Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: informahealthcare.com/journals/iplt20

# Hydrogen sulfide changes adhesive properties of fibrinogen and collagen *in vitro*

Agnieszka Morel, Joanna Malinowska & Beata Olas

**To cite this article:** Agnieszka Morel, Joanna Malinowska & Beata Olas (2014) Hydrogen sulfide changes adhesive properties of fibrinogen and collagen *in vitro*, Platelets, 25:2, 147-149, DOI: 10.3109/09537104.2012.737490

**To link to this article:** https://doi.org/10.3109/09537104.2012.737490

Published online: 13 Nov 2012.

*Platelets*, March 2014; 25(2): 147–149

ISSN 0953-7104 print/ISSN 1369-1635 online
DOI: 10.3109/09537104.2012.737490

informa
healthcare

LETTER TO THE EDITOR

# Hydrogen sulfide changes adhesive properties of fibrinogen and collagen *in vitro*

Agnieszka Morel, Joanna Malinowska & Beata Olas

*Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland*

**Keywords:** Hydrogen sulfide, fibrinogen, collagen, adhesion, blood platelets

Hydrogen sulfide ($H_2S$) is a well-known toxic gas that is synthesized from two amino acids: cysteine (Cys) and homocysteine (Hcy). $H_2S$, like other organic gases–nitric oxide ($NO^{\bullet}$) or carbon monoxide (CO) – is a signaling molecule in the cardiovascular system. Some studies have shown that $H_2S$ is a therapeutic agent in cardiovascular diseases [1–3], but the mechanisms involved in the relationship between the action of $H_2S$ and hemostasis process are still unclear. The main aim of this study was to establish the functional changes of two hemostatic proteins (collagen and fibrinogen) induced by $H_2S$, and also to examine the effects of these changes on the capability of fibrinogen and collagen to interact with human blood platelets (by measuring the platelet adhesion) *in vitro*.

Thrombin, collagen type I, bovine serum albumin (BSA), and bicinchoninic acid (BCA) solution were purchased from Sigma (St Louis, MO). Sodium hydrosulfide (NaHS), which has been well established as a reliable $H_2S$ donor [4, 5], was from Sigma (St Louis, MO). Fibrinogen isolated from pooled citrated human plasma by the cold ethanol precipitation technique was followed by ammonium sulfate fractionation at 26% saturation at 4°C, according to Doolittle [6]. Its concentration was determined spectrophotometrically at 280 nm using an extinction coefficient 1.55 for 1 mg/ml solution. The concentration of fibrinogen in plasma was $2 \pm 0.2$ mg/ml. The concentration of purified fibrinogen in the reaction system was also about 2 mg/ml. The concentration of collagen type I was 40 μg/ml collagen (dissolved in 0.05% $CH_3COOH$). The reaction was initiated by placing a small drop of NaHS, on the side of the tube containing the fibrinogen solution or collagen solution (the incubation time −5, 15, and 30 minutes, 37°C). We have been using NaHS at the final concentrations of 0.00001–10 mM. The physiological concentration of $H_2S$ in plasma and in tissues is about 50 μM; its physiological level in the brain is up to three-fold higher than in plasma.

Human blood was taken from healthy volunteers aged 23–32 (average: 24; SD = 5.5 years) not taking any medications or addictive substances (including tobacco, alcohol, and aspirin or any other anti-platelet drugs) and keeping a balanced diet (meat and vegetables), with similar socio-economic background, using no antioxidant supplementation. Human blood was collected into ACD solution (citric acid/citrate/dextrose; 5:1 v/v) and platelets were isolated by differential centrifugation of blood, as described by Wachowicz and Kustroń [7]. The final platelet concentration was $3 \times 10^8$ platelets/ml. The platelets were counted by the photometric method, according to Walkowiak et al. [8]. Adhesion of blood platelets to native fibrinogen or native collagen and fibrinogen or collagen treated with NaHS was determined according to Tuszynski and Murphy [9]. The absorbance of control platelets (with native fibrinogen or native collagen) was expressed as 100%.

All the values in this study were expressed as means ± SD. The statistical analysis (to calculate the differences among the effect of different concentration of NaHS) was performed with an ANOVA test and *POST Hoc* test (Bonferoni). In order to eliminate uncertain data, the Q-Dixon test was performed.

