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# Activation of Polysaccharides with 2-Iminothiolane and Its Uses†

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ABSTRACT: 2-Iminothiolane, a cyclic thioimidate, is known to react readily with amino groups of proteins to give amidinated derivatives containing reactive sulfhydryl groups. In this report it will be shown that 2-iminothiolane also reacts with hydroxyl and sulfhydryl groups. Such a reaction with hydroxyl groups can be used to introduce sulfhydryl groups into polysaccharides. When this reaction is carried out in the presence of 4,4′-dithiodipyridine, polysaccharides containing 4-dithiopyridyl groups can be prepared. These activated polysaccharides couple easily via intermolecular disulfide bond formation with proteins containing thiol or 4-dithiopyridyl groups. The resulting polysaccharide–protein conjugates have good stability, and they are useful reagents for different biochemical applications such as the purification of proteins containing thiol groups and affinity chromatography.

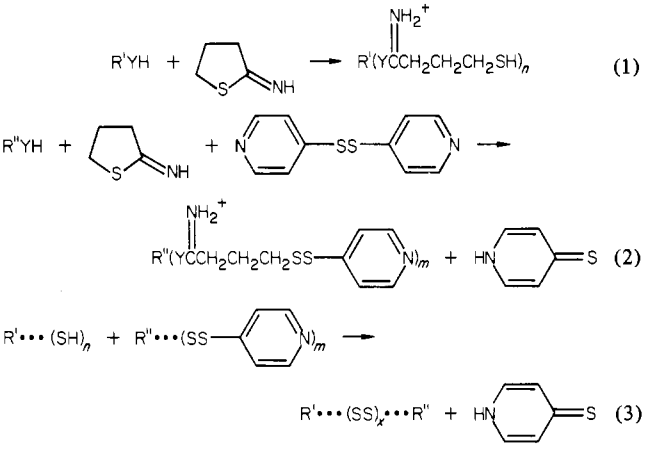
2-Iminothiolane is a useful reagent for chemical modification of proteins to yield amidinated derivatives which contain sulfhydryl groups (Traut et al., 1973; Schramm & Dülfer, 1977; Jue et al., 1978). When modification of proteins with 2-iminothiolane is carried out in the presence of 4,4′-dithiodipyridine, the thiol groups of the amidinated derivatives are converted in situ into 4-dithiopyridyl groups, as shown in Scheme I (King et al., 1978).

When amidinated proteins were separated from an excess of 2-iminothiolane by passage through Sephadex G-25, we frequently obtained variable recoveries of proteins. The losses were higher after repeated use of the same gel. We soon realized that the 2-iminothiolane in the reaction mixture was activating the gel to which the activated proteins could establish a disulfide bond. Under certain conditions, 2-iminothiolane was in fact found to be fairly reactive with a number of polysaccharides and model compounds. In this paper will be reported the findings on the reaction of 2-iminothiolane with hydroxyl-containing compounds and some of their biochemical applications.

## Materials and Methods

2-Iminothiolane was synthesized as described before (King et al., 1978) and purified by vacuum distillation (bp 71–72 °C at 6 torr). The thiol content of a fresh solution of 2-iminothiolane at room temperature and pH 7.2 (0.1 M sodium phosphate buffer) was ≤0.003 residue/mol as determined by spectrophotometric titration with 4,4′-dithiodipyridine (Grassetti & Murray, 1967). 2-Iminothiolane (neat) is stable to storage, but it is hydrolyzed slowly in aqueous solution. The estimated $t_{1/2}$ values for 2-iminothiolane at 25 °C are 390, 210, and 1.8 h, respectively, at pH 9.1, 10, and 11. 4,4′-Dithiodipyridine and DL-*N*-acetylhomocysteine thiolactone were from Aldrich Chemical Co. and Nutritional Biochemical Corp.,

Scheme I




Y = NH, O, or S

respectively. The latter was recrystallized from toluene before use (mp 110–112 °C) and had a thiol content of <0.0015 residue/mol. Methyl acetimidate hydrochloride was prepared as described by Ludwig & Hunter (1967), and it had correct microanalytical data and a mp of 105–107 °C. Sephadex G-25 (medium), Sephadex G-200, Sepharose 4B, and Sepharose 6B were from Pharmacia. Paper disks (6-mm diameter, ~3 mg dry weight/disk) were punched out from Whatman No. 50 filter paper.

