Metal Chelation by Glucose-Ammonia Derivatives

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The sequestration of cupric, calcium, strontium, and ferric ions by D-glucose, sorbitol, maltose, gluconate, glucoheptonate, saccharate, *N*-methylglucamine, mono- and di-D-glucosylamines, and their derivatives was measured in neutral to strongly alkaline solutions. Relatively large amounts of multivalent metal ion were sequestered by two new glucose-ammonia derivatives—disorbitylamine and the anhydro enolization product of di-D-glucosylamine. The results are discussed in terms of carbohydrate structure and metal chelation theory; and some possible benefits to agriculture are indicated.

THE IMPORTANCE of metal chelates in plant and animal nutrition was discussed by Haertl and Martell (*14*). Since then, the need for compatible metal-chelating agents for use in plant and animal therapeutics has been stressed in several symposia (*12*, *22*, *35*, *38*). Because the main problem is to find nontoxic agents that will effectively sequester and translocate metal ions in biological media, sugar derivatives should be considered (*4*, *11*). Sugar derivatives might also be more acceptable for inhibiting metal-catalyzed oxidations in processing food and in controlling trace radioactive elements, such as strontium-90, in foodstuffs.

Gluconates and glucoheptonates are industrially useful chelating agents (*8*, *9*, *13*, *29*, *33*, *41*), and a soluble ferric chelate of sodium glucoheptonate reportedly corrects iron chlorosis in bean plants without phytotoxicity (*19*). Also, glucamines have shown some metal-chelating effects (*1–3*, *20*, *21*, *27*, *28*, *34*, *42*). Gluconate and glucoheptonate chelate iron strongly in neutral solutions (*7*, *19*, *29*, *31*, *33*), but bivalent metallic ions, such as Ca^{+2}, Cu^{+2}, Ni^{+2}, and Pb^{+2}, apparently require strongly alkaline solutions of sugar derivatives (*4*, *6*, *21*, *26*, *29*, *30*). As the function of strong alkali probably is to disengage protons from the hydroxyl groups, thereby creating $-\overset{|}{\underset{|}{C}}-O^{\ominus}$ anionic centers which are known to bind metals strongly in combinations with other electron-donor atoms (*5*), the more readily enolizable sugar derivatives might provide better chelating agents for biological media.

Outstanding in their ease of enolization are the basic aldosylamines and their Amadori rearrangement products (*16*). Moreover, these amino sugars provide nucleophilic nitrogen atoms that tend to bind metal ions more strongly than oxygen atoms (*36*). *N*-Glucosylamino acids and their degradation products already have been reported to form rather stable complexes with multivalent metal ions (*23*, *24*, *32*, *39*); however, the simpler glucosylamines directly derived from ammonia apparently have not been investigated. Consequently, the metal-chelating capacity of several simply prepared glucose-ammonia derivatives were compared with the capacities of known chelating agents, with a view toward possibly serving agricultural nutritional needs.

Materials

Considering the principles of Schwarzenbach (*36*), especially in analogy with the iminodiacetic and nitrilotriacetic acids, 1,2-enolic forms of di- and tri-D-glucosylamine (the former being known and the latter unknown) were considered to be the most promising glucose-ammonia derivatives for chelating meta ions. Mono- and di-D-glucosylamine and their *N*-acetyl derivatives were prepared by known methods (*17*, *18*). However, attempts to condense di-D-glucosylamine with either another molecule of D-glucose or tetra-*O*-acetyl-α-D-glucosylbromide did not succeed in producing isolable amounts of tri-D-glucosylamine. A mixture of mono- (27%) and di-D-glucosylamine (65% of theory) was produced economically and directly from D-glucose and ammonia by a new procedure (*17*, *18*).

Catalytic hydrogenation of the mixture of hydrochloride salts of mono- and di-D-glucosylamine gave mono- and disorbitylamine, from which the di- was fractionally crystallized as the hydrochloride salt (*17*, *18*). Recrystallization of the hydrochloride salt from aqueous alcoholic sodium hydroxide gave the free base. Disorbitylamine (di-1,1′-D-glucitylamine) also was made by high-pressure hydrogenation of pure di-α-D-glucosylamine (*17*, *18*); this product was used in the quantitative analyses. Disorbitylamine was acetylated in water to yield the *N*-acetyl derivative (*17*, *18*).

