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Short Communication

The kinetics of chondroitin 4-sulfate release from stimulated platelets and its relation to thromboxane A_2 formation and granule secretion

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1. In platelet rich plasma (PRP), chondroitin 4-sulfate release from platelets occurred after stimulation with ADP (5 μM), collagen (5-10 μg/ml), or adrenaline (10 μM). Release started within 60 s and maximum release (0.7-2.0 mg/l) was reached within 180 s. TXA_2 formation and dense granule release reached a maximum within 90 s after stimulation.

2. Using washed platelets (1.5 x 10^8 cells/ml), the platelet responses were faster. Release of chondroitin 4-sulfate and TXA_2 started within 20-30 s after thrombin addition (100 mU/ml). Maximum release was reached within 60 s in both cases. Dense granule release started in the first 5 s of stimulation (34.6 ± 12.4%) reaching maximum secretion (74.4 ± 8.7%) within 60 s.

3. Our results demonstrate that maximal chondroitin 4-sulfate release occurs after the dense granule release reaction in both PRP and washed platelets. This observation suggests that chondroitin 4-sulfate is unlikely to be stored in the dense granules but may be stored in the α-granules.

Key words: chondroitin 4-sulfate, thromboxane A_2, 5-HT release, platelet aggregation.

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Introduction

Platelets contain large amounts of proteoglycans in their membranes and in their storage granules. In the latter case, the proteoglycans are present in the same subcellular fraction as platelet factor-4 (Barber et al., 1972). Chondroitin 4-sulfate, which accounts for more than 90% of the platelet proteoglycan content (Barber et al., 1972; Nader, 1991), is thought to be the carrier molecule for platelet factor-4 (Barber et al., 1972; Luscombe et al., 1981, Huang et al., 1982) and has been implicated in cell-cell interactions (Sampaio and Dietrich, 1981). In addition, a high molecular weight chondroitin sulfate (Mr 240,000) is known to be released from platelets during aggregation induced by several agonists including ADP, adrenaline, noradrenaline, serotonin, collagen and thrombin (Nader, 1991).

We describe the time course of chondroitin 4-sulfate release during platelet activation and aggregation induced by various stimuli. We have also studied the correlation between this release and the biosynthesis of thromboxane A_2 (TXA_2) and the liberation of dense granule contents.

Material and Methods

Platelet preparation and stimulation

Human blood was anticoagulated with 3.8% sodium citrate (1:10) and platelet rich plasma (PRP) was prepared by differential centrifugation. Washed platelets were prepared as described by Radomski and Moncada (1983). Platelet aggregation was monitored using a Born-type aggregometer.

Release reaction

Platelets were labelled in PRP by incubation with 0.5 mM [^{14}C]5-HT (50 Ci/mmol) for 30 min at 37°C. Prior to stimulation, the platelets were incubated with imipramine (1 mM) for at least 15 min in order to minimize the reuptake of released 5-HT. After addition of the agonist, aggregation was allowed to proceed for 5 min and the reaction was terminated by adding 100 μl of 100 mM EDTA. The aggregated platelet suspensions were then centrifuged at 12,000 *g* for 2 min. The extent of granule [^{14}C]5-HT release was determined by quantifying the radioactivity in 100 μl of the resulting supernatant. The results are reported as percent of the total [^{14}C]5-HT content in 100 μl of platelet suspension (Holmsen and Weiss, 1979).

TXB_2 determination

TXA_2 formation was quantified by radioimmunoassay of its stable degradation product TXB_2 (Salmon, 1978). Following aggregation, the platelet samples were centrifuged as described above and the supernatants removed and stored at -20°C until assayed. The results are reported as ng TXB_2/ml platelet suspension.

Quantification of chondroitin 4-sulfate

The amount of chondroitin 4-sulfate released was quantified in both the soluble fraction and in the platelet pellet obtained after the centrifugation of stimulated platelets as described above. The procedure for the separation and identification of the glycosaminoglycans obtained from the platelets after proteolysis has been described elsewhere (Nader, 1991).

Results and Discussion

In PRP, chondroitin 4-sulfate release from platelets occurred after stimulation with ADP (5 μM), collagen (5-10 μg/ml), or adrenaline (10 μM). With these stimuli, release started within 60 s and was maximal (0.7-2.0 mg/l) within 180 s. Simultaneous with the increase in the supernatant level of chondroitin 4-sulfate, there was a decrease in the level of this proteoglycan in the platelet pellet. The release of chondroitin 4-sulfate occurred after TXB_2 formation and the secretion of the dense granule contents, as shown by the [^{14}C]5-HT release which reached a maximum within 90 s after stimulation (Figure 1). No chondroitin 4-sulfate, TXB_2 formation or dense granule release was observed when a low dose of ADP (1.5 μM), which was unable to induce the second phase of platelet aggregation, was used.

