International Journal of Biological Macromolecules 55 (2013) 39–46

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Inhibition of hyaluronidase by N-acetyl cysteine and glutathione: Role of thiol group in hyaluronan protection

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ARTICLE INFO

Article history:
Received 25 September 2012
Accepted 31 December 2012
Available online 8 January 2013

Keywords:
Hyaluronidase
N-acetyl cysteine
Glutathione
Snake venom

ABSTRACT

Hyaluronidase inhibitors have immense applications in pathophysiological conditions associated with hyaluronan–hyaluronidase system. The present study demonstrates the inhibitory efficacy of clinically accepted antioxidant N-acetyl cysteine (NAC) against hyaluronidase of serum, testis, and snake and bee venoms. The experimental and molecular dynamic simulation data suggest the non-competitive inhibition and involvement of thiol groups of both NAC and glutathione in exertion of inhibition. The bioavailability, less-toxic and antioxidant nature of NAC and glutathione could become valuable in the management of pathologies triggered by extracellular matrix degradation and to increase the endurance of hyaluronan based biomaterials/supplements, which are highly exciting aspects.

1. Introduction

Hyaluronic acid (HA) is a megadalton, non-sulfated, acidic glycosaminoglycan (GAG) composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked through β 1-3 and β 1-4 glycosidic linkages. The association of HA with proteins and aggrecan monomers throughout its linear structure confers a mechanical support to organize the extracellular matrix (ECM). Being highly hydrated HA regulates cell adhesion, migration and proliferation. Several studies have demonstrated the participation of HA in different physiological and pathophysiological conditions including embryogenesis, angiogenesis, wound healing, tissue turnover, malignancies and inflammatory disorders [1,2].

The homeostasis of HA is mainly regulated by coordinated activities of HA-synthesizing and -degrading enzymes [3,4]. HA is synthesized at the inner face of plasma membrane by three transmembrane glycosyltransferases (HA synthases) HAS1, HAS2 and HAS3, whereas it is enzymatically degraded by hyaluronidases (HAases) in normal physiological conditions and is subjected to non-enzymatic fragmentation by free radicals generated during oxidative stress phenomenon in many pathological conditions like arthritis, cancer, and chronic obstructive pulmonary disease (COPD) [2,5,6]. The HA degradation in ECM, cracks down the structural integrity with an eventual increase in the tissue permeability that attributes the spreading property of hyaluronidase. The spreading property is well noticed in envenomation, acrosomal reaction during ovum fertilization, cancer progression and microbial pathogenesis such as wound infections. The HA fragmentation has dual effects: (1) generation of a wide molecular range of bioactive oligosaccharides with angiogenic, pro-inflammatory and immunostimulatory properties. (2) Impairment of the reservoir capacity of ECM that stores metal ions, growth factors, cytokines and various enzymes for signal transduction [7].

HA degradation is the rate-limiting step in several physiological and pathophysiological processes ranging from embryogenesis and aging. Hyaluronidase inhibitors are thus considered as potent regulators that might serve as anti-inflammatory, anti-aging, antimicrobial, anti-cancer, anti-toxin and contraceptive agents [7,8]. The inhibitors can also be used to increase the endurance of HA-based biomaterial/bioscaffolds for drug/vaccine delivery systems, embryo preservation, organ transplantation, surface coating and moisturizing agents [7].

The role of oxidative stress in up-regulating hyaluronidase expression in addition to non-enzymatic cleavage of HA is well defined in various pathologies. Instead of several naturally occurring and synthetic molecules that are characterized and classified based on hyaluronidase inhibitory action, the low molecular weight antioxidants which are already in clinical practice with promising bioavailability and less toxicity might become an interesting pool of molecules that can be considered for the management

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http://dx.doi.org/10.1016/j.ijbiomac.2012.12.047

of pathologies triggered by hyaluronidases [7,9]. In addition, this could facilitate the transit from the preclinical to the clinical scenario. GSH as an endogenous antioxidant could prevent the HA degradation by scavenging ROS, however no information is available regarding its inhibitory effect on hyaluronidase activity. N-acetyl cysteine (NAC), a precursor of glutathione is being used in clinical therapy from the past 35 years as a mucolytic agent and also as an antidote for acetaminophen and heavy metal poisoning. NAC was shown to have beneficial effects in multiple pathologies associated with oxidative stress including cancer, cardiovascular diseases and COPD. Clinically, a dose of 1200 mg NAC twice a day was shown to be well tolerated by subjects [10,11]. In addition, inhibitory action of NAC against the matrix metalloproteinase (MMPs), a group of ECM degrading enzymes has been well demonstrated [12]. However, no studies report the inhibitory efficacy of NAC on hyaluronidase. In light of above, the present study made the first attempt ever to evaluate the hyaluronidase inhibitory properties of glutathione and its precursor NAC followed by docking and molecular dynamic simulations to investigate the molecular mechanism of hyaluronidase inhibition.

2. Materials and methods

Echis carinatus venom was obtained from Irula Snake Catchers, Chennai, India. Hyaluronic acid, N-acetyl glucosamine, bee venom, N-acetyl cysteine, N-acetyl lysine, N-acetyl methionine, Methionine, Cysteine, and Glutathione (oxidized and reduced) were obtained from Sigma Chemicals, St. Louis, USA. Bovine testicular hyaluronidase was obtained from Worthington Chemicals, NJ, USA. All other chemicals were of analytical grade purchased from SRL (India) and Merck (India).

