In a previous study on heparin biosynthesis, incubation of a mouse mastocytoma microsomal fraction with UDP-N-acetyl-d-glucosamine and UDP-d-glucuronic acid resulted in the formation of two nonsulfated polysaccharides, one of which was fully N-acetylated whereas the other contained about equal amounts of N-acetylated and N-unsubstituted glucosamine residues. In the presence of 3'-phosphodiamidate sulfate the latter species was converted into heparin through a series of reactions initiated by sulfation of the unsubstituted amino groups (Hook, M., Lindahl, U., Hallén, A., and Backström, G. (1975) J. Biol. Chem. 250, 6065-6071).

Incubation of the fully N-acetylated polysaccharide with microsomal enzyme resulted in partial N-deacetylation, as shown by the formation of a product susceptible to deamination with nitrous acid. Characterization of the deamination products by gel chromatography suggested that the N-acetyl groups had been attacked in a random fashion. An assay for the deacetylase was developed, based on the liberation of labeled acetate from a N-[3H]acetylated heparin precursor polysaccharide; the released [3H]acetate was recovered by extraction with ethyl acetate and quantified by liquid scintillation counting. The rate of acetate release was linear with time and directly proportional to the concentration of microsomal enzyme. The reaction required Mn2+ ions and had a pH optimum between 6.0 and 6.5. Salts were inhibitory at relatively low concentration (50% inhibition at 50 mM NaCl concentration). Exhaustive incubation of the labeled substrate with microsomal enzyme caused release of 30 to 35% of the [3H]acetate groups originally present; this value could be increased only by reincubating the substrate after substitution of the N-unsubstituted d-glucosamine residues with (unlabeled) acetyl groups.

Incubation of various labeled glycosaminoglycans with mastocytoma microsomal fraction showed that only polysaccharides structurally related to heparin (microsomal heparin-precursor polysaccharides, and, in addition, a N-desulfated, N-acetylated heparan sulfate) were susceptible to N-deacetylation. Other N-acetylated polysaccharides, such as hyaluronic acid, chondroitin sulfate, and dermatan sulfate were not attacked by the N-deacetylase.

Our knowledge regarding the biosynthesis of heparin derives from experiments with cell-free systems involving mouse mastocytoma microsomal fractions. The process occurs in a stepwise manner and yields a series of polymeric intermediates, the structures of which (Fig. 1) reflect the nature of the individual reactions. A carbohydrate polymer is initially formed by stepwise addition of d-glucuronic acid and N-acetyl-D-glucosamine residues. This polymer subsequently undergoes N-deacetylation, N-sulfation, C5-epimerization of glucuronic acid residues, and finally O-sulfation in two positions (3-7). Assay procedures have been developed for some of the polymer-modifying enzymes, including the uronosyl C5-epimerase (2) and N- and O-sulfotransferases (8). The present report describes an assay for the enzyme that catalyzes the initial polymer modification reaction, the deacetylation of N-acetylglycosamine residues. Certain basic properties of the enzyme have been determined.

MATERIALS AND METHODS

Microsomal fraction from mouse mastocytoma tissue was prepared as described previously (2). Pronase and β-glucuronidase (Grade B-10) were obtained from Sigma Chemical Co., St. Louis, Mo. Heparitinase isolated from Flavobacterium heparinum was kindly given by Dr. A. Linker, Salt Lake City, Utah. [3H]Acetic anhydride (500 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, Bucks., United Kingdom.

Unlabeled Polysaccharides—Chondroitin 4-sulfate was obtained from Dr. A. Wasteson, keratan sulfate from Dr. T. C. Laurent, and hyaluronic acid from Dr. A. Tengblad, all at the Institute of Medical and Physiological Chemistry, University of Upsala, Sweden. Dermatan sulfate was given by Dr. L. Rodén, University of Birmingham, Birmingham, Ala., and further purified as described (9). Heparan sulfate was isolated from human aorta according to the procedure of Iverihs (10). Heparin (Stage I) from pig intestinal mucosa was obtained from Inolex Pharmaceutical Division, Park Forest South, Ill., and purified by repeated precipitation with cetylpyridinium chloride as described (11). Analytical data for some of the polysaccharide preparations are given in Ref. 2; the heparin and heparan sulfate preparations, before and after chemical modification (see below), are described in Table 1.

