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Research Article

ANTICOAGULANT POTENTIAL OF MARINE POLYCHAETE (*NEREIS* SPECIES)

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Abstract

There has been an accelerated rate of mortality and morbidity stemming from various chronic diseases worldwide over recent years. Anticoagulants play a pivotal role as agents for the prevention and treatment of thromboembolic disorder. Glycosaminoglycans (GAGs) are linear polysaccharides found in the extracellular matrix and biological fluids of animals where they interact with hundreds of proteins and perform a variety of critical roles. The present study reports the isolation and characterization of glycosaminoglycans from marine polychaete (*Nereis* sp). The isolated glycosaminoglycans were quantified as 12 g/kg and 0.83 mg/kg from the crude and purified samples respectively. Polychaete showed the anticoagulant activity of the 58 USP units/mg from crude; whereas purified samples showed 114 USP units/mg. The structural characterization of anticoagulant GAG was carried out by Fourier Transform Infrared spectroscopy (FTIR) by comparing with that of the standard GAG. The results of this study could pave the way for further research and exploration of GAGs from marine polychaetes in particular *Nereis* sp. as an alternative source of heparin.

Keywords: Polychaetes, Anticoagulant, Glycosaminoglycans, Clotting

INTRODUCTION

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting[1]. There has been an accelerated rate of mortality and morbidity stemming from various chronic diseases worldwide over recent years. In view of this, the contribution of vascular coagulopathies to the alarming number of deaths yearly cannot be overlooked because thrombosis was identified as one of the leading causes of myocardial infarction, stroke and pulmonary embolism[2]. Hence, anticoagulants play a pivotal role as agents for the prevention and treatment of thromboembolic disorder[3,4]. For more than five decades, anticoagulant drugs consisting of heparins, vitamin K-antagonists, and their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented[5,6]. At present, lavish focus is being given to potent anticoagulant sources hailing from natural origin. In this regard, marine organisms could be of immense interest as they are living in a very exigent, competitive and aggressive surrounding different in many facets from the terrestrial environment, a situation that demands the production of quite unique and potent active molecules[7]. Heparin is a compound that has been widely used as an anticoagulant and antithrombotic agent for more than 50 years. This drug is also employed during extracorporeal circulation[8]. However, several adverse effects of heparin have been identified, such as the development of thrombocytopenia, hemorrhage and low platelet count[9]. Thus, new compounds with similar properties to heparin are needed, and the sulfated polysaccharides of marine resources, which have important anticoagulant and anti-thrombotic actions, are an attractive alternative[10]. There are five classes of animal Glycosaminoglycans (GAGs): heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronan (HA). Many biological functions can be monitored directly by their impact on GAG quantity. Heparin has been used for anticoagulant therapy for many years. Its anticoagulant effect appears to be mediated mainly through antithrombin III (AT III), which is a plasma protein and the main coagulation inhibiter in the blood. AT III, or heparin cofactor, as it is also called, inhibits thrombin and the activated forms of the coagulation factors IX, X, XI and XII. In the absence of heparin the inhibition reactions are slow, but the addition of heparin strongly accelerates them. Several models have been proposed to explain the effect of heparin on the inhibition of thrombin by ATIII. Heparin is known to bind to ATIII; one widely accepted model assumes that heparins forms a complex with AT III and transforms it into a more rapidly acting inhibitor[11]. However heparin and heparin-like substances are not found only in higher life forms. It is factual that the importance of marine organisms as a source of novel bioactive compounds is an emergent discipline, and it has been classified as the leading remaining reservoir of natural molecules to be evaluated for drug activity. Over three decades ago, it was appreciated that a variety of tissues from marine life also contain heparin-like substances with high anticoagulant activity. Since these initial observations, numerous reports have appeared demonstrating that heparin and heparin-like substances are also present in tissues from a variety of molluscs/clams, in blood, muscle and viscera of many species of fish[12]. Polychaetes play an important role in the functioning of benthic communities[13]. They have been shown to be good indicators of species richness and community patterns in benthic invertebrate assemblages[14]. Polychaetes have been

proposed as surrogates for marine biodiversity[15]. They have been extensively studied for their ecology, taxonomy, bio-indicator, aquaculture, and pharmacological potential[16-20]. However no attempt has been made to explore the anticoagulant properties of marine polychaetes. Therefore, the present study was executed with the objective to isolate and enumerate the anticoagulant potential of polychaetes.