2.1. Hyaluronidase activity

Hyaluronidase activity was assayed according to the method of Reissig et al. [13]. Snake venom sample (0.3 mg/mL), bee venom (0.13 mg/mL), bovine testicular hyaluronidase (BTH: 4 IU) and human serum (2.5 mg/mL) were incubated independently with 0.15 mg/mL of hyaluronic acid in 300 μL of 0.2 M sodium acetate buffer pH 5.5 containing 0.15 M NaCl at 37 °C for 2½ h. Reaction was terminated by the addition of 50 μL of Potassium tetra borate (PTB) and the change in absorbance was read at 585 nm. Activity was expressed as nmoles of N-acetyl glucosamine released/min/mg of protein. For inhibition studies, venom/enzyme/serum was pre-incubated independently with different concentrations (0–3 mg/mL) of NAC, glutathione (reduced and oxidized) and different amino acids and their derivatives for 10 min at 37 °C. The IC_{50} value for BTH was determined by analyzing the enzyme activity in the presence of different concentrations of NAC (0–1.8 mg/mL) under standard assay conditions.

2.2. Determination of the type of inhibition

To determine the type of inhibition and for calculating the Michaelis–Menten constant (K_m), velocity maximum (V_{max}) and inhibitory coefficient (K_i), variable concentrations of substrate and inhibitor were used. Testicular hyaluronidase (4 IU) and different concentrations of NAC (4 and 8 mM) were pre-incubated for 10 min. Reactions were initiated by adding different concentrations of hyaluronic acid (0–1.5 mg/mL) in the final reaction volume of 300 μL of 0.2 M sodium acetate buffer pH 5.5 containing 0.15 M NaCl at 37 °C for 2.5 h. The change in absorbance was monitored at 585 nm. Activity was expressed as mM of N-acetyl glucosamine released/2.5 h at 37 °C.

2.3. Reversibility of inhibition

Reversibility of hyaluronidase inhibition was determined according to the method of Ackermann and Potter [14]. The extent of hyaluronidase inhibition by NAC (4 mM) was determined by varying the amounts of enzyme (0–12 IU).

2.4. Autodock

In order to understand the binding mode of NAC or GSH to human hyaluronidase (2PE4) the docking simulations were performed using Lamarckian genetic algorithm implemented in AutoDock 3.0.5 [15]. Further, to facilitate a better understanding of the role of thiol group in exerting inhibition, docking was also performed for NAC or GSH in the absence of thiol (SH) group. The proteins were prepared using AutoDock Tools. The initial population was 100 randomly placed individuals. A $60 \times 60 \times 60$ Å point grid was used, centered at Asp 129 (one of the binding sites) of human hyalurodinase. Docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest free energy cluster returned for each compound was used for further analysis. Docking results were analyzed and visualized using Pymol [http://www.pymol.org/].

2.5. Molecular dynamics simulations

MD simulations for protein ligand complex of human hyalurodinase (2PE4) with NAC or GSH in the presence and/or in the absence of thiol group [2PE4 with NAC, 2PE4 with NAC-without-SH, 2PE4 with GSH, 2PE4-GSH-without-SH] were performed using the GROMACS 4.5.1 package with GROMOS96 force field [16]. The proteins and the complexes were solvated with the TIP3P water model using a triclinic box [17]. Periodic boundary conditions were enforced and the systems were neutralized by replacing water molecules with sodium and chloride counter ions depending upon the charge. A twin-range cutoff was applied to long-range interactions using the PME [18] method: 10 Å for van der Waals and electrostatic interactions. Bonds involving hydrogen atoms were constrained using LINCS [19]. The weak coupling method V-rescale was used to regulate the temperature, while the Parrinello–Rahman method [20] was used to set the pressure of the system. Equilibration MD for both temperature (300 K) and pressure (1 atm) were carried out for 100 ps. The temperature, pressure, density and total energy of the system were well equilibrated. Topology file and other force field parameters except the charges of ligands were generated using the PRODRG program (http://davapc1.bioch.dundee.ac.uk/prodrg/). Finally, a maximum of 50,000 energy minimization steps were carried out for the protein and complexes using the steepest descent algorithm with a tolerance of 1000 kJ/mol/nm. Then, a 100 picoseconds (ps) position restraining simulation was carried out to restrain the complexes by a 1000 kj/mol A 2 harmonic constraint to relieve close contacts before the actual simulation.

2.6. Statistical analysis

Results were expressed as mean ± SEM of five independent experiments. Statistical significance among groups was determined by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. $p < 0.05$(*).