Di-α-D-glucosylamine was heated in glacial acetic acid with the expectation of producing imino-bis-1-deoxy-D-fructose. Although elemental analyses on the product agreed for an acetate salt of the expected compound (*17*), it was later believed that the prepared substance was the monohydrate of an

anhydro compound, i.e., $C_{14}H_{25}NO_{11}\cdot H_2O$ (*17*, *18*). This monohydrated anhydro compound, of as yet unknown structure, is referred to below as the anhydro enolization product (AEP) of di-D-glucosylamine.

Isoglucosamine acetate (1-amino-1-deoxy-D-fructose acetate salt) was prepared by the method of Druey and Huber (*10*). Also, crude mixtures of this compound with the AEP of di-D-glucosylamine were prepared in glacial acetic acid by enolization of the mixture of mono- and di-D-glucosylamines (*17*).

Anhydrous dextrose, β-maltose hydrate, sorbitol, sodium gluconate, sodium α-glucoheptonate dihydrate, and the disodium salt of ethylenediaminetetraacetic acid (EDTA) were samples of the purest available commercial grades. *N*-Methylglucamine was a product-development sample kindly provided by Commercial Solvents Corp.

Methods

The sequestration of cupric and ferric ions was determined quantitatively by the ferrocyanide precipitation procedure of Mehltretter *et al.* (*29*), except pH was kept constant on a meter during the addition of cupric or ferric solution by simultaneously adding dilute sodium hydroxide. In determining the chelation of copper, the ferrocyanide solutions of the additive were buffered with sodium acetate (0.5*M*) to nullify any effect of the added acetate salts of two of the chelating agents. At the end point, cupric ferrocyanide precipitated sharply at pH 8.5 but not so sharply at higher alkalinities.

Qualitative procedures for demonstrating the chelation of copper, calcium, and strontium at high alkalinities are described under Results. Sequestration of strontium was measured quantitatively at pH 9.0 by the oxalate precipitation method Zussman used on calcium (*43*).

Water hardness was determined by a standard method (*37*). Local tap water of total hardness 390 p.p.m. of calcium carbonate (magnesium content not known) was diluted 1 to 4 with distilled water, and this stock solution was brought to pH 9.0 with sodium hydroxide for the blank determinations. After adding the sequestering agent, the test solution was adjusted to pH 9.0 before titrating with soap solution.

All analyses were run at 25 ± 1° C. in duplicate, and the soap titrations for water-hardness were run in triplicate. Four different preparations of the crude mixture listed in Table V were analyzed. The pH values selected are the lowest at which a satisfactory spread in the results was obtained.

Results

The first comparative tests were run with copper in strongly alkaline solution. Copper is known to chelate more strongly than other common bivalent metal ions and to be sequestered by sugars, sugar acids, sugar alcohols, and glycamines in strongly alkaline solutions but to a much lesser extent in neutral or acidic solutions (*3*, *4*, *26*, *27*, *29*, *30*).

When 0.002 to 0.006*M* cupric acetate solutions were kept at pH meter reading of 11.7 with added sodium hydroxide solution, cupric hydroxide did not precipitate in the presence of 0.002*M* concentrations of the glucose derivatives listed in Table I. Whereas all these derivatives prevented cupric hydroxide precipitation up through a 3 to 1 molar ratio of Cu^{+2} to glucose derivative, only the sugar acids and the nitrogenous derivatives extended the sequestration through a 4 to 1 molar ratio. Then, at a 5 to 1 molar ratio of copper to glucose derivative, only the anhydro enolization product of di-D-glucosylamine was effective. Equal final volumes of solution were maintained in all these tests.

In quantitative determinations of copper, Table II shows that glucose and most of its derivatives sequester very little cupric ion at constant pH 8.5. However, three nitrogenous derivatives gave significantly large chelation values: —disorbitylamine, the Amadori rearrangement product of mono-D-glucosylamine, and the anhydro enolization product of di-D-glucosylamine. The results in Table II were reproducible within 0.01 atom per mole below 0.10 and within 0.02 atom per mole above 0.10.