Ragweed antigen E (AgE)[1] was prepared as described (King, 1972). Rabbit anti-AgE serum was prepared as previously described (King et al., 1977). Mouse serum albumin was purified from pooled normal mouse serum (Miles Laboratories) by ammonium sulfate precipitation at 50–65% saturation, followed by ion-exchange chromatography on a column (25 × 0.9 cm) of Whatman DE-32 cellulose in 0.05 M

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[1] Abbreviations used: AgE, antigen E; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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Tris-HCl buffer (pH 7.95) with a NaCl gradient from 0 to 0.3 M in 400 mL, and then rechromatographed on a column (25 × 0.9 cm) of Whatman CM-32 cellulose in 0.02 M ammonium acetate buffer (pH 4.7) with a linear salt gradient from 0.02 to 0.27 M NaCl in 400 mL.

Slab gel electrophoresis was carried out in polyacrylamide gel as described by Laemmli (1970) and stained with Coomassie Blue (Swank & Munkres, 1971). Quantitation of ammonia was done on a Beckman Model 120 B amino acid analyzer modified for high sensitivity (5 nmol full scale). Infrared spectra of native and modified Sepharose (100 μmol of 4-dithiopyridyl groups/g) were taken in KBr disks (5 mg of lyophilized gel/250 mg of KBr).

The rate of hydrolysis of 2-iminothiolane and DL-*N*-acetylhomocysteine thiolactone was monitored spectrophotometrically by measuring the appearance of free sulfhydryl groups in the presence of 4,4′-dithiodipyridine (Grassetti & Murray, 1967). Decomposition of 4,4′-dithiodipyridine was followed by measurement of the appearance of 4-thiopyridone on the assumption that it decomposes into 2 equiv of 4-thiopyridone. These rates of hydrolysis or decomposition followed first-order kinetics and are therefore expressed as half-life times.

*Polysaccharides Containing Thiol or 4-Dithiopyridyl Groups.* Swollen Sephadex or Sepharose gels were washed with water and equilibrated with the proper buffer. After suction filtration, the moist gel was mixed with an equal volume of buffer. To obtain the thiolated form of the carrier, we added the desired amount of 2-iminothiolane at zero time directly to the above suspension at room temperature. The activation was done in stoppered glass tubes which were rotated end over end during the process. The suspension was filtered and washed thoroughly with 0.1 M sodium phosphate buffer (pH 7.2) containing 1 mM EDTA to stop the reaction. For paper disks the same procedure was followed, but the washing buffers were removed by aspiration, and a ratio of 10–20 disks/mL of buffer was used during the activation reaction. The number of thiol groups introduced was determined by titration with 1 mM 4,4′-dithiodipyridine, using 1-mL aliquots of the gels or five paper disks.

To introduce 4-dithiopyridyl groups into the carriers, we added a solution of 4,4′-dithiodipyridine in acetonitrile immediately after the addition of 2-iminothiolane. The final reaction mixture contained 10% acetonitrile. The number of 4-dithiopyridyl groups introduced was determined by following the release of 4-thiopyridone at 324 nm on addition of 2-mercaptoethanol (25 mM) to the activated carrier in pH 7.2 phosphate buffer.

*Coupling of Thiolated Antigen E with Sepharose Containing 4-Dithiopyridyl Groups.* Sepharose 4B containing AgE was prepared by coupling 4.34 mg (0.114 μmol) of 4-mercaptobutyrimidinyl-AgE (3.8 thiol groups/mol) (King et al., 1978) with 4 mL of Sepharose containing 4-dithiopyridyl groups (0.159 μmol/mL) in 0.1 M phosphate buffer (pH 7.2) containing 1 mM EDTA. The reaction mixture (9.7-mL total volume) was rotated end over end for 18 h at room temperature. The unreacted 4-dithiopyridyl groups were removed by washing the AgE-containing gel with 0.5 mM 2-mercaptoethanol. Under these conditions, 2.89 mg (0.076 μmol) of AgE was coupled, based on the decrease of protein and the appearance of 4-thiopyridone in the supernatant at the end of the reaction. When this sample of Sepharose 4B–antigen E was used as an immunosorbent column under the conditions previously described (King & Kochoumian, 1979), its capacity was found to be ~3 mg of specific antibody (0.019 μmol). The capacity of the column decreased to ~1 mg of antibody after storage for 9 months at 4 °C in 0.05 M Tris-HCl buffer (pH 7.95) containing 1 mM EDTA and 0.1% $NaN_3$.