MATERIALS AND METHODS

Sample collection

Polychaetes (*Nereis* species) were procured from Sona hatchery, Marakkanam, Pondicherry, Tamil Nadu, India and washed thoroughly with distilled water to remove the unsolicited sediment particles.

Isolation of glycosaminoglycans (GAGs)

Tissue samples were blended in 0.4 M sodium sulfate solution (Na_2SO_4; 3.5 l/kg of the tissue) and kept at 55°c for 90 minutes. The pH was adjusted to 11.5 by adding 10 % sodium hydroxide (NaOH) solution. Aluminium sulfate ($Al_2(SO_4)_2$) crystals (80 mg/kg tissues) were added to this solution, and the suspension was heated to 95°C for 1 h. Cetyl pyridinum chloride (CPC) solution (3 g/100 ml of 0.8 M NaCl) was used to precipitate the crude white heparin complex. The precipitate was re dissolved in 150 ml of sodium chloride solution (2.0 M) and was incubated at 30°c for 30 minutes. The precipitate was washed, with ethanol and methanol through centrifugation, and vacuum dried.

Purification of GAGs

GAGs were purified on a 5 × 90 cm column of Sephadex G-100 (Sigma). The elution rate was approximately 60 ml/h and 15 ml fractions were collected. The active fractions were pooled and extensively dialyzed against distilled water and freeze dried[21].

Anticoagulant activity

The anticoagulant activities of crude and purified heparin samples were determined by comparing with the concentration necessary to prevent the clotting of sheep plasma using USP (United State Pharmacopoeia) method.

FTIR - (Fourier Transform Infra-Red spectrum analysis)

The crude and purified GAG samples were pelleted and compressed to prepare a salt disc (10 mm diameter) with potassium bromide (KBr) in the ratio of 1:10, and subjected for FTIR spectroscopic measurement (Nicolet IS5, Thermo Scientific). The wavenumber ranged from 450–2500 cm^{-1} with the resolution of 4 cm^{-1} and were analyzed by subtracting the spectrum of pure KBr.

Table 1: Yield of glycosaminoglycans and its anticoagulant activity from *Nereis* sp

Sample	Net yield GAGs (g/kg)		Anticoagulant activity USP units/mg	
	Crude	Purified	crude	purified
Nereis sp.	12	0.83	58	114