As shown in Table I, both resting as well as thrombin-activated blood platelets demonstrate a reduced ability to adhere to NaHS-treated collagen and NaHS-treated fibrinogen *in vitro*. The distinct inhibitory effect on the platelet adhesion to modified adhesive proteins (collagen and fibrinogen) was observed when collagen or fibrinogen was treated with NaHS, even at the lowest concentration of NaHS (0.00001 mM) (Table 1). The inhibitory properties of NaHS appears to be concentration-dependent for both adhesive proteins. Moreover, we observed the time-dependent (5, 15, and 30 minutes) inhibition of adhesive properties of proteins either by NaHS (0.01 and 10 mM) (for collagen–$p < 0.01$ (for resting platelets), $p < 0.01$ (for stimulated platelets); for fibrinogen–$p < 0.02$ (for resting platelets), $p < 0.001$ (for stimulated platelets)) (Table I).

Our initial results have also shown that, the adhesion to collagen and to fibrinogen of platelet preincubated with NaHS was reduced *in vitro* [10]. Moreover, $H_2S$ caused the reduction of anion radicals generation in these cells [10]. Other latest experiments have demonstrated the inhibitory properties of $H_2S$ on the platelet aggregation *in vitro*. The total inhibition of platelet aggregation (stimulated by various agonists: collagen, arachidonic acid and ADP) was observed at

Correspondence: Beata Olas, Department of General Biochemistry, University of Lodz, Pomorska 141/3 90-236 Lodz, Poland. Tel/Fax: +48 42 635 44 84. E-mail: olasb@biol.uni.lodz.pl

(Received 2 September 2012; revised 1 October 2012; accepted 3 October 2012)

Table I. Platelet adhesion of resting and thrombin-stimulated platelets to microplate wells coated with native adhesive proteins (fibrinogen and collagen) and NaHS-treated fibrinogen or collagen (5 min, 15 min, and 30 min, 37°C).

| Concentration of NaHS [mM] | Adhesion of resting platelets [%] | Adhesion of thrombin–activated platelets [%] |
|---|---|---|
| Incubation time–5 minutes | | |
| Native collagen [0 mM NaHS] | 100 | 100 |
| Collagen + 0.00001 mM NaHS | 96.2 ± 7.4 | 95.7 ± 7.8 |
| Collagen + 0.0001 mM NaHS | 92.3 ± 7.6 | 92.1 ± 5.9 |
| Collagen + 0.001 mM NaHS | 87.3 ± 8.8 | 87.4 ± 6.9 |
| Collagen + 0.01 mM NaHS | 81.5 ± 7.6 | 83.3 ± 10.1 |
| Collagen + 0.1 mM NaHS | 79.3 ± 8.1 | 79.4 ± 7.7 |
| Collagen + 1 mM NaHS | 75.4 ± 7.0 | 70.3 ± 6.5 |
| Collagen + 5 mM NaHS | 71.4 ± 9.0 | 65.2 ± 5.9 |
| Collagen + 10 mM NaHS | 66.6 ± 4.9 | 60.4 ± 4.9 |
| Native fibrinogen [0 mM NaHS] | 100 | 100 |
| Fibrinogen + 0.00001 mM NaHS | 93 ± 5.4 | 87.5 ± 10.8 |
| Fibrinogen + 0.0001 mM NaHS | 86.9 ± 9.9 | 82.0 ± 8.6 |
| Fibrinogen + 0.001 mM NaHS | 83.7 ± 12.1 | 79.8 ± 13.7 |
| Fibrinogen + 0.01 mM NaHS | 80.7 ± 10.6 | 74.2 ± 12.7 |
| Fibrinogen + 0.1 mM NaHS | 76.3 ± 10.5 | 69.7 ± 13.6 |
| Fibrinogen + 1 mM NaHS | 74.9 ± 8.9 | 63.2 ± 12.2 |
| Fibrinogen + 5 mM NaHS | 70.4 ± 10.2 | 51.8 ± 8.2 |
| Fibrinogen + 10 mM NaHS | 61.5 ± 5.6 | 49.0 ± 8.9 |
| Incubation time–15 minutes | | |
| Native collagen [0 mM NaHS] | 100 | 100 |
| Collagen + 0.01 mM | 74.7 ± 8.2 | 75.9 ± 5.9 |
| Collagen + 10 mM | 53.2 ± 5.5 | 54.3 ± 4.1 |
| Native fibrinogen [0 mM NaHS] | 100 | 100 |
| Fibrinogen + 0.01 mM | 70.5 ± 4.9 | 67.9 ± 7.2 |
| Fibronogen + 10 mM | 49.4 ± 7.8 | 40.4 ± 6.6 |
| Incubation time–30 minutes | | |
| Native collagen [0 mM NaHS] | 100 | 100 |
| Collagen + 0.01 mM | 70.2 ± 5.9 | 69.9 ± 7.3 |
| Collagen + 10 mM | 44.3 ± 7.1 | 47.8 ± 4.5 |
| Native fibrinogen [0 mM NaHS] | 100 | 100 |
| Fibrinogen + 0.01 mM | 63.7 ± 4.9 | 60.2 ± 6.6 |
| Fibrinogen + 10 mM | 41.3 ± 6.6 | 38.5 ± 7.4 |

