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Biosensor analysis of the molecular interactions of pentosan polysulfate and of sulfated glycosaminoglycans with immobilized elastase, hyaluronidase and lysozyme using surface plasmon resonance (SPR) technology

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Abstract

Pentosan polysulfate (NaPPS) and chondroitin sulfates (ChSs) have recently been shown to exhibit both symptom and disease modifying activities in osteoarthritis (OA), but their respective mechanisms of action are still the subject of conjecture. Excessive catabolism of joint articular cartilage is considered to be responsible for the initiation and progression of OA but the abilities of these drugs to mitigate this process has received only limited attention. Human neutrophil elastase (HNE) is a proteinase, which can degrade the collagens and proteoglycans (PGs) of the cartilage directly or indirectly by activating latent matrix metalloproteinases. Hyaluronidase (HAase) is an endoglycosidase, which degrades glycosaminoglycans including hyaluronan, which provides the aggregating component of the PG aggrecan complex. In the present study the molecular interactions between the NaPPS, ChSs and some other sulfated polysaccharides with immobilized HNE, HAase or lysozyme (a cationic protein implicated in PG metabolism) were studied using a SPR biosensor device-BIAcore2000. The above three enzymes were covalently immobilized to a biosensor chip CM5 separately using amine coupling. The binding affinity of each sulfated polysaccharide and the kinetics of NaPPS over the concentration range of 0.3–5.0 μg/ml were determined. The inhibition of HNE by the sulfated polysaccharides as determined using the synthetic substrate succinyl-Ala-Ala-Val-nitroanilide (SAAVNA) in a functional assay was compared with their respective binding affinities for this proteinase using the BIAcore system. The results obtained with the two independent techniques showed good correlation and indicated that the degree and ring positions of oligosaccharide sulfation were major determinants of enzyme inhibitory activity. The observed difference in order of binding affinities of the drugs to the immobilized HNE, HAase and lysozyme suggests a conformational relationship, in addition to the charge interactions between the sulfate esters of the polysaccharides and the cationic

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amino acids of the enzymes. Significantly, the SPR biosensor technology demonstrated that small differences among sulfated polysaccharides, even subtle variations among different NaPPS batches, could be readily detected. The SPR technology therefore offers not only a sensitive and reproducible method for ranking noncompetitive enzyme inhibitors for drug discovery but a rapid and quantitative bioassay for monitoring batch consistency of manufacture.

Keywords: Osteoarthritis; Surface plasmon resonance; Pentosan polysulfate; Chondroitin sulfates; Enzyme inhibition

1. Introduction

Musculoskeletal disorders, particularly osteoarthritis (OA) and rheumatoid arthritis (RA) are the major cause of morbidity worldwide and the prevalence in developed countries is rising progressively as the life span of the population increases [1,2]. These diseases have a major impact on patients' productivity and quality of life and inflict an enormous financial burden on health systems. It has been estimated that the costs of managing OA and related disorders in the USA exceeded $70 billion in 1996 [3].

The etiology of OA is multifactorial and while aging is the most strongly associated risk, mechanical, hormonal and genetic factors all contribute to varying degrees. OA emerges as a clinical syndrome when these etiological determinants result in sufficient joint damage to cause impairment of function and the appearance of symptoms. This clinical syndrome is manifest radiologically by joint space narrowing (due to loss of cartilage) and extensive re-modeling of subchondral bone with proliferation at the joint margins (osteophytosis) [1,4].

One of the earliest pathological features of OA is disruption of the collagen network particularly in the superficial zone of articular cartilage accompanied by depletion of proteoglycans (PGs) from the extracellular matrix [5–7]. These events not only lead to a decline in the functional properties of the articular cartilage, but also contribute to synovial inflammation since breakdown products of cartilage are antigenic and, when released into synovial cavity, can activate macrophage and neutrophils [8,9]. Proinflammatory cytokines, prostaglandins, proteinases and free radicals derived from these activated cells suppress chondrocyte anabolic processes as well as promote cartilage catabolism [10–12]. A major proteinase present in RA and OA joints is human neutrophil elastase (HNE) [13,14]. This proteinase has been shown not only to bind to chondrocytes [15] and degrade the articular cartilage extracellular matrix [16–20] but also to cleave many other connective tissue components, such as type I, III, IX, XI collagens [21,22], PGs [23,24], fibronectin [25] and activate the complement, fibrinolytic and kininogen systems [26]. HNE also inhibits the synthesis of articular cartilage PGs [27], inactivates tissue inhibitors of metalloproteinases [28] and activates matrix metalloproteinases such as stromelysin and gelatinase [29], which independently degrade collagens and PGs.

