carbon position.

Figure 1 shows a fragment of a polypeptide chain composed of united atoms while compared to the real full-atom structure. The location of these united atoms and their orientation in space were restricted to vertices of a quasi-crystalline lattice. This lattice approximation was used in order to speed up the calculations. The lattice used was based on the following vectors [± 3, ± 1, ± 1], [±3, ±1, 0], [±3, 0, 0], [±2, ±2, ±1], [±2, ±2, 0]. It was shown that the model chains were represented with the accuracy of 0.6–0.7 Å compared with real polypeptides. This best fit for polypeptide chains was found for the lattice unit equal to 1.22 Å [12].

The model chain was put into a large Monte Carlo box with edge $L = 200$. Since the aim of this work was to study the process of the passage of the chain through a hole, we made the walls of the box impenetrable. A square hole $d \times d$ was made in one wall (the plane $x = 0$). Figure 2 presents the model chain on the [310] lattice near the impenetrable wall on

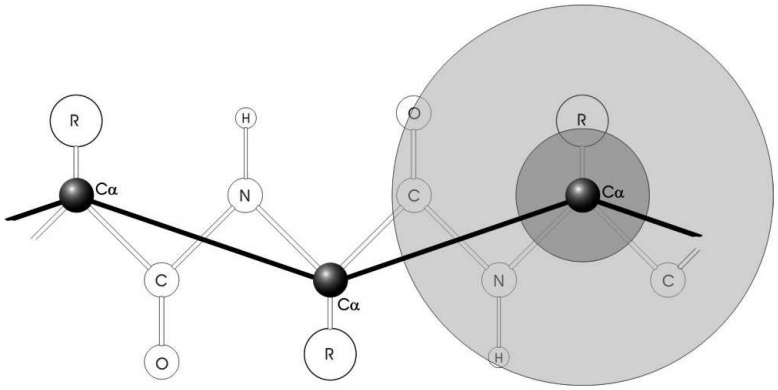

Fig. 1. The idea of a simple representation of a polypeptide chain. The spheres located on alpha carbon atoms correspond to the repulsive (internal) and soft attracting (outer sphere) values of an interaction potential.

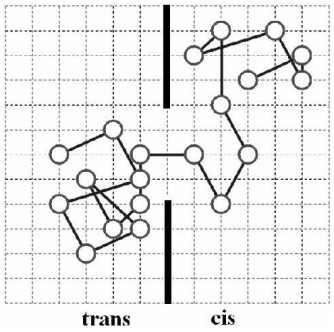

Fig. 2. An example of a model chain on [310] lattice. A fragment of the impenetrable wall dividing the Monte Carlo box with a hole is also shown.

both *trans* (x <0, *i.e.* out of the box) and *cis* (x >0, *i.e.* inside the box) sides. The force field used in our model was very simple. Each pair of non-bonded amino acid residues interacted with a contact potential V_{ij} that has the following form:

$$V_{ij} = \begin{cases} \varepsilon_{\text{rep}} & \text{for} \quad r_{ij} < r_1\,, \\ \varepsilon_a & \text{for} \quad r_1 \le r_{ij} \le r_2\,, \\ 0 & \text{for} \quad r_{ij} > r_2\,, \end{cases} \tag{1}$$

where r_{ij} is a distance between i^{th} and j^{th} residues under consideration. The repulsive part of the potential $\varepsilon_{\text{rep}} = 5$ was assumed and the radius $r_1 = 3^{1/2}$ (in lattice units). The cut-off radius of the attractive part of the potential was chosen $r_2 = 5$ (in lattice units). The attractive part of the

potential is the only parameter that defined this kind of residue as hydrophobic (denoted as H) and hydrophilic (denoted as P). The attractive part of the potential ε_{a} took the following values: $\varepsilon_{\mathrm{HH}} = -2$, $\varepsilon_{\mathrm{PP}} = -1$, $\varepsilon_{\mathrm{HP}} = 0$ for a pair hydrophobic residues, a pair of hydrophilic residues and a pair of hydrophobic–hydrophilic residues, respectively. This choice of the potential values was done following the concept presented by Shakhnovich and Gutin [15], who stated that this approach, though not very realistic for proteins, is much better than the pure "hydrophobic" potential and provides the correct ground state of the system. It was previously shown that the correct content of helical structures in low-temperature polypeptide chains could not be achieved without the preferentially of forming the helices. Therefore, we introduced a local helical potential. The right-handed α-helical state formed by three consecutive vectors could be identified from a value of the following expression:

$$r*^2_{i-1,i+2} = (\nu_{i-1} + \nu_i + \nu_{i+1})^2 \operatorname{sign}((\nu_{i-1} \times \nu_i) \cdot \nu_{i+1}) \,, \tag{2}$$

where ν_{i-1}, ν_i,ν_{i+1} were three consecutive vectors connecting $(i-1)^{\mathrm{th}}$, i^{th} and $(i+1)^{\mathrm{th}}$ residues. A right-handed α-helical state corresponded to the values of $r*^2_{i-1,i+2}$ located between 9 and 25 [12]. The appearance of a right-handed helical state in the chain during the simulation process was hence associated with the energy loss equal to certain $\varepsilon_{\mathrm{loc}}$.

In order to calculate the properties of our model we used the Monte Carlo simulation method. The chain was placed in the box, *i.e.* on the *cis* side. The C-terminus (the first bead) was located one lattice unit apart from the hole in the impenetrable surface. The conformation of the chain was chosen randomly and then it was locally modified using the usually applied set of micromodifications: (a) one-residue motion, (b) two-residue motion and (c) two-residue end reorientations [14]. These micromodifications of conformation were selected randomly along the entire chain. A driving force parallel to the x axis was applied to the model in order to emulate a static electric field. The value of this force was 0.2 what is negligible when compared with the total energy of the chain. A new trial conformation that appeared after a micromodification was accepted according to the Metropolis criterion. A large number of the above mentioned local changes of the chain's conformation were carried out. Whenever the chain diffused apart from the hole the simulation was suspended and the chain was placed back in the vicinity of the hole. When the entire chain passed through the hole the entire simulation process was started from the beginning. Simulation runs were performed several times (usually 30) starting from quite different initial conformations of chains. As we studied the dynamic properties of polypeptide chains the time unit had to be defined. It was previously shown

that the above set of the chain's micromodifications produced the correct dynamics when the time unit consisted of one attempt of each kind of motion per one amino acid [12].

3. Results and discussion

In the present work we studied a chain built of $N = 10$ to 100 residues. The polypeptide chains consisted of typical helical septets $-$HHPPHPP$-$, which can be found in real helical proteins. The helical potential $\varepsilon_{\rm loc}$ was assumed to take values 0, -4 and -8. It was assumed that the temperature on the *cis* side (inside the box) was high ($T = 4$) while on the *trans* side (out of the box) it was low ($T = 1$). The differences in the temperature are equivalent to the differences in the solvent quality on both sides of the surface, as it takes place in the real membrane systems [2]. The detailed analysis of static and dynamic properties as well as the thermodynamic description of chains have been resented in our previous works [12–14]. Therefore, we would like to focus our attention on the problem of the translocation of a chain and its structure.

At first we studied the changes of the chain's size during the translocation process. Therefore, we calculated the radius of gyration which is a parameter commonly used the size of macromolecules. In general, the radius of gyration decreases along with the annealing of the chain [12]. In Figure 3 we present the squared radius of gyration S^2 as a function of time for parts of the

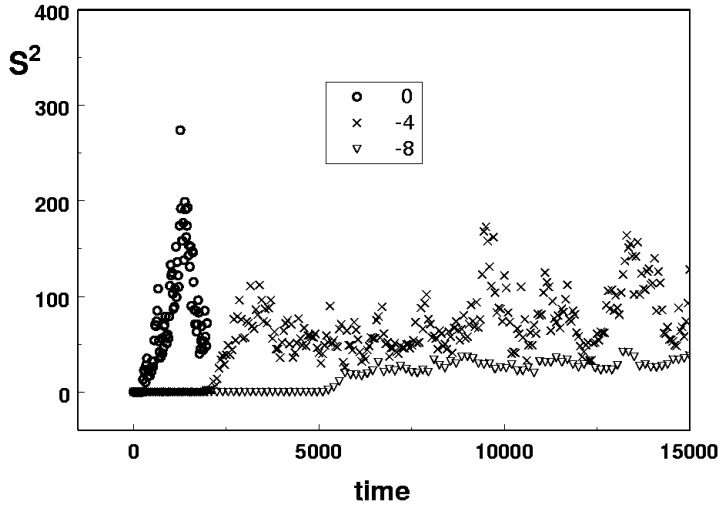

Fig. 3. The changes of the squared radius of gyration S^2 with time. S^2 was calculated for the *trans* side of the surface. The chain consisted of $N = 60$ with the local potential $\varepsilon_{\rm loc}$ shown in the legend.