## Results

*Preparation of Polysaccharides Containing Thiol or 4-Dithiopyridyl Groups.* Reaction of polysaccharides with 2-iminothiolane, in the presence or absence of 4,4′-dithiodipyridine, was studied under different conditions by measuring the incorporation of thiol or 4-dithiopyridyl groups into polysaccharides. The results are summarized in Table I. The degree of reaction was strongly pH dependent, being greater at high pH values (experiments 1–5). This is in accord with the known reactivity of 2-iminothiolane as an amidinating agent of proteins (King et al., 1978). The degree of reaction was proportional to the concentration of 2-iminothiolane (experiments 6–11); the highest concentration of 360 mM represents a nearly saturated solution of 2-iminothiolane at room temperature. Higher degrees of reaction were found when the reactions were carried out in the presence of 4,4′-dithiodipyridine (experiments 16, 19, and 22) than in its absence (experiments 14, 18, and 20). A likely explanation is the oxidation of thiol groups since this side reaction is greatly diminished upon rapid in situ conversion of reactive thiol groups to stable 4-dithiopyridyl groups. Nevertheless, the number of 4-dithiopyridyl groups introduced at pH 11 (experiments 17 and 23) was lower than at pH 10 (experiments 16 and 22). This is due to the fact that 2-iminothiolane hydrolyzes ~100 times faster at pH 11 ($t_{1/2}$ = 1.8 h) than at pH 10 ($t_{1/2}$ = 210 h) to yield 4-mercaptobutyronitrile, which competes with the thiolated polysaccharide for the limited supply of 4,4′-dithiodipyridine. The concentration of 22.5 mM 4,4′-dithiodipyridine used is near saturation in buffer containing 10% $CH_3CN$. 4,4′-Dithiodipyridine is unstable in alkaline solutions; for example, its half-life at pH 10 is ~14 h. Since this compound is likely to be less stable at pH 11 than at pH 10, its stability becomes the limiting factor for the extent of the desired reaction. The reproducibility of the experiments in Table I is good, and the values are usually within 15% when repeated on different occasions.

The rate of reaction of 2-iminothiolane with Sepharose 4B and paper disks is shown in Table II. The results indicate that the highest value of introduced thiol groups is reached between 1 and 2 h; longer reaction times decrease the number of thiol groups, probably as a result of oxidation. The 4-dithiopyridyl groups of activated Sepharose 4B or paper disks have good stability. After 9 weeks at 4 °C in 0.1 M sodium phosphate buffer (pH 7.2) containing 1 mM EDTA, more than 90% of their 4-dithiopyridyl groups remained titrable.

To elucidate the chemical nature of the modified Sepharose 4B, we determined its ammonia content following hydrolysis in 1 N HCl at 105 °C for 2 h. When compared with the unmodified Sepharose 4B, no significant increase in the ammonia content of the modified Sepharose 4B was observed with a freshly prepared or aged sample, nor did we find any release of ammonia accompanying the reaction of Sepharose 4B with 2-iminothiolane. The infrared spectrum of 4-dithiopyridyl-Sepharose 4B showed two new bands at 1575 and 1725 $cm^{-1}$ which were absent in the unmodified Sepharose. These two bands correspond respectively to the stretching frequencies of the C=C and C=O bonds. The C=C bond may be attributed to the pyridyl groups introduced, while the absorption frequency of the C=O bond suggests the presence of an ester group. Studies with model compounds showed that the absorption of the ester group of ethyl acetate is at 1735 $cm^{-1}$