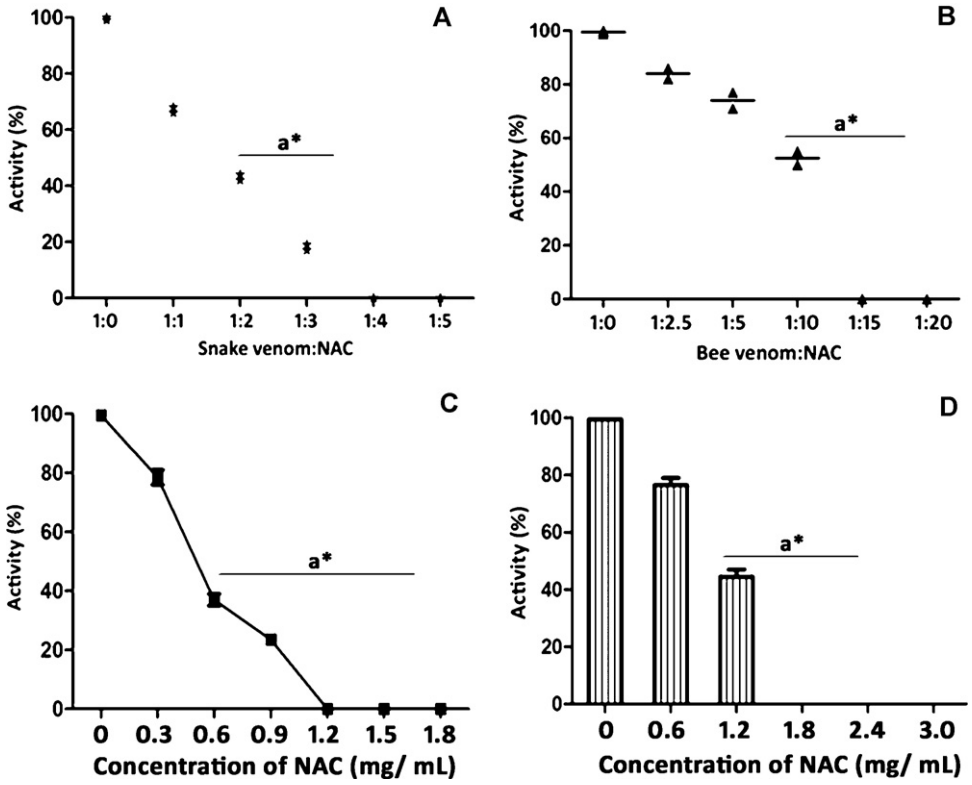

Fig. 1. Effect of NAC on hyaluronidase of different source: (A) EC snake venom (0.3 mg/mL), (B) bee venom (0.13 mg/mL); (C) testicular hyaluronidase (4 IU) and (D) human serum (2.5 mg/mL) were pre-incubated separately with different dose of NAC for 10 min at 37 °C. The hyaluronidase assay was performed as described in Section 2. Values are mean ± SEM of five independent experiments. a* – significant when compared to enzyme alone.

3. Results

3.1. Effect of NAC on hyaluronidase from different source

The effect of NAC on hyaluronolytic activities of venom (snake and bee), testicular and serum hyaluronidase enzyme were determined *in vitro* (Fig. 1). As shown in Fig. 1A, NAC inhibited *E. carinatus* venom hyaluronidase in a dose-dependent manner. The complete inhibition was observed at the ratio of 1:4 (venom: NAC; w/w). Similarly, NAC suppressed the hyaluronidase activity of bee venom in a dose-dependent manner and complete inhibition was observed at the venom to NAC ratio of 1:15 (Fig. 1B). Furthermore, NAC inhibited testicular hyaluronidase in a dose-dependent fashion. NAC at the concentration of 1.2 mg/mL completely inhibited the testicular hyaluronidase with IC_{50} value of 4 mM (Fig. 1C). NAC also induced dose-dependent inhibition of serum hyaluronidase and complete inhibition was observed at 1.8 mg/mL (Fig. 1D).

3.2. Type of inhibition

Double reciprocal plot analysis (Fig. 2A) showed the noncompetitive pattern of inhibition by NAC. V_{max} was altered in the presence of NAC and the K_m remained same (1.085×10^{-3} mg). To determine the inhibitory constant (K_i), reciprocal velocity was plotted against inhibitor concentration and K_i was found to be 2.56 mM (Fig. 2B).

3.3. Reversibility of inhibition

To know the reversible/irreversible nature of inhibition, V_{max} was plotted against total enzyme added to the assay mixture in the presence of 4 mM of NAC. According to the results, NAC showed reversible inhibition toward the enzyme (Fig. 3).

3.4. Effect of GSH on hyaluronidase

The effect of GSH on serum hyaluronidase enzyme was determined *in vitro*. As shown in Fig. 4 GSH inhibited the enzyme in a dose-dependent manner. The complete inhibition was observed at the ratio of 1.8 mg/mL.

3.5. Role of functional group of NAC and GSH in exerting inhibition

In order to investigate the role of functional groups (N-acetyl and Thiol) of NAC and GSH (thiol) on hyaluronidase inhibition, acetyl derivatives of amino acids and thiol-containing compounds were screened (Fig. 5). The thiol containing amino acids and their derivatives such as cysteine, NAC and glutathione (reduced) inhibited enzyme activity dose dependently, while no inhibition was observed with the glutathione (oxidized). Further, acetyl group containing compounds like N-acetyl leucine and N-acetyl methionine did not inhibit the enzyme. These results clearly suggest the involvement of thiol (-SH) in exertion of hyaluronidase inhibition.

3.5.1. Docking and molecular dynamic simulations studies for NAC

The conformation of 2PE4 was checked by analyzing the backbone root mean square deviations (RMSD) from the starting crystal structure over the course of trajectory. RMSD for the complexes of 2PE4 with ligands (NAC and NAC-without-SH) suggested that all the simulated structures are stable except the complex between 2PE4 and NAC-without-SH (Fig. 6A). Results of radius of gyration indicated the compact structure of enzyme and NAC-without-SH (Fig. 6B). In addition, NAC-without-SH group formed only single lowest free energy cluster with 2PE4 out of 10 docking runs