Radioactively Labeled Polysaccharides—14C-Labeled microsomal heparin-precursor polysaccharides were prepared as described (2, 3). In brief, mouse mastocytoma microsomal fraction was incubated with UDP-d-[14C]glucuronic acid and UDP-N-acetyl-d-glucosamine in the presence or absence of 3'-phosphodiamidate sulfate. The resulting 14C-labeled polysaccharide was isolated and fractionated by ion exchange chromatography (2) into two nonsulfated components, [14C]PS-NH2 and [14C]PS-NAC (corresponding to Fractions I and II, respectively, in Fig. 1, Ref. 3) and two sulfated components (formed only in the presence of 3'-phosphodiamidate sulfate). The resulting [14C]labeled polysaccharide was isolated and fractionated by ion exchange chromatography (2) into two nonsulfated components, [14C]PS-NH2 and [14C]PS-N/SO4 (corresponding to Fractions III and IV, respectively, in Fig. 1, Ref. 3; see also Fig. 3 in Ref. 1). The characteristic structural features of the various components are shown in Fig. 1. The partially N-deacetylated component, [14C]PS-NH2, was substituted with [3H]labeled acetyl groups by treatment with [3H]acetic anhydride. The 14C-labeled polysaccharide (0.5 to 1.0 x 106 cpm) was dissolved in 0.1

Biosynthesis of Heparin

ASSAY AND PROPERTIES OF THE MICROSMAL N-ACETYL-D-GLUCOSAMINYL N-DEACETYLASE*
ml of 0.5% NaSO₄ in 10% aqueous methanol and 5 × 2 mCi of [³H]acetic anhydride was added at 0°C over a period of 1 h. After the addition of 4 × 5 μ of unlabeled acetic anhydride the product was passed through a column of Sephadex G-25 equilibrated with 1 M sodium acetate, and desalted by dialysis against water. Final purification was obtained by ion exchange chromatography on DEAE-cellulose; the acetylated material, [³C]PS-N[³H]Ac (ratio of ³H to ¹⁴C, 9:1 to 40:1, depending on preparation), was eluted at the same position as native PS-NAc. The purified [³C]PS-N[³H]Ac was susceptible to degradation by heparinase from F. heparinum (12), as shown by gel chromatography of the digestion products on Sephadex G-50; the major portion of the label appeared at the elution position of di- or tetrasaccharides. The N-acetylated species, [³C]PS-NAc, was also obtained by substituting [³C]PS-NSO₄⁻ with unlabeled N-acetyl groups. Since this product was available in much larger amounts than the native analog (see Fig. 1 in Ref. 3) it was used throughout this study, except where otherwise stated.

Additional heparin-related labeled polysaccharide preparations were obtained by chemical N-desulfation of [³C]PS-NSO₄⁻ and [³C]PS-NSO₃⁻ and [³C]PS-NSO₄⁻ followed by N-acetylation with unlabeled acetic anhydride, and by N-desulfation and N-deacetylation of heparan sulfate followed by N-acetylation with [³H]acetic anhydride. Hyaluronic acid, chondroitin 4-sulfate, and dermatan sulfate with ¹³C labeled N-acetyl groups were prepared by limited N-deacetylation followed by reacetylation of the exposed amino groups with [³H]acetic anhydride. The procedure will be described in detail elsewhere. The labeled products had specific activities as shown in Table II. Biosynthetically labeled chondroitin, containing [³C]glucuronic acid residues, was prepared as described previously (2) except that UDP-¹³C]glucuronic acid was substituted for UDP-[⁵-³H]glucuronic acid.

**Analytical Methods**—The methods used to determine uronic acid, protein, sulfate, radioactivity, and ratios of D-glucuronic acid to L-iduronic acid were as described (2). The N-substitution pattern of polysaccharides was determined by gel chromatography of the products obtained by selective deamination with nitrous acid (2). Paper chromatography was carried out on Whatman No. 1 filter paper developed with 1-propanol, 1.5 M H₄NOH (7:3, Solvent A) or with 1-butanol, 1.5 M H₄NOH (1:1, upper phase, Solvent B). Ion exchange chromatography of glycosaminoglycans on DEAE-cellulose (Whatman DE-52) was performed as described earlier (2).