Pharmacological management in OA has until quite recently treated the symptoms of the disease rather than the underlying cause; analgesics, steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) representing the mainstay of treatment. However, the deleterious side effects associated with many of these agents have led to a more conservative approach to their use in recent years and have stimulated the search for alternative treatments which target the underlying pathology of OA which is responsible for the symptoms [30,31]. Such agents have been identified as structural or disease modifying osteoarthritis drugs (DMOADs) [31–33]. While totally synthetic DMOADs are still in the early phases of development, agents derived directly from natural sources or obtained by their chemical modification (semi-synthetics) are at a more advanced stage. Two of the most widely used natural products for the management of OA are glucosamine and the chondroitin sulfates (ChSs) which are both structural components of the PG complex. Double-blind clinical trials with these agents have indicated that both can provide relief of

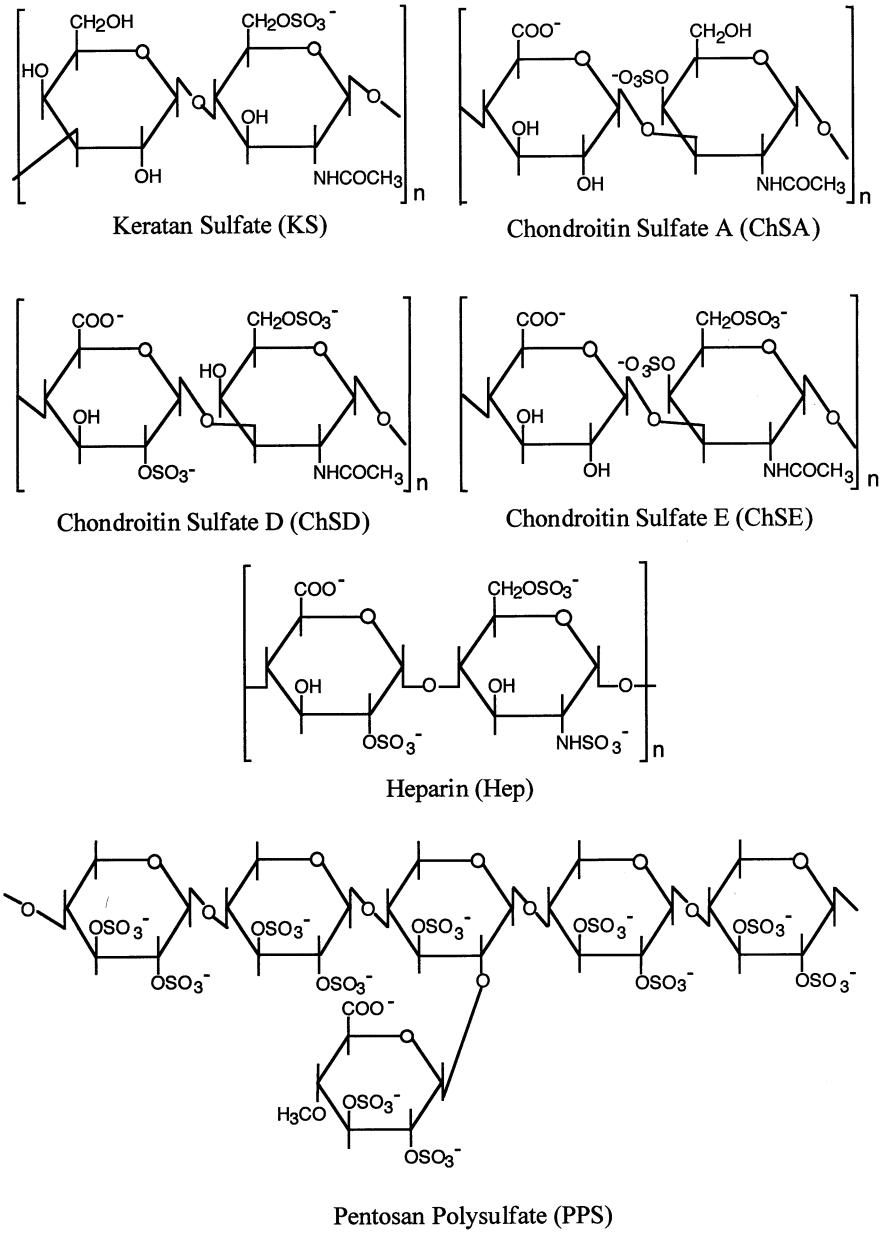

Fig. 1. Structural formulae of the semi-synthetic anti-OA drug, PPS and other naturally occurring sulfated glycosaminoglycans used in this study. NaPPS, sodium pentosan polysulfate; ChSA, D and E, ChS A, D and E; Hep, heparin; KS, keratan sulfate. It should be noted that the structural units shown for the sulfated glycosaminoglycans are the most common repeating units in these polymers. More complete representations of the structural variations, which exist within these polysaccharides can be obtained from the review of Sugahara and Yamada [45]. The structure for NaPPS is also abbreviated in the interest of space since it is reported to contain, on average, nine to ten pentose sugar units for every sulfated glucuronyl residue [31].