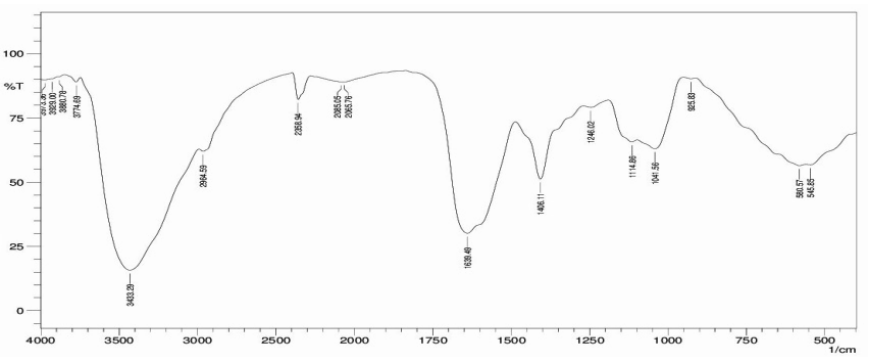

Figure 1: FTIR spectrum of Standard heparin sulphate

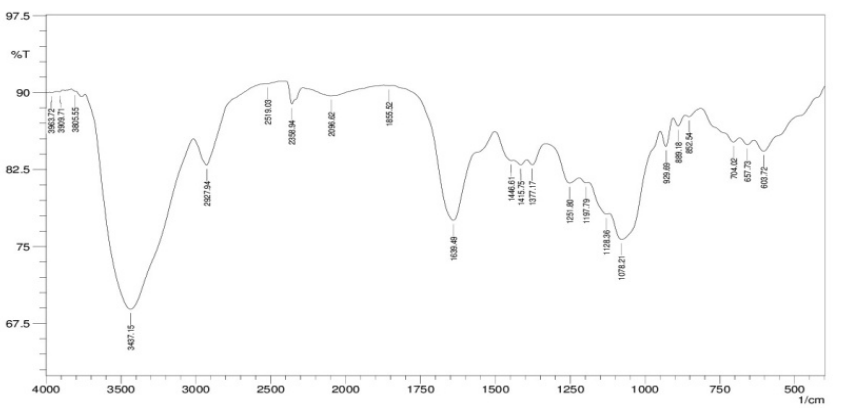

Figure 2: FTIR spectrum of GAGs obtained from polychaete

RESULTS

Estimation of GAGs

The amount of crude GAG was estimated as 12 g/kg of tissue in *Nereis* sp. After purification using gel chromatography, the yield was found to be 0.83 mg/kg (Table 1).

Anticoagulant activity

By United States pharmacopoeia method, the anticoagulant activity of the *Nereis* sp. crude and purified sample was reported to be 58 USP units/mg and 114 USP units/mg (Table 1).

Fourier Transform – Infra Red (FTIR) spectral analysis

FT-IR spectrum of GAGs of *Nereis* sp. was compared with standard heparin sulphate (Figure 1, 2 and 3). The IR spectrum of standard heparin sulphate contains 17 peaks ranging from 3973.36 to 545.85 cm^{-1}. Among them, four major peaks having the wavelength 3433.29, 1639.49, 1406.11 and 1041.56 cm^{-1} (Figure 1); whereas the IR spectrum of crude GAG from *Nereis* sp. presents 23 peaks, among them six are major peaks having the wave length of 3437.15, 1639.49, 1251.8, 1197.79, 1128.36 and 1078.21 cm^{-1} (Figure 2).

DISCUSSION

Heparin and heparin like compounds, reported from marine invertebrate has been showed to possess high anticoagulant activity and share most of the structural properties with mammalian heparins[22]. Similarly, heparin has been prepared from a number of different species including humans[23], clams[24], shrimp and seaweeds[25]. Heparin and heparin like compounds, which are present in polychaetes, showed high anticoagulant activity and share most of the structural properties with mammalian heparins. Anticoagulants have been reported from leech (one of the class of phylum annelida)[26]. Leech salivary glands produce a more diverse pharmacological cocktail of a wide variety of anticoagulants[27,28] that not only assist in phlebotomy by keeping blood flowing in and around an incision wound but that also keep the blood from coagulating inside the leech crop during the long periods of digestion[29]. Homogenization of a 1 kg portion of whole *Nereis* sp. in acetone and subsequent extraction in acetone/petroleum ether resulted in 250 g of defatted tissue. The heparin obtained from defatted polychaete tissue showed similar features with that of heparin. Heparin has been extracted from a number of marine organism's including[23], clams[30,31] and seaweeds. Extraction of the defatted soft body tissue of the giant African snail and subsequent purification of its GAGs showed that this tissue contained a large amount of GAG and free from impurities[32]. The use of CPC for quantitative separation of sulphated polysaccharides in tissue extracts has been preferred by most of the researchers'[33]. In the present investigation, the yield of crude and purified GAGs was found as 12 g/kg and 0.83 g/kg in *Nereis* species. Arumugam *et al.