**TABLE II**

<table>
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<td>[³C]PS-NSO₄⁻</td>
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</table>

*Samples (15,000 cpm of ³H) were incubated according to the standard procedure (see "Materials and Methods"); 0.34 mg of microsomal protein was added/incubation (0.1 ml). The amounts of [³H]acetate released could be increased further by prolonging the incubation time.

**TABLE 1**

| Analysis of polysaccharide preparations |
|---|---|---|---|---|---|
| Preparation | Uronic acid | Hexosamine | L-Muronic acid/ | L-iduronic acid | D-glucosamine residues with |
| | % | % | + D-glucuronic acid | + L-glucuronic acid | Unsubstituted amino groups* | Sulfated amino groups* | Acetylated amino groups |
| Heparin | 24 | 29 | 0.7 | 2.3 | 2 | 70 | 28 |
| Heparin, N-desulfated | 27 | 22 | 2.1 | 39 | 35 | 25 |
| Heparin, N-desulfated and N-acetylated | 25 | 23 | 1.9 | 4 | 29 | 67 |
| Heparan sulfate | 29 | 35 | 0.15 | 0.5 | 3 | 30 | 67 |
| Heparan sulfate, N-desulfated | 24 | 12 | 64 |
| Heparan sulfate, N-desulfated and N-acetylated | 0 | 8 | 92 |

*Percent of dry weight, not corrected for moisture or for losses during hydrolysis.

* Molar ratios with hexosamine as 1.0.

* Percent of total hexosamine. For experimental details, see Ref. 2.

The ratio of glucosamine to total hexosamine was 1.0 for heparin and heparan sulfate.

**FIG. 1.** Representative disaccharide units of microsomal polysaccharidic intermediates. In addition to the N-sulfated glucosamine residues shown in the figure, PS-NSO₄⁻ and PS-N/S/O-SO₃⁻, also contain small amounts of N-acetylated glucosamine units. The occurrence of L-iduronic acid (10 to 20% of the total uronic acid) in PS-NSO₄⁻ was not recognized in a previous study (3), but has since been clearly demonstrated by more refined analysis (2). The intermediates, PS-NAc, PS-NH₃⁺, PS-NSO₄⁻, and PS-N/S/O-SO₃⁻, mentioned in the chronological order of formation during heparin biosynthesis, correspond to the chromatographic Fractions II, III, and IV, respectively, described in a previous publication (3). Fractions I to III appear fairly homogenous in ion exchange chromatography whereas Fraction IV (PS-N/S/O-SO₃⁻) is markedly heterogeneous.
Microsomal protein). After incubation at 37°C for 2 h, 40 and aqueous phases during the extraction procedure. Such contamination, resulting in erroneously high \(^{3}H\) values was revealed by the addition of fresh enzyme (8 mg of microsomal protein).

Degradation of Polysaccharides with Nitrous Acid—Polysaccharides were treated with nitrous acid at pH 3.9 (Reaction B in Ref. 6, initially described by Shively and Conrad (16)). Under these conditions, D-glucosamine residues with unsubstituted amino groups are converted to 2,5-anhydro-D-mannose units, with cleavage of the corresponding glucosaminic linkage (see also Ref. 17).

**Assay of N-Acetylglucosaminyl N-Decaylase**—The assay procedure used was a modification of that employed in the enzymatic N-deacetylation of chitin in Mucor rouxii (18). Reaction mixtures contained the following components in a total volume of 0.1 ml: 0.05 M 2-(N-morpholino) ethanesulfonic acid, pH 6.3; 10 mM MnCl2; \(^{3}H\)- and \(^{14}C\)-labeled polysaccharide substrate ([\(^{14}C\)]PS-N\(^{3}H\)Ac, 5,000 to 10,000 cpm \(^{3}H\)); and enzyme (0.4 mg or less of microsomal protein). After incubation at 37°C for 2 h, 40 \(\mu\)l of 0.2 M HCl, 10 \(\mu\)l of 1 M acetic acid, and 0.2 ml of water were added. The \(^{3}H\)acetate released was then separated from the polysaccharide by extraction with 3 x 1 ml of ethyl acetate, and quantified after addition of 6 ml of Dimilume (Packard Instrument Co.) by scintillation counting. Occasionally, the counted samples were contaminated with the polysaccharide substrate, due to inadequate separation of the organic and aqueous phases during the extraction procedure. Such contamination, resulting in erroneously high \(^{3}H\) values was revealed by the presence of \(^{14}C\) radioactivity, and the samples were eliminated. The standard error of duplicate assays was 53 cpm (70 observations), when the amount of released \(^{3}H\)acetate ranged from 200 to 1000 cpm.