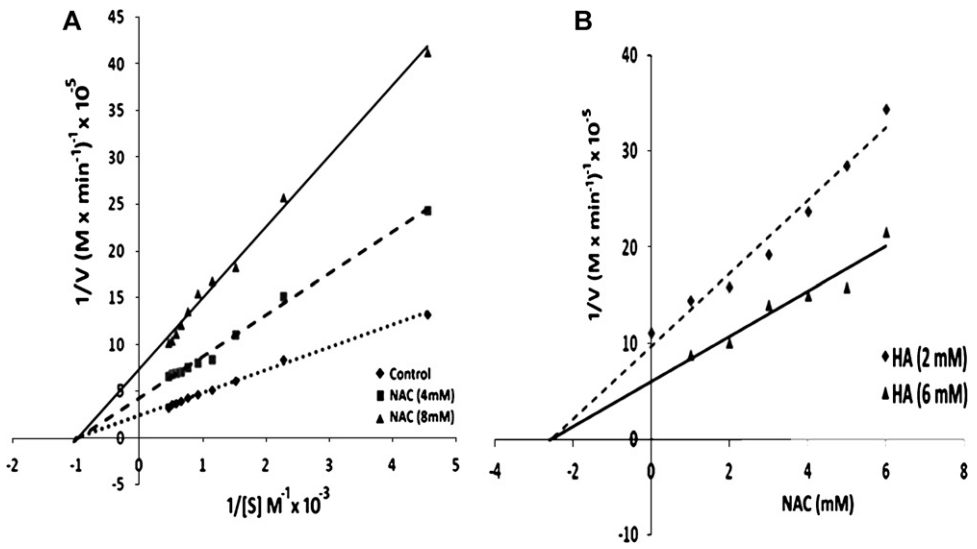

Fig. 2. Inhibition of testicular hyaluronidase by NAC and determination of K_i (A) Lineweaver–Burk plot (B) Dixon plot: testicular hyaluronidase (4 IU) was pre-incubated with different doses of NAC (0, 4 and 8 mM) for 10 min at 37 °C. The reaction was started by adding hyaluronan and assay was performed as described in Section 2. Activity was expressed as mM of N-acetyl glucosamine released/min. To determine inhibitory constant 1/*V* was plotted against varied concentration of NAC in the presence of fixed concentration of substrate (2 and 6 mM).

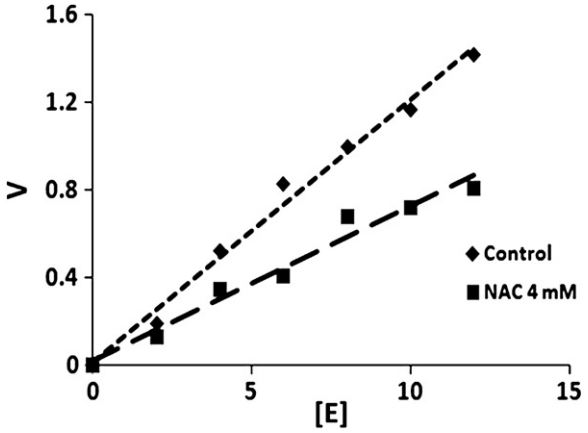

Fig. 3. Reversibility of inhibition of Testicular hyaluronidase by NAC: reversibility of inhibition was determined by plotting rate (V) against varied concentration of enzyme (0–15 IU) in the presence (4 mM) and in the absence of NAC.

whereas NAC formed 4 clusters with 2PE4 (Table 1). The data clearly indicate the requirement of SH group for stability and for better binding.

According to the simulation data of docked complexes, NAC can interact with residues of the active site through hydrogen bonds and hydrophobic interactions (Fig. 7). NAC formed hydrophobic interactions with active site amino acids such as TRP130, TYR202, PHE204, and TYR208. MET183 observed to be interacting with SH group of NAC with the distance ranging between 3.59 and 5.75 Å, but unable to form disulphide bridges (data not shown). Further, NAC also induced changes in Phi and Psi angle of ASP129 in docked complexes suggesting the possible reason for enzyme inhibition (Fig. 8).

3.5.2. *Docking and molecular dynamic simulations studies for GSH*

According to the RMSD results, all the simulated structures are stable except the complex between enzyme and GSH-without-SH (Fig. 6A). The results of radius of gyration indicated increased value

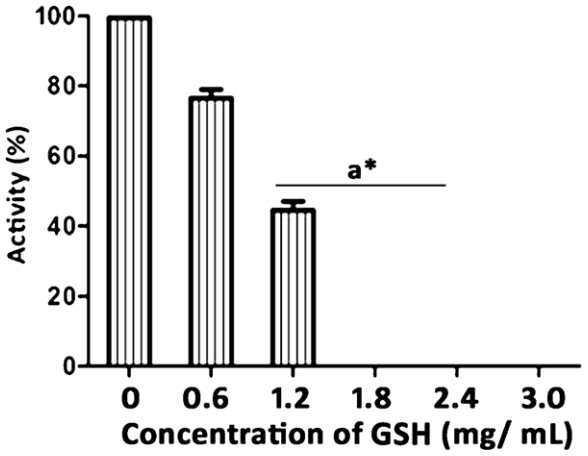

Fig. 4. Effect of GSH on serum hyaluronidase: Human serum (2.5 mg/mL) was pre-incubated with different dose of GSH for 10 min at 37 °C. The hyaluronidase assay was performed as described in Section 2. Values are mean ± SEM of five independent experiments. a* – significant when compared to enzyme alone.

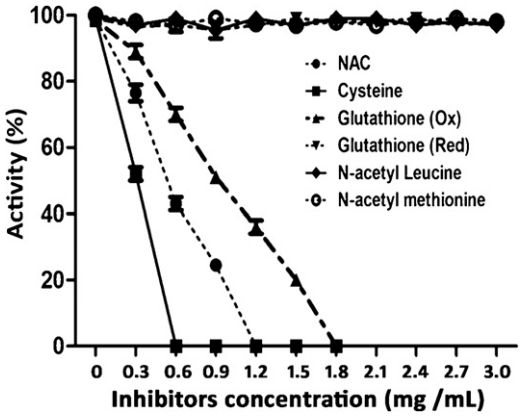

Fig. 5. Effect of acetyl and thiol containing compounds on hyaluronidase activity: testicular hyaluronidase (4 IU) was pre-incubated independently with different doses (0–3 mg/mL) of sulfhydryl containing compounds (Cysteine, NAC, and GSH), acetyl group containing compounds (NAC, N-acetyl methionine, N-acetyl leucine) and GSSG for 10 min at 37 °C. The assay was performed as described in Section 2. Values are mean ± SEM of five independent experiments.