symptoms in OA [34–38] and more recently it has been reported that they may also exhibit DMOAD activities [39,40] although these claims have been challenged [41,42]. Since these latter products are obtained from natural sources, their composition and structure may vary from batch to batch. This applies particularly to the ChSs which are manufactured from cartilaginous tissues from a variety of animal species. The ChSs in cartilages of different species and age and from different anatomical locations have been shown to have variable molecular sizes as well as different numbers and locations of ester sulfation in the glycosaminoglycan rings [43–45]. While the ChSs marketed by the nutraceutical industry are presently not subject to mandatory analytical controls this is not true for the semi-synthetics which are required to comply with the normal pharmaceutical requirements for drug manufacture and sale. One such semi-synthetic, pentosan polysulfate (PPS) which is now commercially available as its sodium and calcium salts, is widely used for the treatment of OA in domestic animals and is in phase II/III of clinical trials for human OA [31,46]. Although the manufacture of PPS is undertaken in compliance with the GMP and GLP requirements for government agency registration, there has been, until quite recently, analytical difficulty associated with characterization of PPS molecular weight distribution and oligomer substructure. This problem has been largely resolved by the use of high resolution capillary zone electrophoresis (CZE) which was able to distinguish NaPPS synthesized by different manufacturers [47,48]. However, as with the ChSs, it was still unproven that the structural variations observed in NaPPS from different manufacturers would be associated with differences in their respective pharmacological activities.

In this report we describe the use of surface plasmon resonance (SPR) technology to address these issues. The results showed that this technique could readily discriminate between sulfated polysaccharides with major or minor differences in structures in terms of their binding to enzymes implicated in the pathogenesis of OA. Moreover, the study illustrates the potential of the technique as a means of rapidly screening novel compounds as enzyme inhibitors and identifying other molecular interactions which may be relevant to disease processes.

2. Materials and methods

2.1. Materials

Bovine testicular hyaluronidase (HAase) (EC 3.2.1.35) was purchased from Sigma (St. Louis, MO, USA). Egg-white lysozyme (EC 3.2.1.17), from CalBioChem (Bad Soden, Germany) and HNE (EC 3.4.21.37), from ICN (Costa Mesa, CA, USA). Sodium pentosan polysulfate (NaPPS) were obtained from two sources (NaPPS-B from Bene Arzneimittel GmbH, Munich, Germany and NaPPS-N from Nature-Vet, Pty, Sydney, Australia). Three different batches of NaPPS from each of these companies were examined. Bovine lung heparin (Hep) and bovine tracheal chondroitin sulfate A (ChSA) were purchased from Sigma. Chondroitin sulfate D and E (ChSD and ChSE) were generous gifts from Dr N. Seno (Ochanomizu University, Tokyo, Japan). Bovine corneal keratan sulfate (KS) was purchase from Seikagaku kogyo (Tokyo, Japan). The structural formulae of these sulfated polysaccharides are shown in Fig. 1. Synthetic elastase substrate succinyl-Ala-Ala-Val-nitroanilide (SAAVNA) was obtained from Bachem (Bubendorf, Switzerland).

2.2. Instrumentation and reagents

The BIAcore2000 instrument, BIACORE CONTROL and BIAEVALUATION software 3.1, sensor chip CM5, amine coupling kit and HBS buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% (v/v) Surfactant P20) were all purchased from Pharmacia Biosensor AB (Uppsala, Sweden).

2.3. Immobilization of enzymes (ligands) to sensor chip

Stock solution (1.0 mg/ml) of the ligands, HNE, HAase and lysozyme were prepared in H_2O and then further diluted 1:10 with 10 mM sodium

acetate buffer at pH 6.0 and individually immobilized to a CM5 sensor chip using the amine coupling reaction as described by the manufacturer. Briefly, the surface of the chip consisting of flow cell (FC)-1, 2, 3 and 4 was activated by exposing them to a mixture of 200 mM *N*-ethyl-*N*′-dimethylaminopropyl carbodiimide (EDC) and 50 mM *N*-hydroxysuccinimide (NHS) for 7 min. FC-1 was used as a reference surface and was directly deactivated by injecting 1 M ethanolamine at pH 8.5 for 7 min. The other three flow cells were injected with either HNE, HAase or lysozyme, respectively, followed by injecting 1 M ethanolamine to block the unreacted groups on the surface. One sensor chip was immobilized simultaneously with the three different ligands. The baseline was allowed to stabilize for at least 2 h in HBS running buffer before injecting test analytes.