*[34], had quantified the heparin yield as 2.27 g/kg and 2.2 g/kg from *Tridacna maxima* and *Perna viridis* respectively. Matsubara *et al.*[35] reported an anticoagulant isolated from the green alga *Codium cylindricum* on thrombin activity in the absence of anti thrombin III and heparin cofactor II using chromogenic substrate. The result of the present study clearly showed average yield of heparin and heparin-like glycoasaminoglycans compared to existing reports. The heparin isolated from marine organisms (clams and mussels) has been reported to possess identical structural features and anticoagulant activity with that of mammalian polysaccharide[30]. The biological activity of heparins in invertebrates remains enigmatic. The anticoagulant activity of the crude and purified sample of GAG from the whole body tissue of *Nereis* sp. was reported as 58 USP units/mg and 114 USP units/mg. This variation might be due to the presence of non-anticoagulant substance in the samples since the activity of heparin depends upon the amount of impurity carried over in the isolated products. In the case of heparin, the structural characterization and their properties depend on the concentrations uronic acid and sulfate. Hence, their biological activities vary with concentrations. Also, the anticoagulant activity of heparin differs from species to species due to their in iteration with enzymes and inhibitors of the coagulation system[36]. The polychaete GAGs were found to be structurally similar to the standard heparin as assessed by the FT-IR spectra. In the present study, the anticoagulant GAGs from whole body tissue of *Nereis* sp. crude and purified sample showed a major peaks at 3437.15, 1639.49, 1251.8, 1197.79, 1128.36 and 1078.21 cm^{-1} which is said to be for the GAGs groups. The acetyl amino group was represented by a band at 1474.78 cm^{-1} and the carboxylic group at 1552.66 cm^{-1}. This was also well supported by the study of Rivera *et al*.[37] who also claimed that the characterization of traces of contaminants in crude heparin by conventional physico-chemical techniques such as size-exclusion or ion-exchange chromatography is relatively difficult. The peak pattern between the standard heparin and the sample was at 3433.29, 1639.49 cm^{-1} and 3437.15, 1639.49 cm^{-1} indicating the presence of GAGs group in the samples analyzed. The FT-IR spectral analysis of the anticoagulant GAGs from *Nereis* sp. showed more or less same number of peaks, lying within the same range of values of the commercial heparin used as a standard. Thus, the result of the present investigation provides information about the isolation, purification ad characterization of the heparin and heparin like glycoaaminoglycans (heparin and heparin sulfate) compound from marine polychaete.