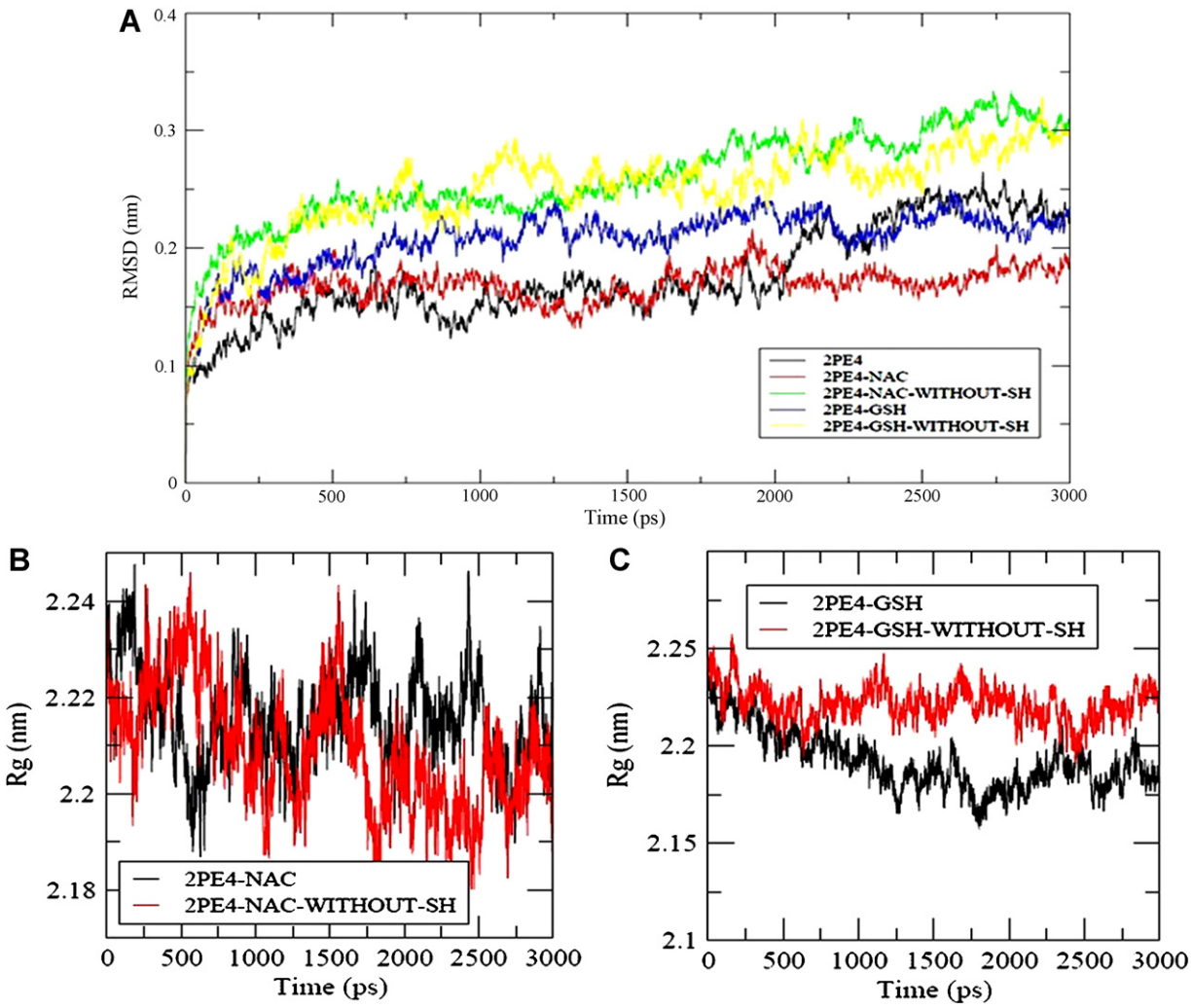

Fig. 6. RMSD and radius of gyration of the simulated docked complexes. (A) RMSD extracted from the docked complexes of human enzyme (2PE4) with ligands (NAC, NAC-without-SH, GSH, GSH-without-SH); (B) change in radius of gyration of the docked complexes of human hyaluronidase (2PE4) with ligands (NAC, NAC-without-SH) during the simulation were calculated and presented. (C) Change in radius of gyration of the docked complexes of human hyaluronidase (2PE4) with ligands (GSH, GSH-without-SH) during the simulation were calculated and presented.

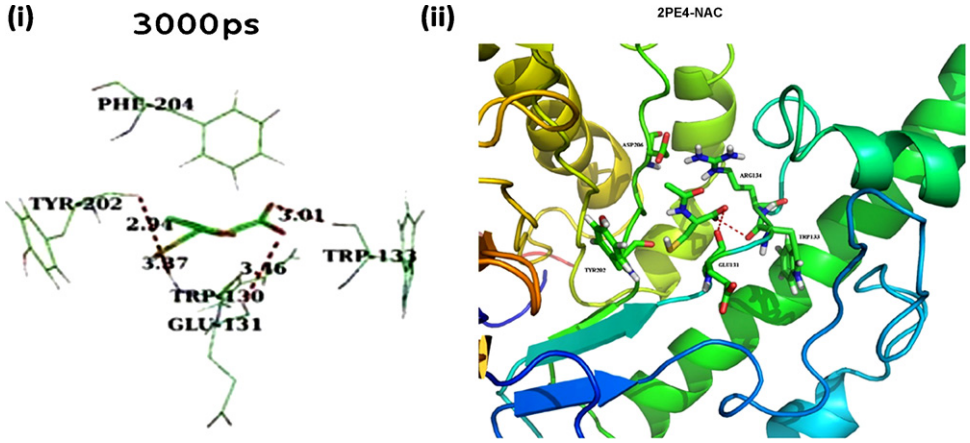

Fig. 7. Binding pose snapshot and interactions of key residues of human enzyme with NAC. Left panel: the docked snapshot of the last ns simulation was shown in the figure along with the distance of interacting residues (C, N, O, H and S shown in green, blue, red, white and orange, respectively). Neighboring residues distances are shown in red dots along with the distance. Right Panel: The interaction of key residues of human hyaluronidase (2PE4) with the NAC is presented and hydrogen bond distances are shown in red dots. Data are presented according to the lowest energy structure from the MD simulation. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