2.4. Assay design

The seven sulfated polysaccharides including sodium NaPPS (NaPPS-B and NaPPS-N), Hep, ChSA, ChSD, ChSE and KS were used as analytes. Binding assays were performed at 25 °C in HBS buffer at a flow rate of 50 μl/min. Injection times of polysaccharides in the HBS buffer were 4 min followed by 4 min of dissociation. Regeneration of the ligands after each binding circle was performed using 1 min pulse of 100 mM acetic acid containing 2.0 M NaCl. Each cycle consisted of a 1 min waiting period to allow monitoring of the baseline stability.

2.5. Data analysis

The real-time reference curve obtained from a nonligand coated flowcell exposed to the HBS buffer was subtracted from binding curves obtained from the flowcells with immobilized ligands. Association and dissociation rate constants were calculated by nonlinear curve fitting of the primary sensorgram data using the two state reaction binding model available in the BIAEVALUATION 3.1 software. The apparent affinity constants (K) for the sulfated polysaccharides were calculated from the association and dissocia-

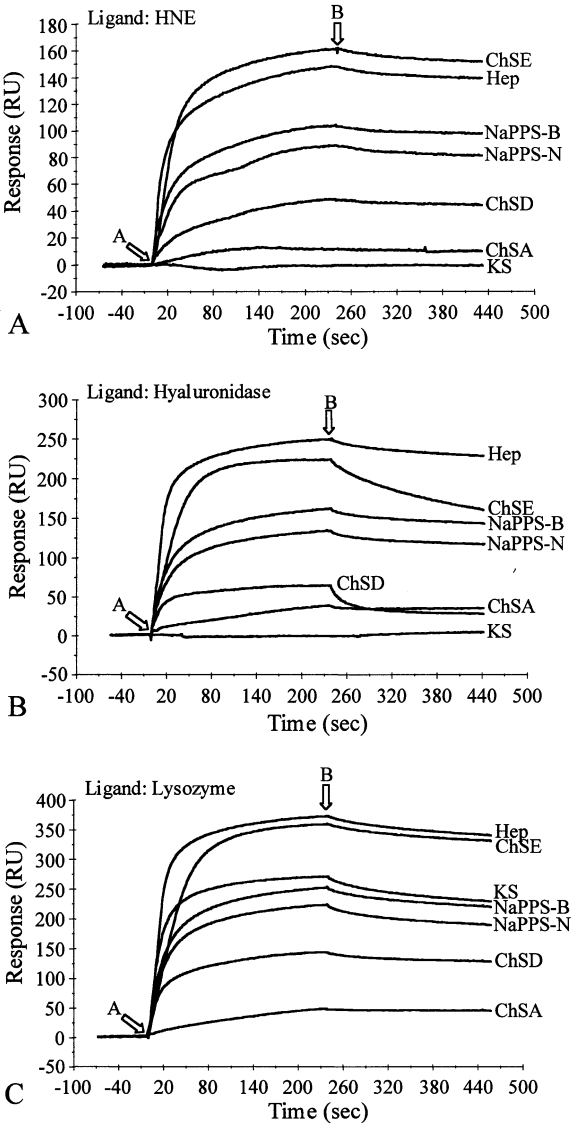

Fig. 2. Comparison of the binding affinity of seven sulfated polysaccharides to HNE (A), HAase (B) or lysozyme (C) immobilized in BIAcore2000 system. The above three enzymes were immobilized separately in three flow cells on a CM5 sensor chip. A 2.0 μg/ml of Hep, ChSE, NaPPS, ChSD, ChSA and KS were individually injected over the immobilized chip surface at a constant flow rate at 50 μl/min with an injection volume of 200 μl. The various resonance units (RU) which were shown at the vertical axis represent the difference in binding affinity of each sulfated polysaccharide to immobilized enzymes. Arrow A represents starting point of sample injection or the beginning of association phase. Arrow B represents the end of sample injection or the beginning of dissociation phase.

tion rate constants using BIAEVALUATION 3.1 software.

2.6. Elastase inhibition assay

Stock solutions of the sulfated polysaccharide samples were prepared at 1.0 mg/ml in HNE assay buffer (50 mM sodium phosphate, pH 7.4, 0.1% (w/v) BSA, 0.025% (w/v) Triton X100) and serially diluted to give solutions with concentrations within the range 0.04–40 μg/ml for KS, ChSA and ChSD, 0.003–3.13 μg/ml for NaPPS-B and NaPPS-N, 0.0015–1.58 μg/ml for Hep and ChSE. Duplicate diluted samples (100 μl) were transferred to wells of a microtitre plate to which was added 50 μl of 2.0 μg/ml HNE in the HNE assay buffer and the plate incubated at 37 °C for 10 min. Substrate solution (100 μl of 0.5 mM SAAVNA in 12.5% (v/v) DMSO and 87.5% (v/v) assay buffer) was added to each well and the absorbance was read at 405 nm four times at 30 min intervals. The elastase inhibition rates were expressed as a percentage of the control rate and plots of percentage residual HNE activity versus inhibitor concentrations in the inhibition assay were prepared. The concentrations of the sulfated polysaccharides inhibiting half-maximal HNE activity (IC_{50}) for each sulfated polysaccharide was determined from these curves.