Semiquantitative tests for chelation of calcium and strontium by the glucose derivatives were run first at pH above 12, because sugar acids are known to sequester calcium strongly in highly alkaline solutions (*6*, *7*, *29*, *33*). Calcium or strontium acetate solutions of the additive were titrated with 1*N* sodium hydroxide until precipitation of calcium or strontium hydroxide occurred. Because of the appreciable solubilities of these hydroxides and their tendency to form supersaturated solutions, some pre-experimentation was necessary to find the optimum Ca^{+2} and Sr^{+2} concentrations for maintaining small volumes of the final solutions and to provide a spread of the results. Vigorous shaking and allowing questionable final mixtures to stand overnight alleviated the supersaturation problem.

Table III shows that the AEP of di-D-glucosylamine excels gluconate and glucoheptonate in sequestering calcium and strontium in strongly alkaline solutions. The AEP solutions were quite brown however, whereas the gluconate solutions were colorless. Disorbitylamine and isoglucosamine sequestered slightly more strontium but less calcium than the sugar acids. The analytical procedure magnified the differences in sequestration among the several agents because, with increasing dilution, the calcium and strontium hydroxides became more soluble. However, even at the highest dilution, the hydroxides precipitated rather promptly in the absence of a chelating agent, and each result in Table III is corrected for a blank determination run at the same dilution.

Table I. Sequestration of Cupric Ion Determined by Hydroxide Precipitation at pH 11.7

(2 m*M*. glucose derivative; 6, 8, 10, and 12 m*M*. cupric acetate)

Glucose Derivative	Cupric Hydroxide Precipitation at Molar Ratios of: 3:1	4:1	5:1	6:1
D-Glucose, maltose, sorbitol	−	+	+	+
Mono- or di-D-glucosylamine	−	+	+	+
N-Methylglucamine	−	+	+	+
N-Acetyldisorbitylamine	−	+	+	+
Sodium gluconate or glucoheptonate	−	−	+	+
Disorbitylamine	−	−	+	+
Isoglucosamine (acetate salt)	−	−	+	+
AEP of di-D-glucosylamine (acetate salt)	−	−	−	+

Table II. Sequestration of Cupric Ion at Constant pH 8.5 Determined by Cupric Ferrocyanide Precipitation

Sequestering Agent	Atoms of Cu^{+2} Chelated per Mole
D-Glucose, maltose, sorbitol, D-glucosylamine, *N*-acetyl-D-glucosylamine, *N*-acetyl-di-D-glucosylamine	<0.01
Di-D-glucosylamine, *N*-acetyldisorbitylamine	0.02
Sodium gluconate, saccharate, or *N*-methylglucamine	0.03
Sodium glucoheptonate	0.05
Disorbitylamine	0.16
Isoglucosamine (acetate salt)	0.22
AEP of di-D-glucosylamine (acetate salt)	0.47
EDTA	1.00

The greater strontium-sequestering action of the AEP of di-D-glucosylamine is still manifest at pH 9 but not at pH 7 (Table IV) although the amounts sequestered in the pH range 7 to 9 are quite low. At pH 7, disorbitylamine, isoglucosamine, the AEP of di-D-glucosylamine, and the sugar acids sequestered only barely detectable amounts of strontium ion but still detectably more than *N*-methylglucamine or di-D-glucosylamine. Reproducibility of the results in Table IV was within 0.01 atom per mole, and all values are corrected for blank determinations.

Calcium was not as easily determined by Zussman's oxalate precipitation method as strontium; therefore, the sequestration of calcium at mild alkalinity was measured by titrating for water hardness with a soap solution in the presence of the sugar derivatives. The results in Table V are for equal weights rather than for equal moles of each additive, and increasing net volumes of soap solution indicate increased sequestration of calcium (and magnesium). Whereas none of the sugar derivatives approached the effectiveness of EDTA in softening water, the crude brown mixtures of acetate salts of isoglucosamine and the AEP of di-D-glucosylamine (*17*) were appreciably more effective at pH 9 than the sugar acids.