chain which were out of the box. These flowcharts concerned the chain with $N = 60$ residues and the local potential $\varepsilon_{\rm loc} = 0,\ -4$ and -8. The changes of the radius of gyration of the part of the chain which was on the *trans* side of the system depended on the values of the helical potential. The stronger potential caused the fluctuations of the dimension of the chain to diminish which resulted in the formation of stable helices.

Experiments showed that the characteristics of the helix-coil transition can change when a protein chain is adsorbed or is positioned near a surface [16]. This was also confirmed by theoretical considerations [17]. Some additional insight into the behavior of the polypeptide chain during the translocation process can be given by the changes of the x contribution (perpendicular to the surface of the hole) of the radius of gyration. In figures 4–6 we present the plots of the x contribution to the squared radius of gyration calculated per one residuum S_x^2/N for different values of the local helical potential $\varepsilon_{\rm loc} = 0,\ -4$ and -8. The analysis of the x contributions of the radius of gyration gives us an information about the extension of the chain along the direction of the translocation on both sides of the surface. One can notice that in the absence of the helical potential the values of S_x^2/N fluctuated with the similar magnitude for *cis* and *trans* location. The presence of the helical potential led to much more stable structures (the fluctuations were less frequent and their amplitude diminished along with the strength of $\varepsilon_{\rm loc}$). Figure 7 presents the changes of the x position of selected residues during the translocation process. In order to monitor

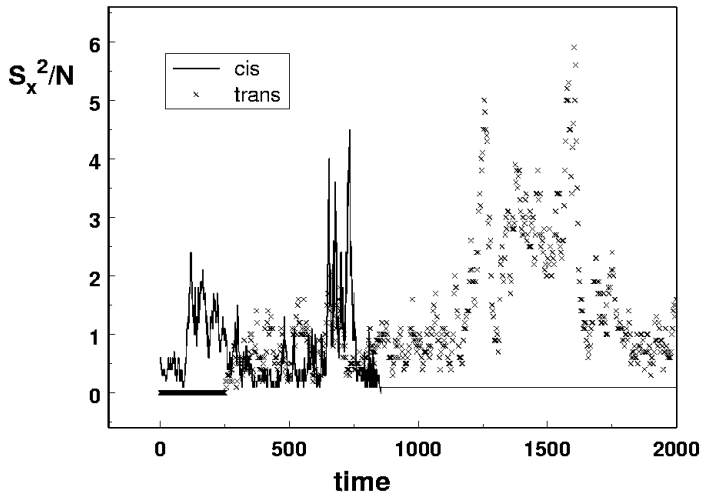

Fig. 4. The changes of the x contribution of the reduced mean-squared radius of gyration S_x^2/N during the translocation for the local potential $\varepsilon_{\rm loc}$ =0.

the process we recorded the x coordinates of the first, medium and the last residue of the chain. The impenetrable surface with the hole had a x coordinate value 0, thus the points under the x axis corresponded to the *trans* positions of the residues. As can be seen for this particular translocation process the medium part of the chain was captured in the hole. Its position did not fluctuate with the magnitude comparable to the terminal residues. The terminal residues passed onto the *trans* side significantly later after the medium part of the chain. After the translocation was completed the fluctuations of the chain position became less intensive. In most cases we observe the threading mechanism starting from one chain's end. The presented mechanism of the translocation process was observed rather seldom (about 0.1 of all cases). We decided to present it due to its curiosity.