Table I: Activation of Polysaccharides with 2-Iminothiolane[a]

| expt no. | support | 2-imino-thiolane (mM) | 4,4'-dithio-dipyridine (mM) | pH | reaction time (h) | thiol groups[b] (μmol/g) | 4-dithiopyridyl groups[b] (μmol/g) |
|---|---|---|---|---|---|---|---|
| 1 | Sephadex G-25 | 240 | | 6 | 1 | 0.04 | |
| 2 | | 240 | | 7 | 1 | 0.23 | |
| 3 | | 240 | | 8 | 1 | 1.24 | |
| 4 | | 240 | | 9 | 1 | 3.22 | |
| 5 | | 240 | | 10 | 1 | 5.29 | |
| 6 | Sephadex G-25 | 6 | | 9 | 1 | 0.03 | |
| 7 | | 30 | | 9 | 1 | 0.15 | |
| 8 | | 60 | | 9 | 1 | 0.28 | |
| 9 | | 120 | | 9 | 1 | 1.14 | |
| 10 | | 240 | | 9 | 1 | 2.72 | |
| 11 | | 360 | | 9 | 1 | 5.91 | |
| 12 | Sephadex G-200 | 360 | | 10 | 1 | 7.32 | |
| 13 | Sepharose 6B | 360 | | 10 | 1 | 9.60 | |
| 14 | Sepharose 4B | 360 | | 10 | 1.5 | 7.38 | |
| 15 | | 360 | | 11 | 1.5 | 9.51 | |
| 16 | | 360 | 22.5 | 10 | 1.5 | | 100.62 |
| 17 | | 360 | 22.5 | 11 | 1.5 | | 36.42 |
| 18 | paper disks | 200 | | 10 | 1 | 2.16 | |
| 19 | | 200 | 10 | 10 | 1 | | 6.46 |
| 20 | | 360 | | 10 | 1.5 | 4.09 | |
| 21 | | 360 | | 11 | 1.5 | 7.94 | |
| 22 | | 360 | 18 | 10 | 1.5 | | 8.83 |
| 23 | | 360 | 18 | 11 | 1.5 | | 6.61 |

[a] Reactions were carried out at room temperature by using, for experiments 1–13, 25 mM sodium borax for pH 9 and 10 buffers and 25 mM sodium phosphate for all other buffers; for experiments 14–17, 100 mM sodium bicarbonate was used for pH 10 and 11 buffers. Experimental details are described under Materials and Methods. [b] Determined as described under Materials and Methods. The weight refers to dry sample and was calculated by taking the swelling factor of Sephadex G-25, Sephadex G-200, Sepharose 6B, and Sepharose 4B to be 5, 35, 40, and 30, respectively. The values were determined experimentally by weighing lyophilized samples of moist gel. For paper disks the value was found to be 1.9.

Table II: Reaction Rate of 2-Iminothiolane (360 mM) with Sepharose 4B and Paper Disks at pH 10[a]

| time (h) | μmol of thiol groups/g | |
|---|---|---|
| | Sepharose 4B | paper disks |
| 0.08 | 1.25 | 0.61 |
| 0.50 | 3.54 | 3.12 |
| 1.0 | 8.19 | 3.92 |
| 2.0 | 8.07 | 4.56 |
| 4.0 | 6.34 | 4.36 |

[a] Reactions were carried out at room temperature in 25 mM sodium borax buffer (pH 10). Other experimental details are the same as in Table I.

while that of the imidate group of methyl acetimidate is at 1635 $cm^{-1}$ with a shoulder at 1715 $cm^{-1}$.

These results suggest that the product formed on reaction of polysaccharide with 2-iminothiolane has an ester linkage (Scheme II). Apparently the imidate ester linkage which is formed initially has hydrolyzed to give an ester linkage with the release of ammonia. The absence of ammonia in the reaction mixture can be accounted for if it is assumed that ammonia reacts rapidly with 2-iminothiolane, which is present in excess.

