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Rigidity and resistance of larval- and adult schistosomes-medium interface



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ABSTRACT

Schistosomiasis is second only to malaria in prevalence and severity, and is still a major health problem in many tropical countries worldwide with about 200–300 million cases and with more than 800 million people at risk of infection. Based on these data, the World Health Organization recommends fostering research efforts for understanding at any level the mechanisms of the infection and then decreasing the social and economical impact of schistosomiasis. A key role is played by the parasite apical lipid membrane, which is entirely impervious to the surrounding elements of the immune system. We have previously demonstrated that the interaction between schistosomes and surrounding medium is governed by a parasite surface membrane sphingomyelin-based hydrogen barrier. In the present article, the elastic contribution to the total motion as a function of the exchanged wave-vector Q and the mean square displacement values for *Schistosoma mansoni* larvae and worms and *Schistosoma haematobium* worms have been evaluated by quasi elastic neutron scattering (QENS). The results point out that *S. mansoni* larvae show a smaller mean square displacement in comparison to *S. mansoni* and *S. haematobium* worms. These values increased by repeating the measurements after one day. These differences, which are analogous to those observed for the diffusion coefficient we previously evaluated, are interpreted in terms of rigidity of the parasite-medium interaction. *S. mansoni* larvae are the most rigid systems, while *S. haematobium* worms are the most flexible. In addition, temperature and hypoxia induce a weakening of the schistosome-medium interaction. These evidences are related to the strength of the hydrogen-bonded interaction between parasites and environment that we previously determined. We have shown that *S. mansoni* worms are characterized by a weakened interaction in respect to the larvae, while the *S. haematobium* worms more weakly interact with the surrounding medium than *S. mansoni*. The present QENS analysis allowed us to characterize the rigidity of larval- and adult *S. mansoni* and *S. haematobium*-host interface and to relate it to the parasite resistance to the hostile elements of the surrounding medium and to the immune effectors attack.

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1. Introduction

Schistosomiasis is a severe parasitic disease endemic in 78 countries of the developing world, with most serious impact on children residing in rural areas. It is caused by several species of the genus *Schistosoma*, namely *Schistosoma mansoni* and *Schistosoma haematobium* [1,2]. Cercariae shed by infected snails invade host skin, and remain in the epidermis for up to 72 h exchanging the classical trilaminar membrane for an outer double

lipid bilayer. This heptalaminar membrane surrounds the parasites, which migrate, develop, mature, copulate, lay eggs and reside exclusively in the mammalian host blood capillaries [3,4]. The outer membrane protects the developing larvae and adult worms from the surrounding hostile immune elements, while allowing entry of nutrients (small molecules such as glucose, amino acids and cholesterol which do not exceed 400 Da) and exit of wastes [5–9]. Indeed, the apical membrane molecules of larvae older than 3–24 h, lung-stage schistosomula and adult worms do not bind antibodies (large molecules exceeding 150,000 Da), while are accessible to the different nutrients [5–8] which interact with their respective binding-proteins or protein transporters. We have hypothesized that sphingomyelin (SM) molecules in the outer lipid bilayer is the main factor responsible for these protective and sieving properties, via formation of a tight hydrogen bond barrier

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with the surrounding water [10–12]. The existence of a SM-based hydrogen barrier around lung-stage schistosomula and adult schistosomes was recently documented using quasi elastic neutron scattering (QENS) [13]. More specifically, we have previously gathered QENS data to determine the nature and the strength of the interaction of both larval and adult parasites with the surrounding medium. The evaluation of the quasi-elastic contribution to the total motion allowed characterization of the mobility features of schistosomes using the obtained diffusion coefficient values. Of note, the outer lipid bilayer and surrounding hydrogen bond network at the host parasite interface was shown to be easily disrupted via heating, or hypoxia-mediated activation of a parasite tegument-associated neutral sphingomyelinase (nSMase) [10–14].

Continuing our previous work, we herein aimed to examine the different characteristics of the formidable interfacial barrier between the host medium and the parasite, via focusing on the correlation between the flexibility and the resistance of the parasites under different external conditions. Since we needed to define these fundamental properties on a molecular level, the larval and adult *S. mansoni* and adult *S. haematobium* are investigated in the same experimental conditions as previously. QENS data were generated in order to use the evaluation of the elastically scattered intensity and the mean square displacement as a function of exchanged vector and temperature for assessing the rigidity of the apical lipid bilayer SM-based hydrogen bond network surrounding larval and adult *S. mansoni* and adult *S. haematobium*. Incoherent neutron scattering was employed to investigate such living systems, since it has already been successfully used on other complex biological samples, such as *Escherichia coli*, *Artemia* cysts, mammalian cells, extremophile bacteria [15–18], where the very large presence of hydrogen atoms distributed nearly homogeneously within these structures predominantly contributes to the scattered signal and gives access to detailed information about atomic motions on the picosecond-nanosecond time domain. This represents an important advantage since it is possible to study the dynamics of hydrogenated molecules also in samples which are not crystalline neither monodisperse, such as living cells. In addition, neutron scattering studies have unambiguously demonstrated that biomolecular motions are affected by environmental conditions where biological systems are immersed [15–23].

