Carbohydrate Research, 59 (1977) 285–288

Note

Some *N*-acyl derivatives of *N*-desulphated heparin*

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(Received December 20th, 1976; accepted for publication, March 21st, 1977)

Heparin comprises (1→4)-linked tetrasaccharide repeating-units made up of α-L-idosyluronic acid[2], β-D-glucosyluronic acid[3,4], 2-amino-2-deoxy-α-D-glucosyl, and, in part, 2-acetamido-2-deoxy-α-D-glucosyl[5] residues with sulphate groups at positions 2 and 6 of the hexosaminyl residues and position 2 of the α-L-idosyluronic acid residues[6]. Heparin has dual biological functions, *viz.*, anticoagulant action on mammalian blood and lipolytic action on blood serum.

Chemical modification of heparin is important for the study of these biological functions[7] and its molecular conformation[8]. *N*-Acetyl[9,10], *N*-benzoyl[11], *N*-(3,5-dimethylbenzoyl)[12,13], *N*-(disulphobenzoyl)[14,15], *N*-succinyl[16], and *N*-(sulphobenzoyl)[14,17] derivatives have been prepared by reaction of *N*-desulphated heparin with acid anhydrides or halides in the presence of alkali in aqueous solutions, or by the reaction of its hyamine complex in the presence of amines in organic solvents.

We now report a novel procedure for the *N*-acylation of *N*-desulphated heparin, and a series of its new *N*-acyl derivatives.

TABLE I

THE D.S. VALUES FOR *N*-BUTYRYL DERIVATIVES OF *N*-DESULPHATED HEPARIN

Butyric anhydride, mol. per hexosaminyl residue (ml)[a]	*Procedure*	*Yield (mg)*	*D.s. value per hexosaminyl residue*[b]
1.5 (0.05)	*A*	68.5	0.58
1.5 (0.05)	*B*	70.5	0.82
3.0 (0.10)	*A*	89.0	0.89
3.0 (0.10)	*B*	84.0	0.95
7.0 (0.22)	*A*	84.5	0.87
7.0 (0.22)	*B*	83.0	0.96

[a]*N*-Desulphated heparin (100 mg) was *N*-acylated with butyric anhydride, in the absence and presence of pyridine (0.5 ml), in formamide solution (3 ml). [b]The values include the d.s. of 0.18 for *N*-acetyl group (see text).

*For a preliminary report, see Ref. 1.

Solutions of *N*-desulphated heparin (0.18 ±0.04 *N*-acetyl group per hexosaminyl residue; S/N ratio, 1.52) in formamide reacted with acid anhydride without addition of catalyst to give *N*-acetyl derivatives. The presence of *N*-acyl groups in the products was reflected by i.r. absorptions at 2900 (CH in fatty acid) and 1650 and 1540 cm^{-1} (CONH), and by *N*-acyl proton signals in the n.m.r. spectra (D_2O). The use of $\lesssim 3$ mol. of acid anhydride per hexosaminyl residue gave the maximal d.s. values shown in Table I.

All of the products, isolated as sodium salts, were soluble in water but insoluble in acetone and ethanol. As shown in Table II, the d.s. values of *N*-acyl groups were 0.86–1.00 for the lower acyl (C_2–C_6) and benzoyl, 0.52–0.63 for the intermediate acyl (C_8–C_{12}) and 2-carboxybenzoyl, and 0.27–0.32 for the higher acyl (C_{14}–C_{18}) derivatives. The variation of the d.s. values may reflect steric effects in the acylation reactions. Slight desulphation also occurred, as evidenced by the sulphate contents of the *N*-acyl derivatives (Table II). *N*-Benzoyl and 2-carboxybenzoyl derivatives showed $\lambda_{max}^{H_2O}$ 227 and 270 nm, respectively. Three procedures of *N*-acylation were used: *A*, formamide–acid anhydride; *B*, *A* + pyridine; and *C*, acid anhydride + Dowex 1 (CO_3^{2-}) resin in aqueous solution. The d.s. values of the products prepared by procedure *A* were slightly lower than those prepared by procedure *B*[18], but were much higher than those prepared by procedure *C*[10]. Procedure *C* was not applicable to the preparation of the higher acyl derivatives (C_{10}–C_{18}). *O*-Acylation occurred[19] by procedure *B* with acetic anhydride, but not with the higher fatty acid anhydrides (C_4–C_{18}) or benzoic anhydride, as the products had no i.r. absorptions for *O*-acyl groups at 1700–1800 cm^{-1}.

EXPERIMENTAL

General. — N.m.r. spectra were recorded at 60 MHz with a Hitachi R-24 spectrometer and solutions in D_2O [internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate]. I.r. spectra were recorded for KBr discs with a Hitachi 215 grating spectrometer. Specific rotations (1-cm path length) were recorded with a Yanagimoto OR-50 polarimeter. The d.s. values for *N*-acyl groups were based on the ratio of signal intensities for *N*-acyl protons and methine and methylene protons of the sugar in the n.m.r. spectra.

Nitrogen analysis was performed at the Elemental Analysis Center of Kyoto University, and sulphate analysis was carried out by a colorimetric method using barium chloroanilate[20]. References to the other analytical methods have been cited previously[21].

Heparin sodium salt (Sigma; from porcine intestinal mucosa) was used.

N-Desulphation of heparin. — Heparin sodium salt (2 g) was *N*-desulphated by treatment[22] with 0.04M hydrochloric acid (100 ml) at 100° for 2.5 h. The hydrolysate was made 5% with respect to sodium acetate and 0.5M with respect to acetic acid, and 2 vol. of 99% ethanol were added. The mixture was stored at ~5° overnight to afford *N*-desulphated heparin as the sodium salt (1.6 g), $[\alpha]_D^{21}$ +57° (*c* 0.76, water); the d.s.