We also studied a structural analogue of 2-iminothiolane, DL-*N*-acetylhomocysteine thiolactone, as a thiolating reagent of polysaccharides. This reagent has also been used for acylation of proteins (Benesch & Benesch, 1956, 1958). The results in Table III indicate that DL-*N*-acetylhomocysteine thiolactone at pH 10 is ~10 times less effective than 2-iminothiolane in introducing thiol groups into different polysaccharides. DL-*N*-Acetylhomocysteine thiolactone is more readily hydrolyzed than 2-iminothiolane; its $t_{1/2}$ at pH 10 is ~14 h, which is 15 times faster than that of 2-iminothiolane. This difference in the hydrolytic properties of the two reagents is not sufficient to account for the low efficiency of DL-*N*-acetylhomocysteine thiolactone in introducing thiol groups into

Scheme II

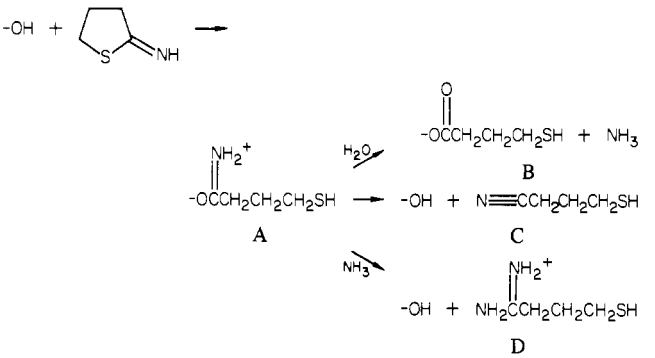


Table III: Comparison between 2-Iminothiolane and DL-*N*-Acetylhomocysteine Thiolactone as Thiolating Agents of Polysaccharides[a]

| carrier | μmol of thiol groups/g | |
|---|---|---|
| | 2-imino-thiolane | DL-*N*-acetyl-homocysteine thiolactone |
| Sephadex G-200 | 6.55 | 0.49 |
| Sepharose 4B | 6.21 | 0.87 |
| paper disks | 4.75 | 0.68 |

[a] Reactions were carried out at room temperature for 1.5 h in 25 mM sodium borax buffer (pH 10). The reagents were used at 360 mM. Other experimental details are the same as in Table I.

polysaccharides. That is, the low efficiency cannot simply be a consequence of reduced concentrations of the thiolactone owing to its hydrolysis.

*Reaction of Model Compounds with 2-Iminothiolane.* Methanol and phenol were studied for their reactivity with 2-iminothiolane. Methanol or phenol (5 mM) in 0.1 M sodium bicarbonate buffer (pH 10) was allowed to react in the presence of 22.5 mM 4,4'-dithiodipyridine, with an excess of

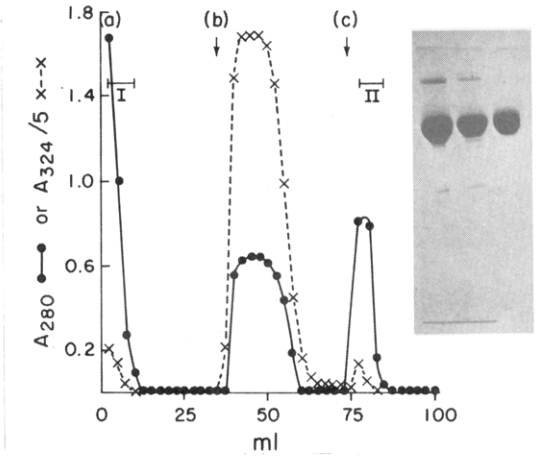


FIGURE 1: Purification of murine serum mercaptalbumin on a column of 4-dithiopyridyl-Sepharose 4B. Activated Sepharose 4B (2.3 mL) containing 2.85 μmol of 4-dithiopyridyl groups/mL was mixed with 18.3 mg of albumin. The reaction mixture, 4-mL total volume in 0.1 M sodium phosphate buffer (pH 7.2) containing 1 mM EDTA, was rotated end over end and, after 19 h at room temperature, was poured into a column (0.9 × 9 cm). The column was washed with 0.2 M NaCl in pH 7.2 buffer (a), followed by 0.5 mM (b) and 50 mM (c) 2-mercaptoethanol in NaCl-phosphate buffer. Fractions of 2.5-mL volume were collected, and the flow rate was 60 mL/h. Cuts I and II contained 10.4 and 6.7 mg of protein, respectively. Inset: 10% polyacrylamide slab gel in $NaDodSO_4$; 10 μg of protein was used per track. From left to right: starting albumin, cut I, and cut II.