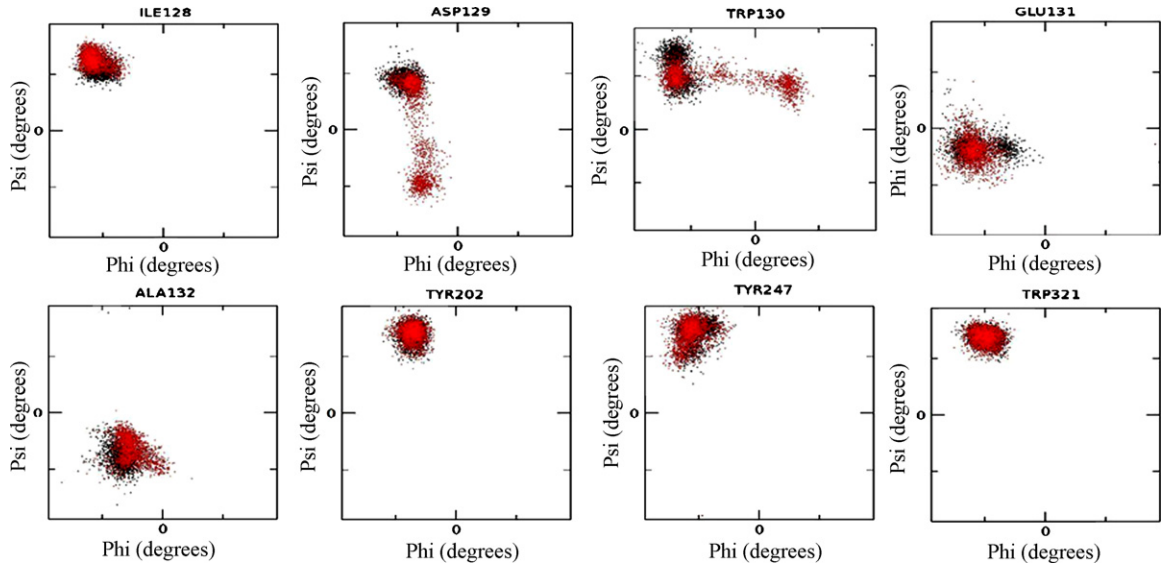

Fig. 8. Ramachandran plot of individual active site amino acid residues of simulated docked complexes. Dihedral (PHI and PSI) angels were calculated for active site residues of simulated human hyaluronidase-NAC docked complex.

Table 1
List of Autodock binding energies of docked complexes.

No.	Complex	Lowest binding energy (kcal/mol)	Number of clusters formed out of 10 docking runs
1	2PE4-NAC	−3.75	4
2	2PE4-NAC-without-SH	−3.59	1
3	2PE4-GSH	−1.03	4
4	2PE4-GSH-without-SH	−4.37	4

in the absence of SH group compared to its presence (Fig. 6C). GSH binds to the active site through hydrogen bonding with GLY63, TYR247, TYR286, TRP321 and TRP324 residues. Further, SH group was found to be situated near to the aromatic amino acids with the distance ranging from 2.62 to 4.53 Å (Fig. 9). Thus the data clearly suggest the interaction between SH group and hydrophobic amino acid residues of enzyme that may assist the ligand to bind and exert the inhibition.

4. Discussion

The hyaluronidases are a group of hyaluronolytic enzymes critical to a number of important regulatory processes. Recently it has been shown that reactive oxygen species (ROS) and hyaluronidases operate in a coordinated fashion to depolymerize HA in several pathological conditions characterized by oxidative stress including arthritis and lung inflammation such as COPD and asthma. Therefore, the cleavage of HA is considered to be the regulatory process in various clinical conditions and the use of hyaluronidase inhibitors could be imperative to control some of the serious pathologies that promote uncontrolled degradation of HA. The inhibitors of hyaluronidase may serve as valuable contraceptive, anti-aging, anti-tumor and anti-microbial agents, and also to complement antivenom as first aid agents in the treatment of venomous bite/sting. In addition, the inhibitors would be of great use in the endurance of HA-based biomaterials and supplements [2,7]. Having a clinically approved antioxidant to reduce both enzymatic and non-enzymatic degradation of HA could become the basic therapeutic approach in the management of hyaluronidase-triggered pathologies. In the present study, NAC a well documented antioxidant