With washed platelets (1.5 x 10^8 cells/ml), the release of chondroitin 4-sulfate and TXB_2 was faster and started within 20-30 s after thrombin (100 mU/ml) addition. The maximum amount released in both cases was reached within 60 s. The dense granule release in the presence of thrombin started within 5 s of stimulation (34.6 ± 12.4%) and reached a maximum of 74.4 ± 8.7% within 60 s (Figure 1). In the first 15 s after stimulation there was no chondroitin 4-sulfate release from washed platelets. Hagen (1972) has previously shown that platelets stimulated with thrombin (4 U/ml) and latex released both adenine nucleotides, which are also stored in platelet dense granules, and glycosaminoglycans with the same time course. This discrepancy most likely reflects the fact that Hagen used

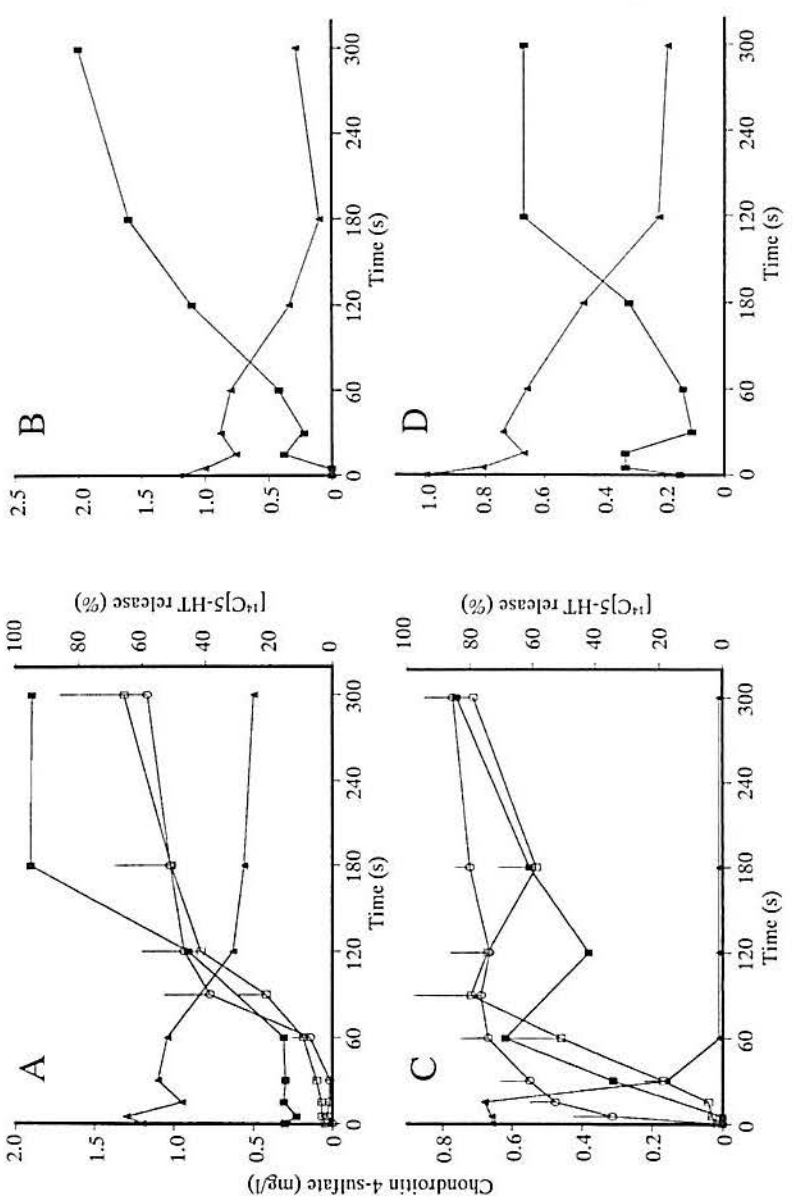

Figure 1 - The time course of chondroitin 4-sulfate release, thromboxane B_2 formation and dense granule release during platelet aggregation. *A*) Platelets aggregated with 5 μM ADP. *B*) Platelets aggregated with 8 μg/ml of collagen. *C*) Washed platelets stimulated with 100 mU thrombin/ml. *D*) Platelets aggregated with 10 μM adrenaline. (■), Chondroitin 4-sulfate in the supernatant; (Δ), chondroitin 4-sulfate in the platelet pellet; (○), [^{14}C]5-HT release into the supernatant; (□), thromboxane B_2 release into the supernatant.

40-fold more thrombin than used here. Under such conditions, the granule release reaction and other biochemical events are very rapid and it becomes impossible to dissociate them in a time-course experiment.

It is known that when platelets are stimulated, the dense granule contents are released first, followed by the α-granule release (Rendu and Dupuy, 1991). Our results demonstrate that maximal chondroitin 4-sulfate release occurs after the dense granule release reaction in both PRP and washed platelets. This observation suggests that chondroitin 4-sulfate is unlikely to be stored in the dense granules but may be stored in the α-granules.

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