3. Results

3.1. Comparison of binding affinity between sulfated polysaccharides and immobilized enzymes

The binding curves for each of the polysaccharides to the three immobilized enzymes were shown as plotted sensorgrams (Fig. 2). These data demonstrated the high sensitivity of the BIAcore technology for detection of binding events involving sulfated polysaccharides. The results obtained showed ranking of binding affinities as ChSE > HEP > NaPPS > ChSD > ChSA > KS for binding to HNE; HEP > ChSE > NaPPS > ChSD > ChSA > KS for binding to HAase and HEP > ChSE > KS > NaPPS > ChSD > ChSA for binding to lysozyme. Interestingly, although the binding of KS to HNE and HAase was undetectable, it showed strong binding affinity to lysozyme.

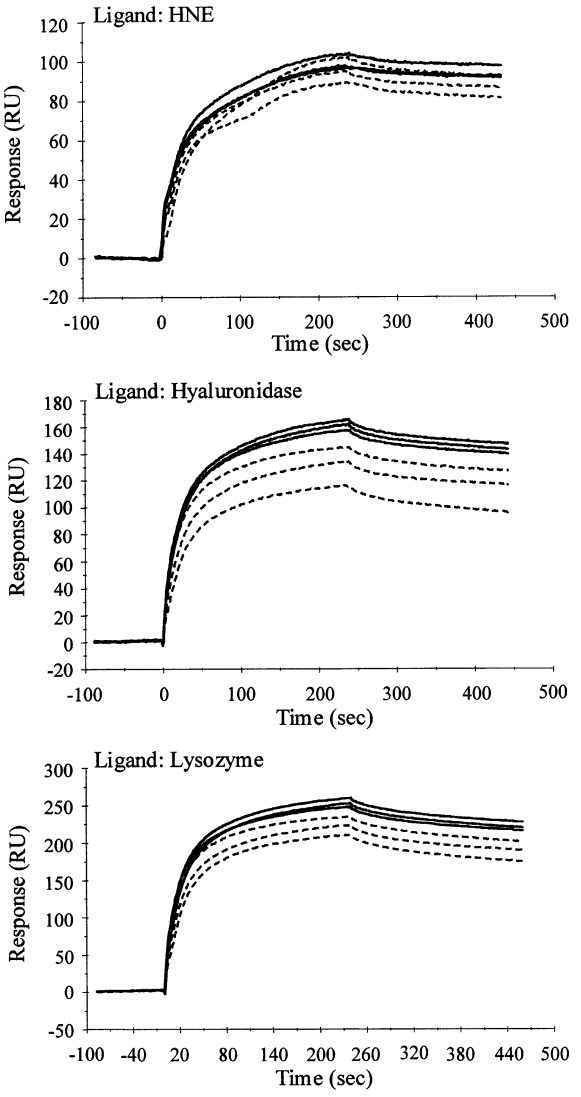

Fig. 3. Overlay plot of sensorgrams of binding of different batches of NaPPS to immobilized HNE (A), HAase (B) or lysozyme (C). Each three batches of NaPPS-B or NaPPS-N were measured using the identical chip and experimental conditions as described in the materials and methods. Solid lines represent data for NaPPS-B and broken lines for NaPPS-N.