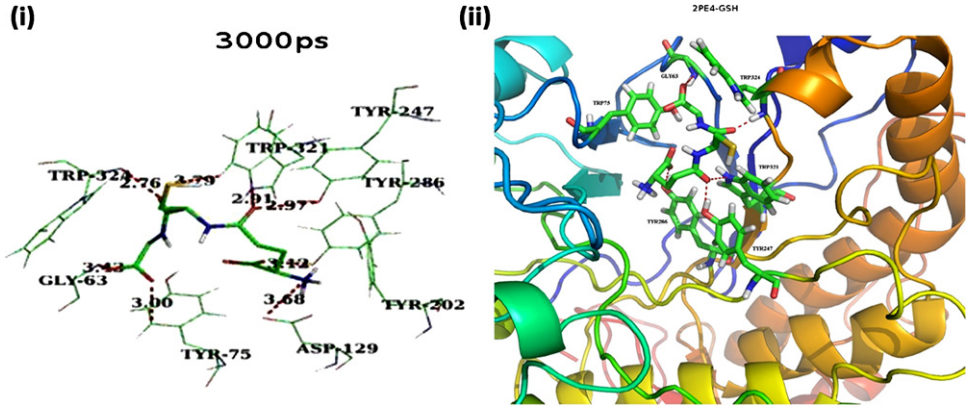

Fig. 9. Binding pose snapshot and interactions of key residues of human enzyme with GSH. Left panel: the docked snapshot of the last ns simulation was shown in the figure along with the distance of interacting residues (C, N, O, H and S shown in green, blue, red, white and orange, respectively). Neighboring residues distances are shown in red dots along with the distance. Right Panel: the interaction of key residues of human hyaluronidase (2PE4) with GSH is presented and hydrogen bond distances are shown in red dots. Data are presented according to the lowest energy structure from the MD simulation. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

effectively inhibited hyaluronidase from different sources including venom (Snake and bee), testicular and serum hyaluronidases. Hyaluronidases are generally referred to as "spreading factors" in snake venom and as potential "inflammatory agents" in bee venom. During natural envenomation, they augment the easy diffusion of target specific toxins from bitten site to circulation by degrading the HA present in basement membrane of connective tissues, which otherwise would have diffused slowly. Furthermore, systemically administered antivenom might be less efficient in reaching the envenomed site resulting in poor or no protection against local tissue damage. Thus, hyaluronidase inhibitors appear to be likely candidates that not only reduce the continuous degradation of local tissue, but also retard the easy dissemination of systemic toxins and hence increase the survival time of the victim [2,9,21,22]. In addition to hyaluronidase inhibition, our previous study demonstrates the inhibitory potency of NAC toward the snake venom metalloproteinase-induced hemorrhage and local tissue degradation [23]. Thus, NAC could be used as a first aid agent to defend the continued local tissue damage and also to reduce systemic toxicity.

Furthermore, NAC inhibited testicular hyaluronidase in a dose-dependent fashion, which is known to play an essential role during normal fertilization. Generally, spermatozoa penetrate the expanded cumulus mass containing the HA-rich viscoelastic matrix to fertilize the egg, but hyaluronidase inhibitors interfere with the transit of sperm through the expanded cumulus mass resulting in the reduction of penetration rate. Thus the inhibition of sperm/enzymes will lead to the disruption in the fertilization process and thereby prevent conception. Moreover, hyaluronidase inhibitors might be of use to control the pathogens involved in sexually transmitted diseases, which are known to secrete microbial hyaluronidase to augment the disease progression [24,25]. Thus, NAC could serve as an anti-microbial as well as contraceptive agent.

Besides, NAC showed significant inhibition against serum hyaluronidase suggesting its potential in inhibiting mammalian hyaluronidases. However, extended studies are necessary to evaluate the inhibitory property of NAC against different isoforms of human hyaluronidase. It has been proposed that the ECM degradation by hyaluronidase and MMPs contribute to the progression of cancer, arthritis, COPD and cardiovascular diseases that are characterized by oxidative stress [26–28]. ROS such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and especially by —OH radicals are known to have direct action on HA to yield several intermediate end products under oxidative conditions at the sites of inflammation, tissue injury and tumorigenesis [6,29–31]. Interestingly, hyaluronidases are endoglucosaminidases, whereas ROS degrade HA randomly at internal glycosidic linkages. The released HA fragments and ROS-induced expression of ECM-degrading enzymes including hyaluronidase and MMPs has been well documented in the progression of several pathologies [28]. Moreover, the inhibitory potency of NAC against MMPs has been described in several studies. Thus, the employment of a common inhibitor for these enzymes with good antioxidant property could be promising as a therapeutic strategy in the management of pathologies instigated by ECM degradation. Further, enzyme kinetic studies have shown the reversible non-competitive pattern of inhibition by NAC. This would be more effective *in vivo* as inhibition is independent of substrate concentration which is abundant in target site.

GSH being a precursor of NAC and to establish the involvement of thiol group in HA depolymerization, the present study evaluated the inhibitory property of GSH against serum hyaluronidase. GSH could block both non-enzymatic and enzymatic degradation of HA suggesting the major role in maintaining the integrity of ECM. Thiol group of both GSH and NAC was shown to play a major role in exerting antioxidant property. In addition, Pei et al. [12] described the inhibitory potency of glutathione and NAC on the activation and function of MMP. Thiol group of these compounds was shown to chelate Zn^{2+} present in the active site and play an important role in exerting inhibition. In order to understand the mechanism of inhibition and to know the involvement of possible functional group of NAC (Acetyl and thiol) and GSH (thiol), the thiol and acetyl derivatives of amino acids were screened. The results demonstrated that GSH, cysteine, NAC but not GSSG and acetyl derivatives, which exhibited the inhibitory effects on hyaluronidase activity, suggesting the involvement of thiol group. These results were further substantiated by docking human hyaluronidase (2PE4) with NAC or GSH in the presence and/or in the absence of thiol group followed by molecular dynamic stimulations studies. Results from RMSD and radius of gyration of docked complexes suggest the requirement of thiol group of ligands (NAC and GSH) to form stable complexes with enzymes. Simulation data indicate the hydrophobic interaction and hydrogen bond formation by NAC with active site amino acids. It also induced changes in phi and psi angles of ASP129 of human hyaluronidase in docked complexes. The crystal structure of human hyaluronidase clearly suggests the direct involvement of ASP and GLU in the catalysis of the enzyme-mediated degradation of hyaluronan [32,33]. Thus the NAC-induced alteration in the dihedral angles of active site amino acids in addition to hydrophobic interaction and hydrogen bonding could play a significant role in exerting inhibition. Similarly the interaction between the thiol group of GSH and hydrophobic amino acid residues of enzymes may assist the ligand to bind and bring inhibition.