2-iminothiolane (360 mM) at room temperature. Following the reaction, the released 4-thiopyridone was measured, after proper correction was made for the concurrent hydrolysis of 2-iminothiolane and 4,4′-dithiodipyridine which also releases thiopyridone under these conditions. The reaction followed apparent first-order kinetics, and the calculated time for 50% of the reaction was 20.1 min for methanol and 10.1 min for phenol. From these results and those reported by Jue et al. (1978) for the reaction of 2-iminothiolane with glycylglycine, glycinamide, and glycine, it can be concluded that hydroxyl groups react at least 100 times more slowly than amino groups. We also have indirect evidence indicating that 2-iminothiolane can react slowly with sulfhydryl groups. For example, the presence of 125 mM 2-mercaptoethanol diminished by 30–50% the introduction of thiol groups into paper disks with 360 mM 2-iminothiolane.

*Uses of 4-Dithiopyridyl-Sepharose 4B.* 4-Dithiopyridyl-Sepharose 4B was used to prepare an immunosorbent on coupling with a protein, antigen E. Rabbit antibodies specific for AgE were purified from rabbit anti-AgE serum by utilizing that same immunosorbent. The results obtained compare well with those obtained through a similar immunosorbent which was prepared by coupling AgE with CNBr-activated Sepharose 4B, as described recently (King & Kochoumian, 1979).

4-Dithiopyridyl-Sepharose 4B was used as a support for the purification of mouse serum mercaptalbumin. The results are shown in Figure 1. On $NaDodSO_4$–polyacrylamide slab gel electrophoresis, the bound mercaptalbumin (cut II) gave a single band, while the unbound albumin (cut I) contained no titrable sulfhydryl group and like the starting material contained several bands. The material which was eluted between cuts I and II with 0.5 mM 2-mercaptoethanol was free of protein and was 4-thiopyridone from unreacted 4-dithiopyridyl groups, as evidenced by its spectrum.

## Discussion

Our results indicate that 2-iminothiolane reacts readily with aliphatic and phenolic hydroxyl groups. Its rate of reaction with hydroxyl groups is about $^1/_{100}$th of that with amino groups. This level of reactivity of 2-iminothiolane is sufficient to be useful for introducing thiol groups into polysaccharides or for introducing 4-dithiopyridyl groups when the reaction medium contains 4,4′-dithiodipyridine.

As indicated in Scheme II, the first product is likely to be an imidate ester (A). However, the instability of imidate esters on aqueous solutions is well-known, and they can undergo two types of decomposition. In the first case they react with water to give esters (B) and ammonia. In the second case they decompose into nitriles and polysaccharides. The ammonia produced in the first case can in turn react with another imidate ester to form an unsubstituted amidine (D) [see Hunter & Ludwig (1962)]. In the latter two cases the molecules containing the nitrile or amidine groups will be separated from the polysaccharides.

Sepharose 4B and paper disks which have been modified with 2-iminothiolane and 4,4′-dithiodipyridine are stable during storage. Following acid hydrolysis, the modified Sepharose was found to have the same content of ammonia as native Sepharose. These two findings on the stability and the ammonia content both suggest that the new linkages present in the modified polysaccharides are of the ester type and not of the imidate ester type. This is further supported by the band at 1725 $cm^{-1}$ found in the infrared spectrum of modified Sepharose. Our inability to demonstrate the release of ammonia during the course of the reaction could be explained by its rapid reaction with 2-iminothiolane, which is present in excess in the reaction mixture.

In addition to the procedure outlined above, there are three other known procedures for preparing modified Sepharose containing thiol or 4-dithiopyridyl groups. One procedure involves the reaction of aminoalkyl-Sepharose with DL-*N*-acetylhomocysteine thiolactone (Cuatrecasas, 1970). As reported under Results, DL-*N*-acetylhomocysteine thiolactone reacts with polysaccharides less readily than 2-iminothiolane, although these two compounds are structural analogues. A second procedure utilizes the reaction of CNBr-activated Sepharose with reduced glutathione (Brocklehurst et al., 1973). A third procedure utilizes epoxidation of Sepharose with epichlorohydrin, followed by treatment with sodium thiosulfate and reduction with dithiothreitol (Axēn et al., 1975). All the reported procedures require more than one reaction step while the present procedure requires only one.