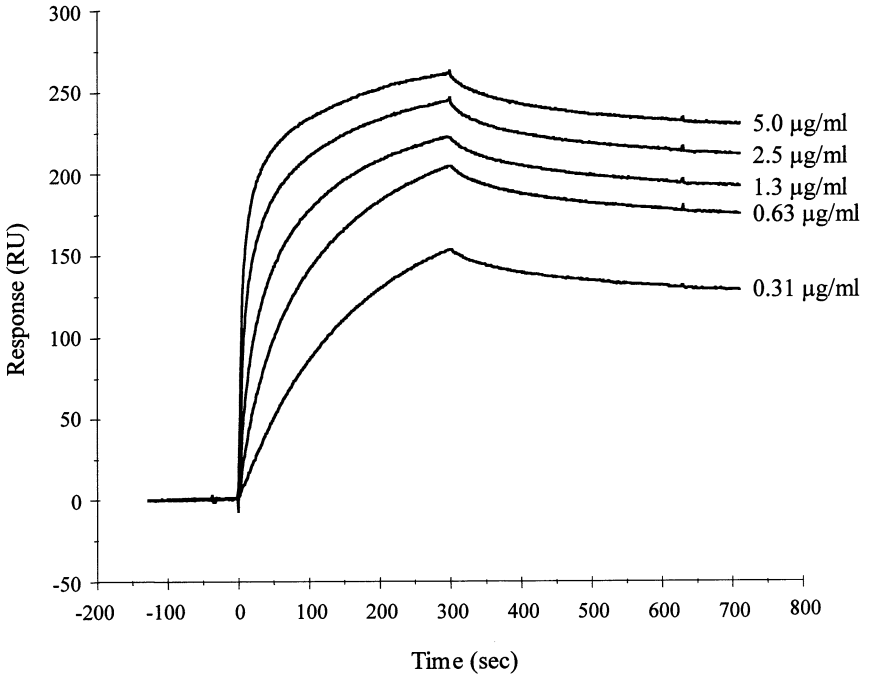

Fig. 4. Overlay plot of sensorgrams of binding kinetics of NaPPS-B to immobilized lysozyme. Each injection volume of sample solution was 200 μl at a constant flow rate of 50 μl/min. Concentrations of NaPPS-B (from top to bottom): 5.0, 2.5, 1.3, 0.63 and 0.31 μg/ml. Dissociation proceeded when NaPPS-B was replaced with HBS buffer.

3.2. Detection of variations of NaPPS binding affinity from two different manufacturers

Three individual batches of NaPPS from the two manufacturers (NaPPS-B and NaPPS-N) were examined by duplicate injection of a single concentration of 2.0 μg/ml of each drug into sensor chip cell. Identical operating experimental conditions were used throughout as samples were run simultaneously over immobilized ligands. Binding

Table 1
Apparent kinetic rate constants (mean ± S.E.M.) and equilibrium binding constants (mean ± S.E.M.) for the interaction of NaPPS with immobilized HNE, HAase or lysozyme

Analytes	ka1 (1/Ms) ($\times 10^5$)	kd1 (1/s) ($\times 10^{-2}$)	ka2 (1/s) ($\times 10^{-2}$)	kd2 (1/s) ($\times 10^{-4}$)	K* (1/M) ($\times 10^8$)
Ligand: HNE					
NaPPS-B	1.83 ± 0.14	3.50 ± 0.57	1.69 ± 0.090	1.78 ± 0.14	6.04 ± 1.07
NaPPS-N	1.80 ± 0.15	5.42 ± 1.65	1.44 ± 0.045	3.92 ± 0.46	2.07 ± 0.32
Ligand: HAase					
NaPPS-B	1.18 ± 0.10	2.14 ± 0.46	1.41 ± 0.065	1.54 ± 0.20	5.38 ± 1.43
NaPPS-N	1.38 ± 0.11	3.46 ± 0.62	1.35 ± 0.059	2.94 ± 0.42	2.31 ± 0.35
Ligand: Lysozyme					
NaPPS-B	1.23 ± 0.043	2.53 ± 0.28	0.91 ± 0.15	2.89 ± 0.49	3.34 ± 0.41
NaPPS-N	1.25 ± 0.038	2.22 ± 0.064	0.95 ± 0.070	2.38 ± 0.23	1.90 ± 0.32

The kinetic data was evaluated using two-state (conformational change) model which can be described briefly as: $A + B \underset{kd1}{\overset{ka1}{\rightleftharpoons}} [AB] \underset{kd2}{\overset{ka2}{\rightleftharpoons}} AB$ * K values present apparent affinity constant which were calculated based on the average molecular mass of 6000 Da for NaPPS [31].

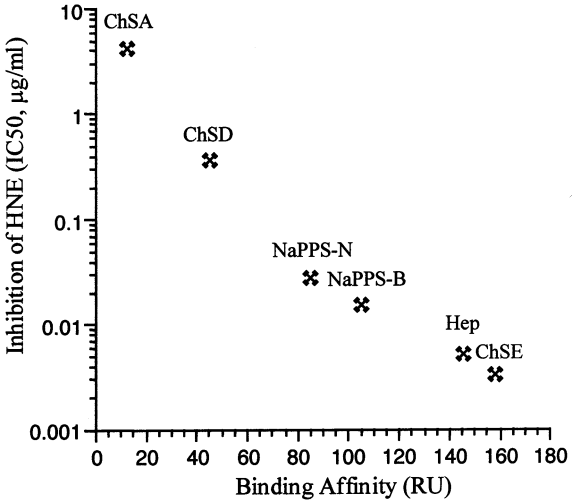

Fig. 5. Correlation between binding affinity (expressed as resonance units, RU) and HNE inhibitory activity of sulfated polysaccharides (expressed as IC_{50}). The data of binding affinity to immobilized HNE were obtained from BIAcore assay and IC_{50} (concentration of inhibitor at 50% inhibition) were determined from HNE inhibition assay using synthetic substrate SAAVNA (see text for details).

curves for the drug batches were shown as plotted sensorgrams (Fig. 3). The results suggest that the inter-batch variation of NaPPS-B was less than for batches derived from the manufacturer of NaPPS-N. Moreover, using this experimental system, the binding affinities of NaPPS-B batches were consistently higher then that of the NaPPS-N batches, particularly for HAase and lysozyme as shown in Fig. 3B and C.