**RESULTS**

**Release of \(^{3}H\)Acetate from \(^{14}C\)PS-N\(^{3}H\)Ac**—Incubation of the \([^{14}C]glucuronosyl- and N-\(^{3}H\)acetyl-heparin precursor, \([^{14}C]PS-N^{3}H\)Ac, with mast cell microsomes resulted in release of \(^{3}H\)-labeled component that was extractable with acid ethyl acetate. On paper chromatography in Solvent A or Solvent B this component co-migrated with a standard polysaccharide, \([^{14}C]PS-N^{3}H\)Ac, before and after treatment with acetic anhydride (15).

**Characterization of Enzymatically N-Decayed PS-NAc**—Removal of N-acetyl groups from polysaccharides such as PS-NAc should yield a product containing D-glucosamine residues with unsubstituted amino groups. Under certain conditions (see "Materials and Methods"), N-unsubstituted D-glucosamine units are susceptible to deamination by nitrous acid, and the presence of such units in a polysaccharide can therefore be conveniently detected by the depolymerization that occurs on treatment with this reagent. This is illustrated in Fig. 6A, showing gel chromatograms (Sephadex G-25) of the partially N-deacetylated microsomal heparin-precursor polysaccharide, \([^{14}C]PS-N^{3}H\)Ac, before and after treatment with nitrous acid.

**Effect of divalent cations on N-acetylglucosaminyl N-deacetylase activity.** Microsomal enzyme was incubated with the doubly labeled substrate, \([^{14}C]PS-N^{3}H\)Ac (10,000 cpm \(^{3}H\)) under standard conditions (see "Materials and Methods"), with 0.05 M 2-(N-morpholino)ethanesulfonic acid (---), 0.05 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (○), or 0.05 M Tris-HCl (Δ—Δ, or 0.05 M Tris-HCl (Δ—Δ) as buffer.

![Fig. 2. Release of \(^{3}H\)Acetate during prolonged incubation of \([^{14}C]PS-N^{3}H\)Ac with mouse mastocytoma microsomal fraction. \([^{14}C]PS-N^{3}H\)Ac (1 x 10\(^{5}\) cpm \(^{3}H\)) was incubated with 8 mg of microsomal protein in 2 ml of 0.05 M 2-(N-morpholino)ethanesulfonic acid, pH 6.3, containing 10 mM MnCl\(_2\). At the times indicated, aliquots of 10 \(\mu\)l were diluted to 100 \(\mu\)l with water and analyzed for released \(^{3}H\)Acetate as described under "Materials and Methods" (○—○, As for 24 h at 37°C, microsomes with unsubstituted amino groups and the polysaccharide was reisolated (2) and incubated in a similar manner for another 24-h period (○—○). The arrows indicate additions of fresh enzyme (8 mg of microsomal protein).](http://www.jbc.org/content/264/10/924/F2)

![Fig. 3. Effect of pH on N-acetylglucosaminyl N-deacetylase activity. Microsomal enzyme was incubated with the doubly labeled substrate, \([^{14}C]PS-N^{3}H\)Ac (10,000 cpm \(^{3}H\), under standard conditions (see "Materials and Methods"), with 0.05 M 2-(N-morpholino)ethanesulfonic acid (---), 0.05 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (○), or 0.05 M Tris-HCl (Δ—Δ) as buffer.](http://www.jbc.org/content/264/10/924/F3)