Gluconate and glucoheptonate are known to be superior chelating agents for iron, even in neutral solution (*7, 29, 31, 33*). Table VI shows that the AEP of di-D-glucosylamine equals gluconate and glucoheptonate in the amount of ferric iron sequestered at pH 7. Disorbitylamine equals EDTA, and isoglucosamine acetate chelates significantly more iron than either *N*-methylglucamine or di-D-glucosylamine. The results for iron are probably not so accurate in an absolute sense as those for copper in Table II. For example, lower results were obtained when the ferric solution was doubled in strength for the longer titrations because of the smaller final volumes of solution in which precipitation occurred. The total variation on six different determinations of the chelating capacity of sodium gluconate was ±0.3 atom per mole. Nevertheless, the order given in Table VI adequately shows the relative chelating capacities of the compounds.

Discussion

The foregoing comparisons of chelating capacity (Tables I to VI) show the relative positions of glucose-ammonia derivatives among the known metal-chelating agents derived from glucose. The comparisons also show that three of the nitrogenous glucose derivatives have much larger chelating capacities than do glucose, maltose, sorbitol, *N*-methylglucamine, or the simple glucosylamines. They are disorbitylamine, isoglucosamine acetate, and an acetate salt of the anhydro enolization product of di-D-glucosylamine. The latter excels gluconate and glucoheptonate in the chelation of copper, strontium, and calcium at pH 9 and equals them in the chelation of iron at pH 7. Structures of these compounds are shown in Figure 1.

D-Glucosylamine (I), di-D-glucosylamine (II), their *N*-acetyl derivatives (Ia, IIa), and the *N*-acetyl derivative of disorbitylamine (IIIa) are not very effective chelating agents. These compounds are weakly basic or neutral, and they hydrolyzed to some extent under the test conditions. Disorbitylamine (III) unlike II is acyclic, strongly basic, does not hydrolyze, and is a better metal-chelating agent. Because the neutral *N*-acetyl derivative of III is acyclic like III, but still is not nearly

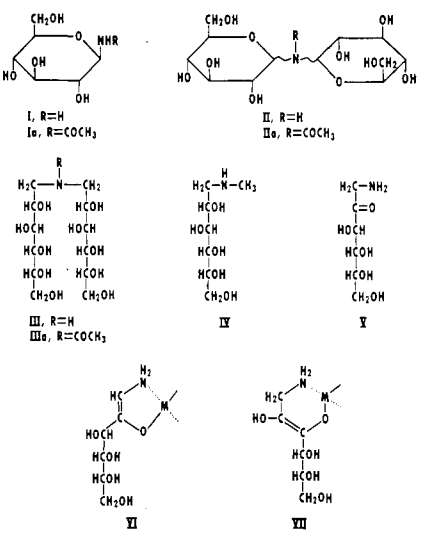

Figure 1. Formulas of compounds discussed

Table III. Sequestration of Calcium and Strontium Ions Determined by Hydroxide Precipitation at pH > 12

(Either 1.00 ml. of 0.33*M* calcium acetate or 0.50*M* strontium acetate and 1.00 ml. of 0.25*M* glucose derivative titrated with 1*N* sodium hydroxide to the point of precipitation of calcium or strontium hydroxide. Titers are corrected for the blank titers at equal volumes of final solution)

Glucose Derivative	Net Ml. of 1N NaOH	
	Calcium	Strontium
D-Glucose, sorbitol, and *N*-methylglucamine	0.15	0.10
Di-D-glucosylamine acetate	0.3	0.15
Isoglucosamine acetate	0.45	0.4
Disorbitylamine acetate	1.1	0.4
Sodium gluconate	1.5	0.3
Sodium glucoheptonate	1.7	0.3
AEP of di-D-glucosylamine acetate	2.2	1.1

Table IV. Sequestration of Strontium Ion at Constant pH Determined by Oxalate Precipitation