TABLE II

SOME *N*-ACYL DERIVATIVES OF *N*-DESULPHATED HEPARIN

N-*Acyl group*	*Procedure*	*Yield* (*mg*)[a]	*Degree of substitution*[b]	$[\alpha]_D^{21}$, *degrees* (c, *water*)	*Calc.*[c]		*Found*	
					N	SO_4^{2-}	*N*	SO_4^{2-}
Acetyl	*A*	206	1.00	+29 (0.97)	2.59	26.7	2.44	24.6
Acetyl	*C*	159	0.86	+33 (0.86)	2.55	26.3	2.40	n.d.[d]
Propionyl	*A*	200	1.00	+35 (0.82)	2.47	25.5	2.33	24.0
Propionyl	*C*	168	0.66	+33 (0.76)	2.56	26.4	2.46	n.d.
Butyryl	*A*	178	0.89	+40 (0.75)	2.46	25.3	2.26	24.1
Butyryl	*B*	161	0.95	+38 (0.85)	2.44	25.1	2.41	23.5
Butyryl	*C*	113	0.65	+33 (1.50)	2.54	26.1	n.d.	24.4
Hexanoyl	*A*	191	0.86	+35 (0.76)	2.39	24.6	2.30	24.0
Hexanoyl	*B*	136	0.91	+37 (0.75)	2.37	24.4	2.26	n.d.
Hexanoyl	*C*	100	0.76	n.d.	2.43	25.0	n.d.	25.6
Octanoyl	*A*	187	0.63	+38 (0.90)	2.43	25.0	2.40	23.6
Octanoyl	*B*	172	0.67	+43 (0.58)	2.41	24.8	2.35	n.d.
Decanoyl	*A*	182	0.57	+51 (0.75)	2.42	24.9	2.31	22.0
Decanoyl	*B*	201	0.60	+40 (0.63)	2.40	24.7	2.37	n.d.
Lauroyl	*B*	210	0.70	+32 (0.90)	2.28	23.5	2.26	20.6
Myristoyl	*A*	189	0.32	+48 (0.58)	2.56	26.2	2.56	24.6
Palmitoyl	*A*	148	0.28	+36.5 (0.69)	2.58	26.5	n.d.	26.0
Palmitoyl	*B*	199	0.27	+37 (0.88)	2.59	26.6	2.47	n.d.
Stearoyl	*A*	120	0.27	n.d.	2.58	26.5	2.55	25.6
2-Carboxybenzoyl	*A*	210	0.52	+67 (0.78)	2.42	24.9	2.40	24.0
Benzoyl	*A*	197	0.90	+40 (0.84)	2.35	24.2	2.29	23.6
Benzoyl	*B*	208	0.98	+40 (0.85)	2.32	23.9	2.10	23.0
Benzoyl	*C*	128	0.50	+36 (0.72)	2.53	26.1	n.d.	25.0

[a]These values are based on the reaction of *N*-desulphated heparin (200 mg). [b]The values include the d.s. of 0.18 for *N*-acetyl group (see text). [c]These are calculated for $[(C_{24}H_{31}N_2O_{29}S_3Na_5)\ (C_4H_6O_2)_{0.18}$ (formula of two molar acyl)$_x$ $(H_2)_y]_n$, in which x is the observed d.s. of the corresponding acyl group and $x+y=0.82$. [d]Not determined.

value for *N*-acetyl per hexosaminyl residue, as determined by n.m.r. spectroscopy, was 0.18 ±0.04.

Anal. Calc. for $[(C_{24}H_{31}N_2Na_5O_{29}S_3)(C_4H_6O_2)_{0.18}(H_2)_{0.82}]_n$: N, 2.69; SO_4^{2-}, 27.7; hexosamine, 27.5; uronic acid, 30.6. Found: N, 2.58; SO_4^{2-}, 26.8; hexosamine, 25.3; uronic acid (carbazole method[21]), 36.6%.

N-*Acylation of* N-*desulphated heparin.* — *Procedure* A. *N*-Desulphated heparin (200 mg) was dissolved in formamide (4–6 ml) by stirring at room temperature overnight. The acid anhydride (3 mol. per hexosaminyl residue) was added to the solution which was then stored at room temperature overnight. With higher fatty acid anhydrides (C_{10}–C_{18}), the mixture was heated for ~10 min in a boiling water bath to afford a homogeneous solution, and the solution was then stored at 40–50°. Acetone (~50 ml) was added and the products were precipitated by the addition of a few drops of 5% sodium acetate in 0.5M acetic acid (solvent *A*). The mixture was stored at room temperature overnight, and the precipitates were then collected by centrifugation and washed with acetone and ether. The precipitates were dissolved in solvent *A* (2 ml), and 99% ethanol (4 ml) was added. The mixture was stored at ~5° overnight, and the precipitates were then collected by centrifugation, and washed with 99% ethanol and ether to afford the corresponding *N*-acyl derivative as the sodium salt.

Procedure B. *N*-Desulphated heparin was dissolved in formamide. *N*-Acylation was performed in the presence of pyridine at 40–50° according to the method of Wolfrom[18]. The reaction products were isolated as described above.

Procedure C. *N*-Desulphated heparin was *N*-acylated with acid anhydride in the presence of Dowex 1 (CO_3^{2-}) resin in aqueous solutions according to the method of Danishefsky[10,22]. The products were isolated as described above.

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