Notes: Results represent the means of eight values. The effect of nine different concentrations of NaHS (0.00001, 0.0001, 0.001, 0.01 0.1, 1, 5, and 10 mM) *vs.* control was statistically significant according ANOVA test and *Post Hoc* test–(for collagen–$p < 0.01$ (for resting platelets), $p < 0.001$ (for stimulated platelets); for fibrinogen–$p < 0.02$ (for resting platelets), $p < 0.0001$ (for stimulated platelets)).

10 mM NaHS [11, 12]. The authors of these articles have observed that the $H_2S$ had the effect of inhibiting platelet aggregation without toxic effects (up to 10 mM). Results of Zagli et al. [12] demonstrated that the inhibitory effects of $H_2S$ were not dependent upon the effects of $NO^{\bullet}$ synthesis, adenylyl or guanylyl cyclase activation, or $K^+$ channels. Essex [13] assumed that the thiol-disulphide reactions may play some important role in platelets treated with $H_2S$. Hu et al. [14] and Morel et al. [10] suggest that phosphoinositides 3-kinase may be involved in the mechanism of $H_2S$ action in these cells. The present experiments for the first time demonstrated that $H_2S$ may change not only the platelet functions, including adhesive properties, but also may modify the adhesive activity of two tested proteins (collagen and fibrinogen). However, significance of these changes caused by $H_2S$ needs more explanation. We assume that the interaction of modified adhesive proteins may cause impaired adhesion. Further studies that may characterize changes in fibrinogen and collagen simulated in $H_2S$ and also their role in hemostasis process are still in progress in our laboratory.

***Declaration of interest:*** This work was supported by grant 506/810 and 545/506 from the University of Lodz. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## References

1. Predmore BL, Lefer DJ. Development of hydrogen sulphide-based therapeutics for cardiovascular diseases. J Cardiovasc Transl Res 2010;3:487–498.
2. Scharbert G, Kalb ML, Duris M, Marschalak C, Kozek-Langenecker SA. Garlic at dietary doses not impair platelet function. Anesth Analg 2007;105:1214–1218.
3. Chuah SC, Moore PK, Zhu YZ. S-allylcysteine mediates cardioprotection on an acute myocardial infarction rat model via a hydrogen sulphide-mediated pathway. Am J Physiol Heart Circ Physiol 2007;293:H2693–H2701.
4. Deplanecke B, Gaskins HR. Hydrogen sulphide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells. FASEB J 2003;17:1310–1312.
5. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. EMBO J 2001;20:6008–6016.

6. Doolittle RF, Schubert D, Schwartz SA. Amino acid sequence studies on artiodactyl fibrinopeptides I Dromedary camel, mule deer, and cape buffalo. Arch Biochem Biophys 1967;118:456–467.
7. Wachowicz B, Kustroń J. Effect of cisplatin on lipid peroxidation in pig blood platelets. Cytobios 1992;70:41–47.
8. Walkowiak B, Michalak E, Koziołkiewicz W, Cierniewski CS. Rapid photometric method for estimation of platelet count in blood plasma or platelet suspension. Thromb Res 1989;56:76–86.
9. Tuszyński GP, Murphy A. Spectrophotometric quantitation of anchorage-dependent cell numbers using the bicinchoninic acid protein assay reagent. Anal Biochem 1990;184:189–191.
10. Morel A, Malinowska J, Olas B. Antioxidative properties of hydrogen sulfide may be involved in its antiadhesive action on blood platelets. Clin Biochem 2012;1–5. (in press).
11. Zaicho NV, Pentiuk OO. Influence of hydrogen sulfide, dithionite, sulfite, thiosulfate and sulfate anions on human platelet aggregation. Ukr Biokhim Zh 2009;81:105–113.
12. Zagli G, Patacchini R, Trevisani M, Abbate R, Cinotti S, Gensini GF, et al. Hydrogen sulfide inhibits platelet aggregation. Eur J Pharmacol 2007;559:65–68.
13. Essex DW. The role of thiols and disulfides in platelet function. Antioxid Redox Signal 2004;6:736–746.
14. Hu Y, Chen X, Pan TT, Neo KL, Lee SW, Khin ES, Moore PK, Bian JS. Cardioprotection induced by hydrogen sulfide preconditioning involves activation of ERK and PI3K/Akt pathways. Pflugers Arch 2008;455:607–616.