The methodological approach used in the data analysis is based on the determination of the elastically scattered intensity on the exchanged vector Q and then on a model proposed by Zaccai and co-workers [16,17,19] to evaluate the “pseudo-force constant”, which is related to the “resilience” of biological systems, i.e. the capability to maintain the stability as a response to a thermal stress, by the mean square displacement behavior. Zaccai and co-workers [16,17,19] used this model to associate the biomolecular dynamics to the thermo-adaptation in many systems and in particular on extremophile biomolecules, demonstrating that proteins extracted from thermophile organisms are characterized by a higher rigidity and a lower flexibility than those extracted from psychrophile organisms that possess a quite high flexibility. These features allow these organisms to maintain a perfect balance between the flexibility fundamental to exert their functions and activities and the structural resistance to the thermal stress [15,17,19].

In line with these studies, here we compare the rigidity of larval and adult *S. mansoni* and adult *S. haematobium* in order to relate it to their adaptive strategies activated as a response to different external stress, i.e. hypoxia and temperature. We emphasise that the different flexibility degree of the parasites is directly related to their different mobility as characterized in our previous work [13]. In other terms, the previous determination of the nature of the parasite-medium barrier, i.e. the role played by the hydrogen bond network in this interaction, provides a clear explanation to

the present findings dealing with the rigidity and then with resistance of parasites against immune system attack and harsh external conditions, such as thermal stress and hypoxia.

2. Materials and methods

2.1. Parasites

Male Syrian hamsters and cercariae of *S. mansoni* and *S. haematobium* were obtained from the Schistosome Biological Materials Supply Program, Theodore Bilharz Research Institute, Giza, Egypt. Hamster Infection and perfusion were performed under anaesthesia, following the recommendations of the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, U.S.A. Lung-stage schistosomula were recovered six days after hamster percutaneous infection with 2000 cercariae of *S. mansoni* as described [13], and then suspended in RPMI medium containing 20% fetal calf serum (FCS), placed in sterile polypropylene tubes (Costar), and transported to Grenoble, France, within 18 h. Adult worms were obtained from hamsters 6 and 13 weeks after exposure to 200 cercariae of *S. mansoni* and *S. haematobium*, respectively. Worms were washed in RPMI medium, resuspended in RPMI medium/20% FCS, incubated for 6 h at 37 °C/3% CO₂, placed in sterile, vented, 25 ml tissue culture vessels (Costar) filled to the rim with medium/20% FCS, and reached Grenoble, France, within 18 h. Intact and hypoxia-exposed larvae were examined by indirect membrane immunofluorescence using radiation vaccine serum as described [10,11].

2.2. Experimental

The IN4 spectrometer at the Institute Laue Langevin (ILL, Grenoble, France) was used to carry out QENS measurements as a function of energy, exchanged wave-vector Q and temperature. The instrumental configuration was: an incident wavelength of 2.96 Å, an energy resolution of 450 μ eV, a sample holder orientation of 135° relative to the incident beam. Measurements have been performed on *S. mansoni* larvae and adult worms and *S. haematobium* worms at a temperature value of 300 K, by repeating after 24 h the measurements on *S. mansoni* larvae and adult worms. In addition *S. mansoni* larvae have been also investigated at a temperature value of 320 K.

The usual corrections, such as empty cell subtraction and vanadium normalization as well as the multiple scattering and multiphonon contribution evaluations, were performed before the data analysis by using the Lamp software.

3. Results and discussion

3.1. Analysis of the elastically scattered intensity and the mean square displacement

The behavior of the elastic contribution to the total motion of lung-stage *S. mansoni* larvae and *S. mansoni* and *S. haematobium* worms has been characterized as a function of Q in the 0.6–3.4 Å^{−1} range at the temperature value of $T = 300$ K, as shown in Fig. 1. By observing Fig. 1, a Gaussian contribution that is related to the vibrational motions, and a non-Gaussian contribution, which is linked to the diffusive motions, can be considered [24–26]. This latter contribution provides a measure of the dynamic heterogeneity degree of the investigated systems and furnishes information about their rigidity and flexibility. In order to point out these spectral features, in the insert of Fig. 1 we consider the logarithm of the elastic contribution to the total motion as a function of Q^2 .

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