Sequestering Agent	Atoms Sr^{+2} per Mole	
	At pH 9.0	At pH 7.0
Di-D-glucosylamine, *N*-methylglucamine	0.01	0.00
Disorbitylamine (hydrochloride)	0.02	0.01
Isoglucosamine (acetate salt)	0.02	0.01
Sodium gluconate or glucoheptonate	0.02	0.01
AEP of di-D-glucosylamine (acetate salt)	0.09	0.01
EDTA	1.03	1.01

Table V. Softening of Hard Water Determined by Soap Titrations at Initial pH 9.0

Sequestering Agent (0.6% by Weight)	Standard Soap Solution, Ml. (Blank Titer — Sample Titer)
D-Glucose, mono- or di-D-glucosylamine	0.0–0.1
Disorbitylamine	0.2–0.4
Sodium gluconate or glucoheptonate	0.4–0.5
AEP of di-D-glucosylamine (acetate salt)	0.6–0.8
Potassium acid saccharate	1.2
Isoglucosamine (acetate salt)	1.5–2.0
Crude brown mixtures of AEP of di-D-glucosylamine and isoglucosamine	1.5–2.4
EDTA	6.0

Table VI. Sequestration of Ferric Ion at Constant pH 7.0 Determined by Ferric Ferrocyanide Precipitation

Sequestering Agent	Atoms Fe^{+3} per Mole
D-Glucose, sorbitol, *N*-acetyl-D-glucosylamine	0.01
N-Methylglucamine	0.07
Di-D-glucosylamine	0.08
Isoglucosamine (acetate salt)	0.7
Disorbitylamine	1.3
EDTA	1.3
Sodium gluconate or glucoheptonate	3.6 ± 0.3
AEP of di-D-glucosylamine (acetate salt)	3.7 ± 0.3

as effective (Tables I and II), the strongly basic nitrogen atom in III is shown to participate in the metal binding. The fact that isoglucosamine (V) and the AEP of di-D-glucosylamine form stable acetate salts shows that they also are strongly basic. Disorbitylamine is much more effective than the equally basic *N*-methylglucamine (IV), so two polyhydroxylated, free-rotating carbon chains in the molecule are much better than one. Three such radicals, as in the yet unknown trisorbitylamine, probably would be still better. Presumably the acyclic radicals can curl around a metal ion and sequester it more effectively than a relatively fixed ring system. The pyranose rings of glucose, I, and II open in strongly alkaline solution; enolization and deprotonation of the hydroxyl groups occur, and sequestration is promoted to the extent shown in Table I. These reactions do not occur extensively for glucose, I, and II in neutral and weakly alkaline solutions, and sequestration is slight (Table II).

Amadori compounds, such as isoglucosamine (V), exist in both open-chain and ring forms (*15, 25, 40*). The 2-keto structure of V allows 2,3-enolization in addition to 1,2-enolization, thereby providing for both 5- and 6-membered metal chelate rings (VI and VII). Whereas saturated 5-membered chelate rings are said to be more stable than 6-membered (*36*), this statement may not be true when the ring contains a double bond. The ability to form 6-membered enolic chelate rings, such as VI, may possibly explain the greater sequestration of metals by isoglucosamine over the glucosylamines.

Unfortunately, the structure of the most promising compound, the AEP of di-D-glucosylamine, is not yet known. Whereas initially this product was considered to be the acetate salt of iminobis-1-deoxy-D-fructose (*17*), it is now known to contain one less mole of water than that compound. Work continues to determine its structure. Because it does reduce 2,6-dichloroindophenol in 0.1*N* sodium hydroxide at 25° C. at more than double the rate of isoglucosamine acetate per mole, it probably contains more than one enolizable keto group. With two enolizing keto groups in the molecule and with corresponding enolate anionic groupings present in weakly alkaline solutions, it would be expected to show the superior metal-chelating properties observed.

Crude mixtures of the acetate salts of isoglucosamine and the AEP of di-D-glucosylamine can readily be prepared from D-glucose and ammonia (*17*). Also, mixtures of di- and monosorbitylamines are readily prepared from D-glucose and ammonia (*18, 28*), and the disorbitylamine can be crystallized preferentially from the mixture as the hydrochloride salt (*18*). Because it is likely that such crude mixtures could be produced economically, their metal complexes should be tested for ability to correct trace-metal deficiencies in agricultural products.

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