![Fig. 4. Effect of divalent cations on N-acetylglucosaminyl N-deacetylase activity. Microsomal enzyme was incubated with the doubly labeled substrate, \([^{14}C]PS-N^{3}H\)Ac (10,000 cpm \(^{3}H\)) in the presence of CaCl\(_2\) (Δ—Δ), MgCl\(_2\) (○—○), or MnCl\(_2\) (○—○). Except for the salts added the incubations were carried out under standard conditions (see "Materials and Methods").](http://www.jbc.org/content/264/10/924/F4)
with nitrous acid, respectively. Chemical N-acetylation of $[^{14}C]$PS-NH$_3^+$, yielding $[^{14}C]$PS-NAc, eliminated the susceptibility to nitrous acid (Fig. 6B). Incubation of this product with mastocytoma microsomal fraction restored the susceptibility to nitrous acid (Fig. 6B). Calculations (2) based on the gel chromatography pattern in Fig. 6B indicated that exhaustively incubated $[^{14}C]$PS-NAc had 38% N-unsubstituted D-glucosamine residues; this value is in fair agreement with the amount of $[^3H]$acetate lost during similar incubation of the doubly labeled substrate, $[^{14}C]$PS-N$[^3H]$Ac (see above).

Treatment of partially N-deacetylated $[^{14}C]$PS-NAc with nitrous acid should yield oligosaccharides with 2,5-anhydro-D-mannose units in reducing and D-$[^1C]$glucuronic acid units in nonreducing position. In order to ascertain that the deamination products derived from the enzymatically deacetylated polysaccharide conformed to this pattern the tetrasaccharide fraction, isolated by gel chromatography (Fig. 6B), was digested with $\beta$-glucuronidase. Analysis of the digestion products by gel chromatography on Sephadex G-15 showed, according to prediction, that the tetrasaccharide had been converted to free D-$[^1C]$glucuronic acid and a labeled trisaccharide, presumably N-acetyl-D-glucosaminyl + D-$[^1C]$glucurono- yl + 2,5-anhydro-D-mannose (Fig. 7). Incubation with microsomal enzyme followed by nitrous acid treatment gave the same degradation pattern irrespective of whether chemically N-acetylated $[^{14}C]$PS-NH$_3^+$ or native $[^{14}C]$PS-NAc was used.

**Figure 5.** Effect of sodium chloride on N-acetylglucosaminyl N-deacetylase activity. Microsomal enzyme was incubated with the doubly labeled substrate, $[^{14}C]$PS-N$[^3H]$Ac (10,000 cpm $[^3H]$), under standard conditions (see "Materials and Methods"), with sodium chloride added as indicated.

**Figure 6.** Gel chromatography on Sephadex G-25 of (A) polysaccharide $[^{14}C]$PS-NH$_3^+$, before (O--O) and after (●●●) treatment with nitrous acid; and (B) polysaccharide $[^{14}C]$PS-NAc, treated with nitrous acid before (△--△) and after (△--△) incubation with mastocytoma microsomal fraction. The sample of $[^{14}C]$PS-NAc (15,000 cpm) was incubated for 24 h at 37°C with 1.7 mg of microsomal protein in a total volume of 0.5 ml of 0.05 M 2-(N-morpholinolethanesulfonic acid, containing 10 mM MnCl$_2$. The incubation mixture was then heated at 100°C for 5 min and mixed with 0.125 ml of 0.5 M Tris-HCl, pH 8.0, containing 0.01 M CaCl$_2$ and 2.5 mg of pronase/ml. After incubation at 50°C for 12 h the $[^{14}C]$labeled polysaccharide was reisolated by gel chromatography (2) and treated with nitrous acid, as described under "Materials and Methods." All samples were analyzed by gel chromatography on a column (1 x 195 cm) of Sephadex G-25, eluted with 0.2 M NaCl at a rate of 5 ml/h. Fractions of about 2.5 ml were collected and analyzed for radioactivity. The arrow in Fig. 5A indicates the peak elution position of nonacetylated glucuronosyl-2,5-anhydroxamnitol disaccharides (19).