CONCLUSION

In conclusion, the findings of the study highlighted the pharmacological importance of marine polychaete (*Nereis* sp.) as a potential source of anticoagulant with characteristics comparable to standard heparin. The isolated glycosaminoglycans were quantified as 12 g/kg and 0.83 mg/kg from the crude and purified samples respectively. Polychaete showed the anticoagulant activity of the 58 USP units/mg from crude; whereas purified samples showed 114 USP units/mg. Further investigations are underway to isolate the bioactive compound(s) responsible for the anticoagulant activity as well as the determination of the coagulation factor(s) affected. This natural source may be a cheaper yet potential alternative anticoagulant agent in the future owing to its abundant availability of raw material and simple extraction methods. The present findings could pave the way for further research and exploration of GAGs from marine polychaetes in particular *Nereis* sp. as an alternative source of heparin.

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REFERENCES

1. Donnell JO. Anticoagulants: Therapeutics, Risks, and Toxicity- Special Emphasis on Heparin-Induced Thrombocytopenia (HIT). J. Phar. Practice 2012; 25(1): 22-29. http://dx.doi.org/10.1177/0897190011431146 PMid:22491766
2. Mackman N. Triggers, targets and treatments for thrombosis. Nature 2008; 451: 914-918. http://dx.doi.org/10.1038/nature06797 PMid:18288180 PMCid:PMC2848509
3. Hirsh J, Donnell MO, Weitz JI. New anticoagulants. Blood 2005; 105: 453-63. http://dx.doi.org/10.1182/blood-2003-12-4195 PMid:15191946
4. Hirsh J, Donnell MO, Eikelboom JW. Beyond unfractionated heparin and warfarin: current and future advances. Circulation 2007; 116: 552-560. http://dx.doi.org/10.1161/CIRCULATIONAHA.106.685974 PMid:17664384
5. Stone WM, Tonnessen BH, Money SR. The new anticoagulants, Perspect Vasc. Surg. Endovasc. Ther 2007; 19: 332-335. http://dx.doi.org/10.1177/1531003507304441 PMid:17911567
6. Bounameaux H. The novel anticoagulants: entering a new era. Swiss. Med. Wkly 2009; 139: 60-64. PMid:19204837
7. Aneiros A, Garateix A. Bioactive peptides from marine sources: pharmacological properties and isolation procedures. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 803: 41–53. http://dx.doi.org/10.1016/j.jchromb.2003.11.005 PMid:15025997
8. Amorim RCN, Rodrigues JAG, Holanda ML, Mourao PAS and Benevides NMB. Anticoagulant properties of a crude sulfated polysaccharide from the red marine alga *Halymenia floresia* Clemente C. Agardh. Maringa 2011; 33(3): 255-261.
9. Thomas DP. Does low molecular weight heparin cause less bleeding? Thrombosis and Haemostasis 1997; 78(6): 1422-1425. PMid:9423787
10. Pereira MG, Benevides NMB, Melo MRS, Valente AP, Melo FR, Mourao PAS. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? Carbohydrate Research 2005; 340(12): 2015-2023. http://dx.doi.org/10.1016/j.carres.2005.05.018 PMid:16023626
11. Holmer E, Soderstrom G and Andersson LO. Studies on the mechanism of the rate-enhancing effect of heparin on the thrombin-antithrombin III reaction. Eur. J. Biochem 1979; 93(1): 1-5. http://dx.doi.org/10.1111/j.1432-1033.1979.tb12787.x PMid:436823
12. Mayer AMS, Rodriguez AD, Berlinck RGS, Fusetani N. Marine compounds with antibacterial, anti tuberculosis, and antiviral activities, affecting the immune and nervous system and other miscellaneous mechanisms of action. Comparative Biochemistry and Physiology, Part C 2011; 153(2): 191-222.
13. Hutchings P. Biodiversity and functioning of polychaetes in benthic sediments. Biodiversity Conservation 1998; 7(1): 1133-1145. http://dx.doi.org/10.1023/A:1008871430178
14. Giangrande A, Licciano M, Luigi M. Polychaetes as environmental indicators revisited. Marine Pollution Bulletin 2005; 50: 1153-1162. http://dx.doi.org/10.1016/j.marpolbul.2005.08.003 PMid:16197965
15. Olsgard F, Brattegard T, Holth T. Polychaetes as surrogates for marine biodiversity: lower taxonomic resolution and indicator groups. Biodiversity and Conservation 2003; 12: 1033–1049. http://dx.doi.org/10.1023/A:1022800405253
16. Grall J and Glemarec M. Using biotic indices to estimate macrobenthic community perturbation in the bay of Brest. Estuarine Coastal and Shelf Science 1997; 44: 43–53. http://dx.doi.org/10.1016/S0272-7714(97)80006-6
17. Solis Weiss V, Aleffi F, Bettoso N, Rossin P, Orel G, Fonda Umani S. Effects of industrial and urban pollution on the benthic macrofauna in the Bay of Muggia (industrial port of Trieste, Italy). Science of the Total Environment 2004; 328: 247–263. http://dx.doi.org/10.1016/j.scitotenv.2004.01.027 PMid:15207588