3.3. Kinetic analysis of NaPPS binding to elastase, hyaluronidase and lysozyme

Real-time kinetic binding of NaPPS-B and NaPPS-N batches to immobilized HNE, HAase and lysozyme was performed over the concentration range of 0.3–5.0 μg/ml. A high flow rate of 50 μl/min was used to minimize mass transport limitations. Typical concentration-response curves of NaPPS-B binding to lysozyme is shown (Fig. 4). All the kinetic data obtained by global fitting curves of NaPPS-B and NaPPS-N binding to these three immobilized enzymes is summarized in Table 1.

Table 2
Inhibitory activity (IC_{50}) of sulfated polysaccharides on HNE using the synthetic substrate SAAVNA under the conditions outlined in the methods section

Sulfated polysaccharides	IC_{50} (μg/ml)
Chondroitin sulfate E (ChSE)	0.0036 ± 0.00005
Heparin (Hep)	0.0057 ± 0.0002
Pentosan polysulfate-B (NaPPS-B)	0.017 ± 0.001
Pentosan polysulfate-N (NaPPS-N)	0.031 ± 0.0005
Chondroitin sulfate D (ChSD)	0.40 ± 0
Chondroitin sulfate A (ChSA)	4.6 ± 0.61
Keratan sulfate (KS)	> 100

IC_{50}s shown are the mean ± S.E.M. of duplicate values.

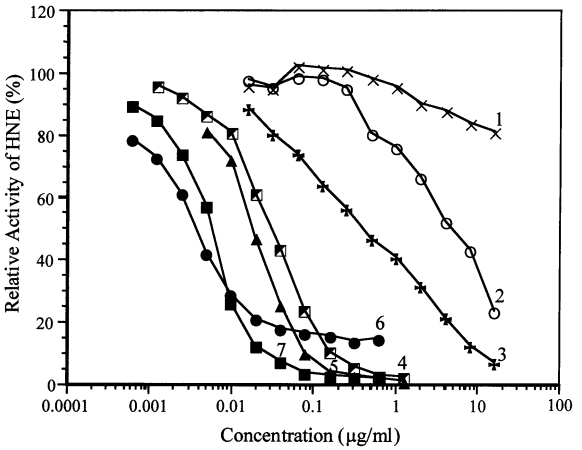

Fig. 6. Inhibition of HNE by the sulfated polysaccharides. The final concentrations of sulfated polysaccharides were 0.016–16.0 μg/ml for KS, ChSA and ChSD, 0.0012–1.25 μg/ml for NaPPS-B and NaPPS-N, 0.0006–0.63 μg/ml for Hep and ChSE. The substrate was SAAVNA at a fixed concentration of 0.2 mM. The HNE concentration was constant at 13.6×10^{-9} M. KS, 1; ChSA, 2; ChSD, 3; NaPPS-N, 4; NaPPS-B, 5; Hep, 6; ChSE, 7.

3.4. Non-competitive inhibition of elastase activity by drugs

When the elastase inhibitory activity of each sulfated polysaccharides was determined using the synthetic substrate succinyl-Ala-Ala-Vla-nitroanilide, Hep and ChSE were found to be the most potent compounds. NaPPS showed strong and ChSD, ChSA and KS weak inhibitory activity against this proteinase (Fig. 5 and Table 2). The

results obtained with the functional elastase inhibition assay showed a good correlation ($r^2 = 0.95$) with BIAcore binding assays ranking (Fig. 6).

4. Discussion

The major advantages of SPR biosensor technology over other techniques for detecting molecular interaction are that it allows real-time monitoring, label-free analytes can be used and it is highly sensitive, efficient and reproducible. The most common types of interactions which have been studied are antigen–antibody [49,50] and protein–receptor interactions [51,52] but more recently this technique has been used to examine drug–receptor interactions [53–55]. In contrast there has been limited use [45] of SPR biosensor technology to study the molecular interactions of sulfated polysaccharides with enzymes and other proteins.

In the present study, the semi-synthetic anti-OA drug, NaPPS and several other naturally occurring sulfated glycosaminoglycans which were also reported to exhibit anti-OA activity were ranked with regard to their binding to HNE, HAase and lysozyme using the SPR technology. While these three enzymes are all present in cartilage and joint fluids and have been shown to contribute to matrix destruction and disease progression [31,56,57] we are aware that there are many other proteinases which may be considered more relevant such as the matrix metalloproteinases and aggrecanase family [11]. However, most of these proteinases are not presently available commercially and the primary goal of the present studies was to evaluate a novel prototype which could be used to rank putative DMOAD activities, then later expand the ligand repertoire to include other proteinases and cytokines.