**Figure 7.** Gel chromatography on Sephadex G-15 of $^{14}C$-labeled tetrasaccharide before (O--O) and after (●●●) incubation with $\beta$-glucuronidase. $[^{14}C]$PS-NAc was incubated with mastocytoma microsomal fraction for 24 h under conditions similar to those described in the legend to Fig. 6, with repeated addition of fresh enzyme after 12 h. After reisolation of the polysaccharide, incubation with microsomal enzyme was repeated for an additional 24-h period. The final reisolated labeled polymer was treated with nitrous acid and the products were fractionated by gel chromatography on Sephadex G-25, as described in the legend to Fig. 6. The tetrasaccharide fraction (peak elution position at 90 ml of effluent volume in Fig. 6) was recovered and desalted by passage through a column of Sephadex G-25, equilibrated with 10% aqueous ethanol. Samples of 3000 cpm were subjected to analytical gel chromatography on a column (1 x 196 cm) of Sephadex G-15, either directly (O--O) or after digestion with $\beta$-glucuronidase (19) (●●●). The gel column was eluted with 0.2 M NH$_4$HCO$_3$ at a rate of 4.5 ml/h. Fractions of about 1.5 ml were collected and analyzed for radioactivity. The most retarded component of the $\beta$-glucuronidase digest co-chromatographed with free glucuronic acid that was added as internal standard and detected by the carbazole reaction.
as substrate. Taken together with the observed liberation of \(^{3}H\)acetate from \(^{14}C\)PS-\(^{3}H\)Ac these results demonstrate the presence in the mastocytoma microsomal fraction of an enzyme that removes \(N\)-acetyl groups from internal positions of the heparin precursor polysaccharide, PS-NAc, leaving \(N\)-unsubstituted \(\beta\)-glucosamine residues. Further support for this conclusion was obtained by chromatography of \(^{14}C\)PS-\(^{3}H\)Ac on DEAE-cellulose, before and after incubation with microsomal enzyme. The incubation product showed a lower \(^3\)H: \(^{14}\)C ratio and a lower net negative charge than the substrate (Fig. 8), in agreement with the postulated \(N\)-deacetylation and exposure of positively charged amino groups.

The action of the \(N\)-deacetylase was studied with regard to the mode of propagation of enzymatic attack along the polysaccharide chain. Samples of \(^{14}C\)PS-NAc were incubated with microsomal \(N\)-deacetylase for varying periods of time and the products were reisolated, treated with nitrous acid, and analyzed by gel chromatography on Sephadex G-100 (Fig. 9). The oligosaccharides formed on deaminative cleavage of exhaustively \(N\)-deacetylated material consisted largely of tetra-, hexa-, and octasaccharides (Fig. 6B), and appeared as a retarded peak (elution volume about 87 ml) on Sephadex G-100 (Fig. 9). The fully \(N\)-acylated (and thus nitrous acid-resistant) substrate was essentially excluded from the same gel. If the \(N\)-deacetylation operated strictly from one end of the polysaccharide chain to the other, the most retarded peak would be prominent already after short periods of incubation. However, the deamination products showed progressively diminishing size with increasing time of \(N\)-deacetylation, without any apparent initial formation of small oligosaccharides (Fig. 9). These results are consistent with a process of random attacks by the \(N\)-deacetylase at internal positions of the polysaccharide chains.

Incubation of mastocytoma microsomal fraction with UDP-\(\beta\)-glucuronic acid and UDP-\(N\)-acetyl-\(\beta\)-glucosamine yields a partially (about 50%) \(N\)-deacetylated polysaccharide product, PS-NH\(^{+}\) (3). The mechanism behind this incomplete deacetylation of an endogenous, membrane-bound polysaccharide has been unclear. Exhaustive incubation of exogenous \(^{14}C\)-PS-\(^{3}H\)Ac with microsomal enzyme similarly removed only a fraction, about 30 to 35%, of the \(N\)-\(^{3}H\)acetate groups. Only a minor portion of the remaining groups was released on reisolation and reincubation of the polysaccharide (Fig. 2). These results raised the question whether acetyl groups at certain predetermined positions in PS-NAc were resistant toward the enzyme. That this was not the case was demonstrated by the following experiment. After exhaustive incubation of \(^{14}C\)-PS-\(^{3}H\)Ac with microsomal enzyme, as described in the legend to Fig. 2, the labeled polysaccharide was reisolated and used as substrate in the standard assay procedure, either directly or after substitution of free amino groups with unlabeled acetyl groups. While only 2% of the \(^{14}C\)acetyl groups were released from the once deacetylated polysaccharide, this figure increased to 11% after acetylation of the free amino groups, the latter value being similar to that obtained with the starting material. \(^{14}C\)-PS-\(^{3}H\)Ac. These observations suggest that the resistance of \(N\)\(^{3}H\)acetate groups was due to the presence of unsubstituted amino groups in the vicinity. The \(N\)-deacetylation process is thus self-limiting and comes to a stop when about half (as in PS-NH\(^{+}\)) or less of the \(N\)-acetyl groups have been removed.