The highest density of thiol or 4-dithiopyridyl groups introduced into Sepharose is ~800 μmol/g by using the epoxidation procedure. The degree of substitution obtained with the procedures using aminoalkyl-Sepharose or CNBr-activated Sepharose is in the range of 20–50 μmol/g. This range of substitution is easily attained by using the present procedure. As shown by the two examples given above for affinity or thiol-covalent chromatography of proteins, a degree of substitution of 5–50 μmol/g of Sepharose is quite adequate. This degree of substitution of protein antigen per gram of paper disks should also be adequate for their use as reagents in radioimmunoassay (Ceska & Lundkvist, 1972) or in enzyme immunoassay (King & Kochoumian, 1979) for specific antibodies.

The above comparison of the ease of modification of polysaccharides with 2-iminothiolane, the degree of substitution, and the stability of the modified polysaccharides all indicate that the present procedure is a useful addition to the known procedures for introduction of thiol or 4-dithiopyridyl groups into polysaccharides. We have shown that protein antigens can be coupled to such modified polysaccharide supports. It

should be possible also to use these reactions for coupling of polysaccharide antigens to polysaccharide supports.

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# Linkage and Sequence Analysis of Mannose-Rich Glycoprotein Core Oligosaccharides by Proton Nuclear Magnetic Resonance Spectroscopy†

Robert E. Cohen‡ and Clinton E. Ballou*

ABSTRACT: The anomeric proton (H-1) chemical shifts of D-mannopyranosides in aqueous solution are affected both by the aglycon and by substitution on the ring [Lee, Y. C., & Ballou, C. E. (1965) *Biochemistry 4*, 257]. We have examined the $^1$H NMR spectra for a variety of linear and branched mannooligosaccharides and have assigned the H-1 resonances to the component sugars. The chemical shifts, which range from $\delta$ 4.76 to 5.36, provide information regarding the linkages, sequences, and anomeric configurations of mannose residues in an oligomer. Thus, $^1$H NMR spectroscopy can complement enzymatic hydrolysis, methylation analysis, and acetolysis for the structural characterization of oligosaccharides. Furthermore, small structural differences between otherwise identical oligosaccharides are often accompanied by long-range chemical shift changes for the anomeric protons. Because sugars three or more residues away from the structural alteration can be affected, the changes must reflect conformational differences. We have placed emphasis on the mannose-rich oligosaccharides from glycoproteins, particularly those produced by endo-$\beta$-*N*-acetylglucosaminidase digestion. Two mannose-rich glycopeptides were isolated from a monoclonal human IgM and their positions of origin on the polypeptide chain were determined. The oligosaccharides were released with endo-$\beta$-*N*-acetylglucosaminidase and fractionated into several size classes. Our structural studies show that each glycopeptide possessed a unique set of oligosaccharides, in agreement with a recent report [Chapman, A. & Kornfeld, R. (1979) *J. Biol. Chem. 254*, 816]. The NMR spectra were particularly valuable in detecting and quantitating isomeric fragments not observed previously, and our results suggest a modification of the scheme presented by Chapman and Kornfeld for the processing of mannose-rich IgM oligosaccharides.

Despite the diversity of glycoproteins, their carbohydrate components have common structural features. For the asparagine-linked carbohydrates, two major classes are known. These have been termed *complex* and *high mannose* (or *simple*) to reflect their sugar compositions (Kornfeld & Kornfeld, 1976). The complex oligosaccharides typically contain fucose, galactose, *N*-acetylneuraminic acid, and *N*-acetylglucosamine attached to asparagine by way of a pentasaccharide core of mannose and di-*N*-acetylchitobiose, $Man_3GlcNAc_2$.[1] Mannose-rich (high-mannose) oligosaccharides lack these other sugars but contain additional mannose that is linked to protein through the $Man_3GlcNAc_2$ core.

Two facts make the mannose-rich structures of particular interest. First, these oligosaccharides appear to have been conserved through evolution, as evidenced by the resemblance

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[1] Abbreviations used: GlcNAc or GNAc, *N*-acetylglucosamine; GlcNAc-$H_2$ or GNAc-$H_2$, *N*-acetylglucosaminitol; reduced oligosaccharides may be indicated with the prefix "r" (rGP-563-I is reduced oligosaccharide GP-563-I); $T_1$, spin–lattice relaxation time.

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