In conclusion, clinically approved compounds as hyaluronidase inhibitors could serve as a better pharmacological tool in the management of hyaluronidase-induced pathogenesis like spreading of venom, cancer, COPD and arthritis. In addition, as HA-based biomaterials are acquiring potential value in the field of tissue engineering, drug delivery systems, organ transplantation, *in vitro* fertilization and visco supplementation of joints, hyaluronidase inhibitors could be used to increase the resident time of HA-based biomaterials. Further research regarding the influence of GSH level on hyaluronidase and hyaluronan synthase expression in various pathological conditions will be highly stimulating and could become a diagnostic marker/strategy to address the state of pathologies triggered by ECM metabolism.

Acknowledgements

Dr. Girish K.S. and Dr. M. Suresh Kumar thank the University Grant Commission, New Delhi, India, for financial assistance (F-36/276/2008 (SR) dated 26/3/2009).

References

[1] T.C. Laurent, J.R. Fraser, FASEB Journal 6 (1992) 2397–2404.
[2] K.S. Girish, K. Kemparaju, Life Sciences 80 (2007) 1921–1943.
[3] P.H. Weigel, V.C. Hascall, M. Tammi, Journal of Biological Chemistry 272 (1997) 13997–14000.
[4] S. Adamia, C.A. Maxwell, L.M. Pilarsk, Current Drug Targets 5 (2005) 3–14.
[5] M. Grootveld, E.B. Henderson, A. Farrell, D.R. Blake, H.G. Parkes, P. Haycock, Journal of Biological Chemistry 273 (1991) 459–467.
[6] M.E. Monzon, N. Fregien, N. Schmid, N.S. Falcon, M. Campos, S.M. Casalino-Matsuda, R.M. Forteza, Journal of Biological Chemistry 285 (2010) 26126–26134.
[7] K.S. Girish, K. Kemparaju, S. Nagaraju, B.S. Vishwanath, Current Medicinal Chemistry 16 (2009) 2261–2688.
[8] K.S. Girish, K. Kemparaju, Current Topics in Medicinal Chemistry 11 (2011) 2494–2508.
[9] K.S. Girish, K. Kemparaju, Life Sciences 78 (2006) 1433–1440.
[10] G.S. Kelly, Alternative Medicine Review 3 (1998) 114–127.
[11] P.J. Millea, American Family Physician 80 (2009) 265–269.
[12] P. Pei, M.P. Horan, R. Hille, C.F. Hemann, S.P. Schwendeman, S.R. Mallery, Free Radical Biology & Medicine 41 (2006) 1315–1324.
[13] J.L. Reissig, J.L. Stominge, L.F. Leloir, Journal of Biological Chemistry 217 (1995) 959–969.
[14] W.W. Ackermann, V.R. Potter, Proceedings of the Society for Experimental Biology and Medicine 72 (1949) 1–9.

[15] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Journal of Computational Chemistry 19 (1998) 1639–1662.
[16] H. Berk, K. Carsten, V.S. David, L. Erik, Journal of Chemical Theory and Computation 4 (2008) 435–447.
[17] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, Journal of Chemical Physics 79 (1983) 926–935.
[18] M. Kawata, U. Nagashima, Chemical Physics Letters 340 (2001) 165–172.
[19] B. Hess, H. Bekker, J. Fraaije, H.J.C. Berendsen, Journal of Computational Chemistry 18 (1997) 1463–1472.
[20] R. Martonák, A. Laio, M. Parrinello, Physical Review Letters 90 (2003) 075503.
[21] S. Yingprasertchai, S. Bunyasrisawat, K. Ratanabanangkoon, Toxicon 42 (2003) 635–646.
[22] K. Kemparaju, K.S. Girish, Cell Biochemistry and Function 24 (2006) 7–12.
[23] K. Sunitha, M. Hemshekhar, M.S. Santhosh, M.S. Kumar, K. Kemparaju, K.S. Girish, Current Topics in Medicinal Chemistry 11 (2011) 2589–2600.
[24] O. Matsushita, A. Okabe, Toxicon 39 (2001) 1769–1780.
[25] A. Garg, R.A. Anderson, L.J.D. Zaneveld, S. Garg, Journal of Andrology 26 (2005) 414–421.
[26] F.E. Lennon, P.A. Singleton, American Journal of Physiology – Lung Cellular and Molecular Physiology 301 (2011) 137–147.
[27] Y. Sasaki, M. Uzuki, K. Nohmi, H. Kitagawa, A. Kamataki, M. Komagamine, K. Murakami, T. Sawai, International Journal of Rheumatic Diseases 14 (2011) 313–319.
[28] D. Jiang, J. Liang, P.W. Noble, Physiological Reviews 91 (2011) 221–264.
[29] K.P. Vercruysse, M.R. Ziebell, G.D. Prestwich, Carbohydrate Research 318 (1999) 26–37.
[30] K. Yamazaki, K. Fukuda, M. Matsukawa, F. Hara, T. Matsushita, N. Yamamoto, K. Yoshida, H. Munakata, C. Hamanishi, Arthritis & Rheumatism 48 (2003) 3151–3158.
[31] H. Uchiyama, Y. Dobashi, K. Ohkouchi, K. Nagasawa, Journal of Biological Chemistry 265 (1990) 7753–7759.
[32] S. Arming, B. Strobl, C. Wechselberger, G. Kreil, Journal of Biochemistry 247 (1997) 810–814.
[33] K.L. Chao, L. Muthukumar, O. Herzberg, Biochemistry 46 (2007) 6911–6920.