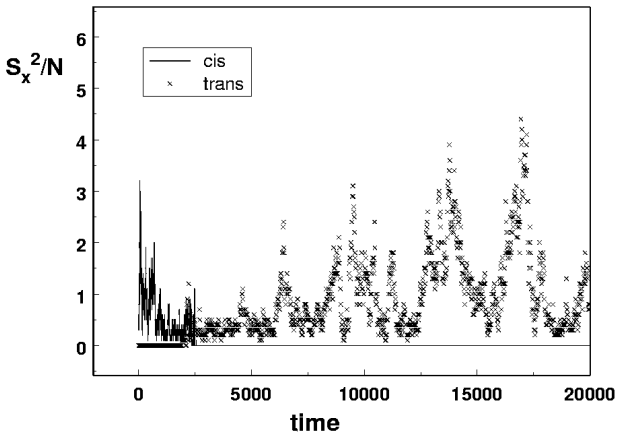

Fig. 5. The changes of the x contribution of the reduced mean-squared radius of gyration S_x^2/N during the translocation for the local potential $\varepsilon_{\text{loc}} = -4$.

One of the parameters describing the translocation process was the time needed for the molecule to pass through the pore, *i.e.*, the mean time (averaged over a large number of experiments) counted from the moment of the appearance of the molecule in the pore window and lasting to the moment in which the last residue passed that window. In these calculations all the cases in which the molecule trying to escape from the system did not succeed were not taken into account. Figure 8 presents the plot of the translocation times for the chains consisting of various numbers of residues and different values of the local potential ε_{loc}. In all cases the dimension of the pore was the same ($d = 15$). The double-logarithmic plot shows a straight line with a slope $\gamma = 2.18 \pm 0.06$ (for $\varepsilon_{\text{loc}} = -4$) and 2.21 ± 0.02 without the local helical potential. The value of this scaling exponent was in good agreement with the theoretical findings of Binder *et al.* [18] who found that $\tau \sim N^{2.18}$. The

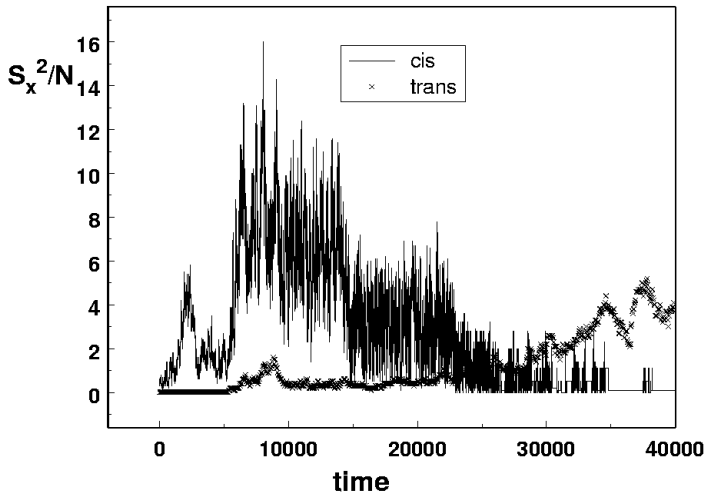

Fig. 6. The changes of the x contribution of the reduced mean-squared radius of gyration S_x^2/N during the translocation for the local potential $\varepsilon_{\mathrm{loc}} = -8$.

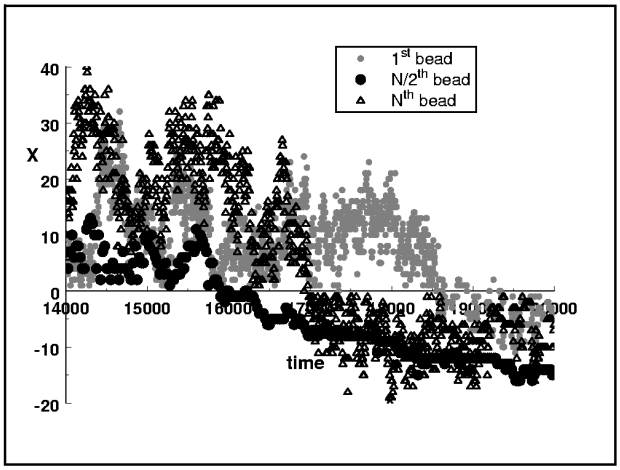

Fig. 7. The position of selected residues during a typical translocation process.

introduction of the helical potential caused the changes of the translocation times, as can be observed in figure 8. The increase in strength of the helical potential led to much longer times of passage through the pore. This can be explained by the fact that the secondary (helical) structures formed on a trans side of the box were stable and the diffusion of the system was very slow [19]. Therefore the part of the chain which was still in the box could not escape freely from it, waiting for room to move forward to the trans side.

