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AN INDIRECT KINETIC METHOD FOR ESTIMATING THE AFFINITY BETWEEN HEPARIN AND HEPARIN COFACTOR II.

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The heparin-dependent inactivation of alpha-thrombin by heparin cofactor II was studied in buffer media with a pH ranging from 6 to 9 and an ionic strength from 0.05 M to 0.80 M. We used a heparin fraction with a mean M_r of 16,000 .The log dose response curves (logarithm of the 2nd order inactivation constant vs. the logarithm of heparin concentration) display a sigmoidal behavior. The lower and upper limiting plateau and the steepness of the ascending limb are characteristic for every pH and ionic strength. Similar log dose response curves can be observed for the heparin-mediated inactivation of factor Xa and plasmin by ATIII, indicating that enhancement of the inhibition only depends on heparin-inhibitor binding despite the presence of heparin-enzyme complexes. This type of inactivation mechanism is clearly different from the one observed for the thrombin- and factor IXa - ATIII interactions which are characterized by a maximum in the log dose response curves. Therefore we can make the assumption that heparin-inhibitor binding is of major importance in the heparin-mediated thrombin-HCII interaction. Our experimental data were fit to a model which describes the dependence of the observed inactivation constant upon the concentration of heparin-HCII complex. This complex concentration is a function of the initial heparin and HCII concentrations and the dissociation constant K_0 of the heparin-HCII complex. The model allows the estimation of K_0 in various buffer media. A decrease of pK_0 with increasing buffer concentration can be observed. The upper limiting plateau value for k_{obs} which is often referred to as the intrinsic activity of heparin also decreases with increasing ionic strength. At pH 7 and 8 and an ionic strength of 0.4 M we found K_0 values of 1.00E-07 M. At pH 6 and an ionic strength of 0.8 M K_0 equals 4.00E-04 M indicating a markedly decreased affinity of heparin for HCII. Through a detailed analysis of the pH profile for K_0 we might gain insight in the nature of the binding sites for heparin on the inhibitor.

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EFFECT OF VARIOUS HEPARIN(OID)S ON HEPARIN COFACTOR II MEDIATED ANTI-THROMBIN ACTIVITY AND INHIBITION OF THROMBIN GENERATION IN VITRO.

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The effects of various heparin(oid)s, standard heparin VII (SH), dermatan sulphate (DS), a low molecular weight fraction of heparin (LMW-H), Fragmin^R (FRA), Org 10172 = low molecular weight heparinoid, the fraction of Org 10172 with high affinity for AT-III (HA-10172) and the low affinity fraction (LA-10172) respectively were examined on in vitro thrombin generation and inactivation.
Thrombin inactivation in the presence of either heparin cofactor II (HC-II) or anti-thrombin III (AT-III) was assessed with two newly developed assays using the purified cofactors, thrombin and chromogenic substrate S2238 on microtiterplates.
Thrombin generation in the presence of HC-II and AT-III was studied using purified factor Xa, prothrombin and blood platelet lysate and the residual thrombin activity was assessed amidolytically.
The inhibition of the compounds on thrombin activity are summarized in the table

HEPARIN(OID)	anti-IIa activity (ID_{50} in µg/ml) via HC-II	via AT-III	Ratio HC-II/AT-III
SH	1	0,03	33
FRA	14	0,1	140
LMW-H	42	0,2	210
DS	5	>2000	<0,0025
10172	24	20	1
HA-10172	37	0,5	74
LA-10172	32	325	0,1

The following conclusions can be drawn:
- SH, LMW-H, HA-10172 and FRA potentiate the AT-III mediated inactivation of IIa more strongly than the HC-II mediated inactivation.
- DS and LA-10172 show the reverse pattern of inactivation, while Org 10172 potentiates both inactivaton pathways to a similar extent.

Thrombin generation in the presence of HC-II is inhibited by LMW-heparin(oid)s at approx. 2-5 times lower concentrations than the HC-II mediated thrombin inactivation, while the inhibiting effect of SH in both assays is comparable.
AT-III mediated thrombin generation inhibition and AT-III mediated thrombin inactivation is comparable as well for SH, LMW-H and FRA. In contrast, Org 10172 and its subfractions are approx. 10 times more potent on AT-III mediated thrombin generation inhibition than on AT-III mediated thrombin inactivation.

Org 10172 shows low anti-thrombin activity and this activity is mainly mediated via HC-II.

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STRUCTURE ACTIVITY RELATIONSHIPS OF DERMATAN SULFATE : EFFECT OF MOLECULAR SIZE AND CARBOXYL GROUP ESTERIFICATION ON HEPARIN COFACTOR-II ACTIVATION.

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Dermatan is a high molecular weight glycosaminoglycan which has been shown to enhance the inhibition of thrombin by heparin-cofactor II. The aim of this study was to establish the influence of the molecular size and the role of the carboxyl group on the in vitro activity of Dermatan Sulfate. Pig skin Dermatan Sulfate was fractionated according to molecular size by gel-chromatography on Ultrogel Ac 44. Each fraction was characterized by its sulfur content and by its mean molecular weight measured on a TSK - 4000 column in reference to standard heparin fractions. Methyl esters of the unfractionated Dermatan Sulfate with varying degree of esterification, where prepared via activation of the carboxyl groups with a carbodiimide and reaction with methanol. The results of this study show that the heparin - cofactor II mediated anti-thrombin activity of Dermatan Sulfate is increasing with the molecular weight and is abolished by esterification of the carboxyl groups. Moreover, it can be speculated that each fraction contains the same amount of high affinity fraction and that, like heparin, the potency of the high affinity component is increasing with the molecular weight.