**Substrate Specificity**—The substrate specificity of the microsomal \(N\)-deacetylation was studied in three types of experiments. First, various polysaccharides containing \(^7\)H-labeled \(N\)-acyetyl groups were incubated with the enzyme and liberated \(^{3}H\)acetate was determined (Table II). Significant release of \(^{3}H\)acetate occurred only with heparin-related polysaccharides, such as \(^{14}C\)PS-\(^{3}H\)Ac and a \(N\)-\(^{3}H\)acylated derivative of heparan sulfate; \(^{3}H\)hyaluronic acid, \(^{3}H\)chondroitin 4-sulfate, and \(^{3}H\)dermatan sulfate were all inactive as sub-


TABLE III

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<th>Unlabeled polysaccharide added</th>
<th>[3H]Acetate released %</th>
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<td>Keratan sulfate</td>
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* Percentage of label released under standard incubation conditions in the absence of unlabeled polysaccharide. To each incubation (0.1 ml) was added 10 μg of unlabeled polysaccharide.

strates. Secondly, variously labeled polysaccharides, including chondroitin (containing D-[14C]glucuronic acid residues), hyaluronic acid (containing N-[3H]acetyl groups), [14C]PS-NAc, [3H]PS-NH3+, N-desulfated, N-acetylated [14C]PS-NS03-, and [3H]PS-N-O/NS03-, and the N-[3H]acetylated heparan sulfate derivative, were incubated with microsomal N-deacetylase for 24 h, and then were reisolated, treated with nitrous acid, and subjected to gel chromatography on Sephadex G-100 or (only with incubation products of [14C]PS-NH3+) Sephadex G-25. Depolymerization of incubated samples, indicative of N-deacetylation, was observed in all polysaccharides except the labeled chondroitin and hyaluronic acid.1 Nonincubated controls resisted deamination, except [14C]PS-NH3+, which, as expected, showed the same content of N-unsubstituted n-glucosamine residues before and after incubation with microsomal enzyme. The two types of experiments confirm in showing a strict requirement for a specific structure of the carbohydrate backbone: [(1 → 4)-β-D-glucuronosyl-(1 → 4)-α-D-N-acetylglucosaminyl]n- sequences (as in the heparin precursor, PS-NAc) are deacetylated whereas [(1 → 4)-β-D-glucuronosyl-(1 → 3)-β-D-N-acetylglucosaminyl]n- (hyaluronic acid) or [(1 → 4)-β-D-glucuronosyl-(1 → 3)-β-D-N-acetylglucosaminyl]n- (chondroitin) sequences are not. The presence of ester sulfate groups in the substrate molecule (N-desulfated and N-acetylated [14C]PS-N-O/NS03-) would appear to be compatible with enzyme activity.

These conclusions were corroborated in a third type of experiment in which unlabeled polysaccharides and polysaccharide derivatives were incubated with the enzyme together with the labeled substrate [14C]PS-N-[3H]Ac (Table III). Of the various potentially competitive substrates tested only those structurally related to heparin were able to significantly inhibit the release of [3H]acetate. The N-unsubstituted heparin-related polysaccharides were less efficient inhibitors than were either the N-sulfated or the N-acetylated analogs.

**FIGURE I**

**DISCUSSION**

N-Deacetylases acting on polysaccharides have to our knowledge been found only in bacterial and viral systems (18, 20). In mammalian tissues, enzymatic deacetylation of N-acetylglucosaminyl 6-phosphate and of noncarbohydrate ma-
the present paper may be called "N-acetylheparosan deacetylase."

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