Of significance was the finding that the order of binding of compounds to HNE as determined by the SPR technique correlated very well with their IC_{50}s obtained using a conventional colorimetric assay. This provided support for the assumption that the nature of the binding of drugs to the immobilized HNE was comparable with interactions that would occur when the proteinase was in solution. The reproducibility of the binding assays and our success in kinetic curve fitting also confirmed that immobilization of the enzymes used here did not significantly disturb their normal ability to interact with analytes, as may have occurred during the amine coupling to the chips. Others have investigated the relative inhibitory activities of Hep, ChSA, ChSD, ChSE and NaPPS against elastase using the same or similar synthetic substrates [56,58–61]. In general there was good agreement of data with the polysulfated glycosaminoglycans, with Hep and ChSE being the most potent inhibitors in the group. As reported by Volpi et al. [44] and Bartolucci et al. [58] while the major ChSs in bovine or porcine trachael cartilages and shark cartilage are the Chondroitin A and C isomers they also contain appreciable but variable quantities of ChSD and ChSE. Since ChSs from all these sources are marketed internationally as treatments for OA it would be expected from the present studies, that these preparations could exhibit differences in their clinical effectiveness. Hep is mainly used as a thromboprophylactic following surgery and would not be used as a DMOAD because of the high risk of bleeding.

Although the highly sulfated NaPPS showed weaker anti-elastase activity than Hep and ChSE in both assays it should be noted that this semi-synthetic has a molecular weight of 5500 Da [31] which is approximately 25% of the weight averaged molecular weight of the ChSs and Hep. Since binding to HNE and presumably HAase and lysozyme is both charge and molecular weight dependent [59,60,62,63], this discrepancy in order of activities may be rationalized. In addition, conformational considerations cannot be ignored as illustrated by the observed strong binding of KS to lysozyme but not to the other enzymes. This finding reinforces our view that several ligands should be immobilized in BIAcore cells when attempting to determine relative drug interactions and potencies. This point was also shown with the different batches of NaPPS from the two manufacturers where greater separations were found with HAase and lysozyme than with the immobilized HNE when the drugs were examined at a

single concentration (Fig. 3). However, these apparent differences were resolved when the NaPPS preparations were subjected to kinetic analysis using a range of drug concentrations to determine the apparent rate constants and equilibrium binding constants (Table 1).

Analysis of the kinetic data using the BIACORE software also showed that a two-state (conformational change) model provided the best fit for the interaction of these compounds with the three enzymes. This model described an intial 1:1 binding of NaPPS to the immobilized ligands followed by a conformational change of the complex to improve the interaction. The selection of the two-state model was based on two findings. Firstly, the experimental data when processed as a two-state model generated a χ^2 value (standard statistical measure of the closeness of fit) of the same order of magnitude as the noise in the resonance unit. Secondly, the noncompetitive binding of the polysaccharides to HNE, as reported by others [59,60], was expected to cause a conformational change of the enzymes. This analysis indicated that the binding of NaPPS-B and NaPPS-N to HNE, HAase and lysozyme complied with a fast association rate and slow dissociation rate, suggesting a strong interaction and stability of the drug: enzyme complex in the standard HBS running buffer. However, the NaPPS-B batches presented a 1.3–2.9-fold higher affinity to these three enzymes than NaPPS-N, suggesting structural differences in the ester sulfation pattern or polymerization between NaPPS-B and NaPPS-N preparations. This conclusion was consistent with the results of CZE analysis reported previously [47,48]. High flow rates (50 μl/min) and relatively low density of ligand (Rmax < 300 RU) were used to minimize the mass transport limitation which may have influenced the interpretation of these data.

The present investigations has clearly demonstrated the ability of the SPR technique to generate kinetic data on the interactions of sulfated polysaccharides with proteinases implicated in the pathogenesis of tissue destruction in arthritis [31]. In addition the reproducibility and sensitivity of this technique facilitated the derivation of kinetic binding constants which were different for NaPPS from two independent manufacturers (Table 1). This observed variation in binding to HNE, HAase and lysozyme determined with the BIAcore system may be explained by the structural differences between these NaPPS, as identified by CZE [48]. These data lead us to suggest that these two NaPPS preparation could exhibit difference in potencies and/or activities when used clinically. We consider that the combined use of CZE and SPR technology using immobilized proteins which are relevant to the mediation of disease processes offers advantages over other methods for evaluating generic drugs, particularly for the sulfated polysaccharides which are now being used more extensively than ever before.

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