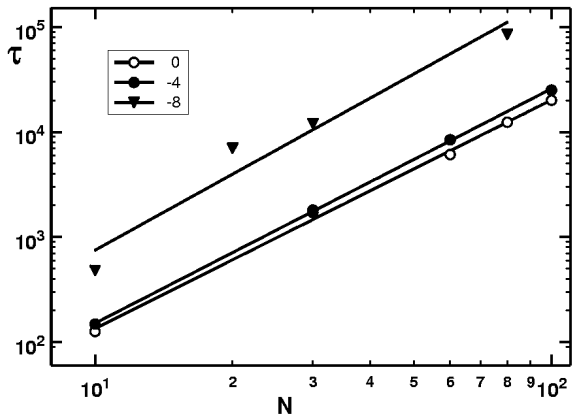

Fig. 8. The mean time of chain's translocation τ as a function of the chain length N. The case of the local potential $\varepsilon_{\rm loc}$=0 and -4 (see legend).

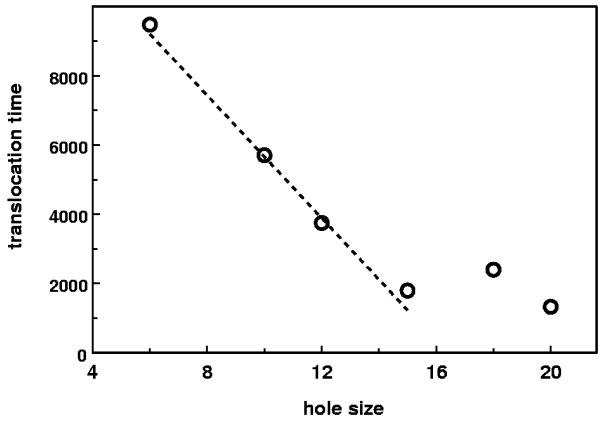

Fig. 9. The mean time of the chain's translocation τ as a function of the size of the hole d. The case of chain consisted of $N = 30$ with the local potential $\varepsilon_{\rm loc} = -4$.

The influence of the size of the pore on the translocation of a chain was also studied. Figure 9 presents the time of translocation as a function of the size of the hole for a chain consisting of $N = 30$ residues. The size of the hole d (*i.e.* the length of a square window) was changed between 6 and 20 lattice units. Since the shape of the window was square we had to take into account that its diagonal was $2^{1/2}d$ and, therefore, some larger elements of the chain could pass through it. The smallest size of the hole corresponded to the smallest possible slit in which the movement of the chain was still likely. The greatest size of the hole corresponded to a size slightly greater than the size of a high temperature coiled polypeptide chain

consisting of $N = 30$ residues (the diameter of the chain can be estimated as $2\langle S^2\rangle^{1/2}$ [19]). Initially the translocation time decreased linearly with the increase in the pore size. The further decrease below $d = 15$ of the edge of the hole did not significantly change the time of translocation τ and all that was observed was some fluctuation. This effect is as expected, since the process of translocation through a window comparable in size with the size of the coil did not depend on the latter.

4. Conclusions

In this paper we presented the Monte Carlo simulations of simple lattice models of a polypeptide chains. The beads representing the polypeptide residues interacted via the a binary contact potential. In our model chains only two kinds of aminoacid residues were distinguished: hydrophobic and hydrophilic. Some local stiffness of the chain was introduced in order to enhance the formation of helical conformations. The chains were put into the Monte Carlo box with walls impenetrable to the polypeptide chains. A hole was made in one wall and a weak force was applied, driving the polypeptide chains to the other part of the box trough this hole. This model can be treated as a crude approximation of the process of translocation of protein chains through a membrane. It was shown that the translocation time of a polypeptide chain depended on the chain length, the value of the helical potential and the size of the hole in the wall. The time of translocation depended on the chain length roughly as $N^{2.2}$ which was very close to other theoretical findings $N^{2.18}$. The scaling exponent was almost independent of the strength of the helical potential. The size of the hole changed the passage time where the length of the window was smaller than the mean diameter of the molecule. As the size of the window increased to a size comparable with the size of the polypeptide chain, this effect on the passage time vanished. The next step of the study should involve the multi-hole wall of the box filled with a multiple chain system, which mimics the dynamics of trans-membrane chains.

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