18. Elayaraja S, Murugesan P, Vijayalakshmi S, Balasubramanian T. Antibacterial and antifungal activities of polychaete *Perineries cultrifera*. I. J. Mar. Sci 2010; 39: 257-261.
19. Stabili L, Sicuro B, Dapra F, Gai F, Abete C, Dibenedetto A, Pastore C, Schirosi R, Giangrande A. The biochemistry of *Sabella spallanzanii* (Annelida: Polychaeta), a potential resource for the fish feed industry. J. World. Aquacul. Soci 2013; 44: 384-395. http://dx.doi.org/10.1111/jwas.12038
20. Singh R, Sahu SK, Thangaraj M. Polychaete fatty acids as potential inhibitor against human glioblastoma multiforme. Inter. J. Rec. Scie. Res 2013; 4(10): 1519-1524.
21. Laurent TC, Tengblad A, Thunberg L, Hok M, Lindahl U. The molecular-weight-dependence of the anti-coagulant activity of heparin. Biochem Journal 1978; 175(2): 691–701. PMid:743219 PMCid:PMC1186120
22. Karthikeyan V, Gopalakrishnan A, Vijayakumar R, Bharathirajan P. Anticoagulant activity of marine bivalve Donax incarnates Lin, 1758 Collected from Thazhanguda, Southeast coast of India. Asian Pacific Journal of Tropical Biomedicine 2012; S1798-S1801.
23. Linhardt RJ, Ampofo SA, Fareed J, Hoppensteadt D, Mulliken JB and Folkman J. Isolation and characterization of human heparin. Biochemistry 1992; 31: 12441-12445. http://dx.doi.org/10.1021/bi00164a020 PMid:1463730
24. Yamada S, Sugahara K and Demir SOM. Evolution of glycosaminoglycan. Communicative and Integrative Biology 2001; 4(2): 150-158. http://dx.doi.org/10.4161/cib.4.2.14547 PMid:21655428 PMCid:PMC3104567
25. Mahanama De Zoysa, Nikapitiya C, Jeon YJ. Anticoagulant activity of sulfated polysaccha-ride isolated from fermented brown seaweed *Sargassum fulvellum*. Appl. Phycol 2008; 20: 67-74. http://dx.doi.org/10.1007/s10811-007-9182-7
26. Rydel TJ, Ravichandran KG, Tulinsky A, Bode W, Huber R, Roitsch C. The structure of a complex of recombinant hirudin and human alpha-thrombin 1990; 249: 277-280.
27. Min GS, Sarkar IN and Siddall ME. Salivary transcriptome of the North American medicinal leech, *Macrobdella decora*. J. Para 2010; 96: 1211–1221. http://dx.doi.org/10.1645/GE-2496.1 PMid:21158638
28. Alaama M, Alnajjar M, Abdualkader AM, Mohammad A and Merzouk A. Isolation and analytical characterization of local Malaysian leech salivary extracts. IIUM Eng. J 2011; 12: 51–59.
29. Salzet M. Anticoagulants and inhibitors of platelet aggregation derived from leeches. FEBS Lett 2001; 429: 187–192. http://dx.doi.org/10.1016/S0014-5793(01)02212-8
30. Pejler G, Danidcon A, Bjork I, Lindahl V, Nadar HB and Dietrich CP. Structural and anti thrombin. Binding properties of heparin isolated from the classes *Anomalocardia brasitiana* and *Tivela mactroides*. J. Biol. Chem 1987; 263: 1413-1421.
31. Dietrich CP, Nader HB, Depavia JF and Santose EA. Heparin in mollusks chemical enzymatic degradation and HNMR spectrocopial evidence for the maintenance of the structure through evolution. Int. J. Biol. Macromol 1989; 11: 361-366. http://dx.doi.org/10.1016/0141-8130(89)90008-1
32. Kim YS, Jo YY, Cheng II M, Toidas T, Parks Y and Linhardts RJ. A new glycosaninoglycan, from the giant African snail *Achatina fulicon*. J. biol. Chem 1996; 211: 11750-11755.
33. Balamurugan K, Kalaichelvan VK, Dey A. Isolation and Identification of Heparin like Substances from the body Tissue of *Conus musicus*. Int. J. PharmTech 2009; 1(4): 1650-1653.
34. Arumugam M, Balasubramanian T, Shanmugam A, Garg H. A comparative study of anticoagulant and anti proliferative activity of fast moving heparin from giant clam *Tridacna maxima* (Roeding,1798) and green mussel *Perna viridis* (Linnaeus,1758). J. Applied Biological Sciences 2008; 2(3): 27-30.
35. Matsubara K, Matsuura Y, Bacic A, Liao M, Hori K, Miyazawa K. Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. Int. J. Biol. Macromol 2001; 28: 395-399. http://dx.doi.org/10.1016/S0141-8130(01)00137-4
36. Mulloy A, Lang R, Reilly MO, Sigafoos J, Lancioni G, Rispoli M. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: A systematic review. Research in Autism Spectrum Disorders 2011; 5(1): 86-88. http://dx.doi.org/10.1016/j.rasd.2010.07.004
37. Rivera V, Levieux A, Levieux D. Characterization of Ag1, the major species-specific contaminant of bovine crude heparin, and its identification as an aprotinin/heparin complex. J. Pharm Biomed Anal 2002; 29(3): 443-58. http://dx.doi.org/10.1016/S0731-7085(02)00084-5

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