^{1}H NMR Elucidation of Observed Stable Sugar-NaCl-water Complexes in Aqueous Solution

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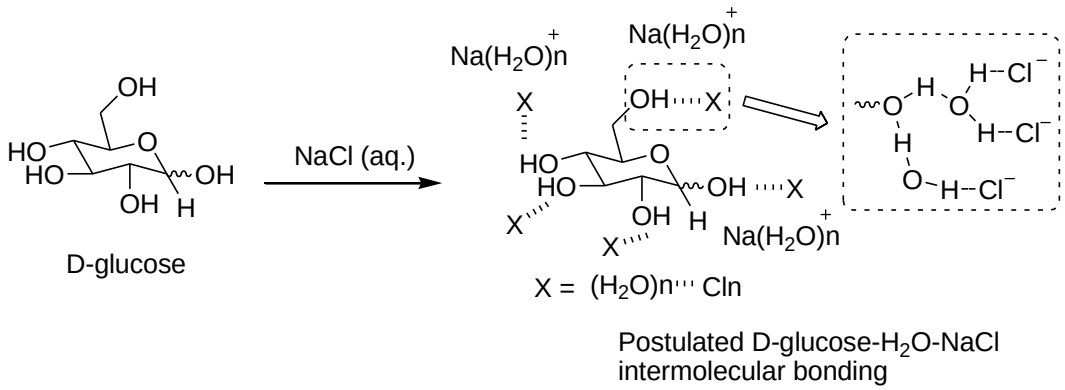
Abstract:

The solvation of sugars in aqueous media matters in the understanding of biological systems and carbohydrate transformations. Generally, 2 – 4 water units were proposed to interact with each hydroxyl group in monosaccharides via different types of hydrogen bondings at room temperature in previous studies. Presence of NaCl was known to perturb hydrogen bondings of sugar hydrates. However, direct evidence to elucidate mechanism at atom level is very rare even though "NaCl Effect" was well known in biomass chemical transformations. Here we report ^{1}H NMR elucidation evidences of mono/disaccharides hydrates in different concentration of NaCl aqueous solutions. We here conclude two new findings: 1) under ideal usage of NaCl, different mono/disaccharides hydrates are likely to be converted into a stable sugar-NaCl-water form; 2) pKa value of different hydroxyls in mono/disaccharides has intangible influence on hydrate form change induced by NaCl. An ideal NaCl usage based on maximum of ^{1}H NMR shift was proposed.

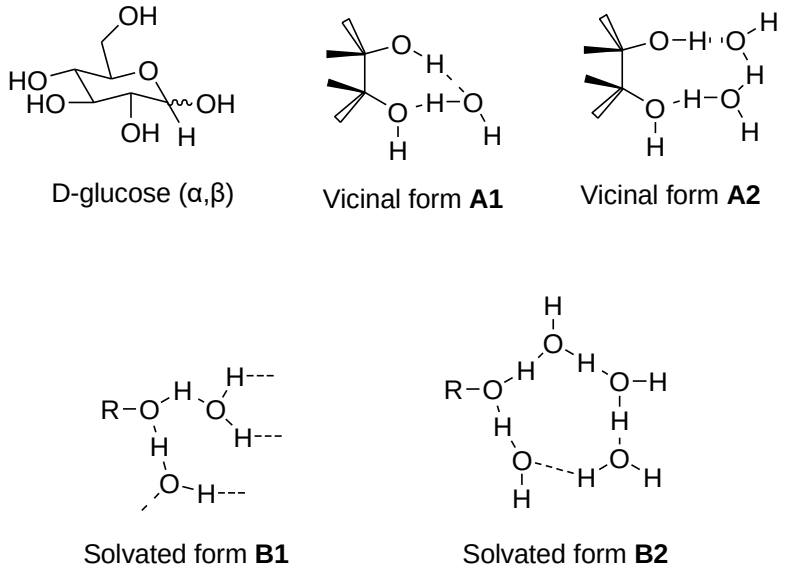


1.0 Introduction

Monosaccharides as basic units of glycogen, starch and cellulose, play very important roles in biological systems and water is the only media[1]; hence solvation of monosaccharides by water was studied for decades for better understanding of molecule mechanism. However, due to lack of direct experimental evidence, exact interaction between monosaccharides like D-glucose and water in solution is not yet fully understood, particularly regarding on how

individual hydroxyl group is locally hydrated. In 1976, J. M. Harvey *et.al* reported that each hydroxyl group of D-glucose forms at least two hydrogen bonds with two water molecules, and four solvated forms with different hydrogen bindings were proposed (**Figure 1**)[2,3], via direct measuring hydroxyl proton at low temperature. T. Suzuki's simulation work also resulted into a similar conclusion in 2008.[4]

Figure 1. D-Glucose and its four different solvated forms in water.



The presence of sodium chloride was proved to perturb the hydrogen bonding network of water[5], resulting into sodium-saccharide interaction in water[6]. Early study via paper electrophoresis and optical rotation by S. J. Angyal[7] showed that as a univalent cation sodium cation is highly hydrated in solution and a single hydroxyl group on monosaccharides cannot compete with water molecular in coordination; only two or three hydroxyl groups in a suitable arranged combination may result in sodium-monosaccharide complex in a very weak manner. A Raman-spectra study by F. Franks[8] *et.al* also indicated that sodium cation-glucose perturbation rather than sodium-glucose complex formation caused observed spectrum changes; and cations effect study also confirmed that the interaction induced by sodium cation is much weaker than other multivalent cations like Ca^{2+}. Recently, NaCl usage in aqueous solution as an additive[9-12] or a promoter[13-15] for biomass transformations into valuable chemicals or biofuels is increasingly popular because they are abundant in nature and are very cheap; and "NaCl Effect" (or "salt effect") became well known in biomass conversion and carbohydrate chemistry in the recent decade. Further system study of the role of metal halide in enhancing the dehydration of xylose to furfural by K. R. Enslow *et.al*[16] and insights from quantum mechanics/molecular dynamics on sodium ion interaction by H. B. Mayes *et.al*[17] both confirmed the previous hypothesis, which states that the cation has a stronger effect than the anion on glucose with the anions acting to stabilize critical intermediates. However, very recent higher yield productions of chemicals from monosaccharides in aqueous solution promoted by NaCl[18-20] challenged the minor role hypothesis of chloride anion in NaCl promoted system. The nature of complex-formation between sodium and monosaccharides

is not yet well understood, particularly at atom level. No direct experimental evidence mapping bonding interaction between monosaccharides like glucose and NaCl in water was reported to our best of knowledge, probably because of the instability nature of complex in solution and NMR spectrum was different depending on many factors[5,6,16]. There are two important concerns yet to be answered: 1) would the presence of NaCl change reactivity order (pKa) of all carbons in mono/disaccharides via perturbing hydrogen bonding networks in aqueous solution? 2) could maximum promotion (or activation) usage of NaCl be predictable? To answer these two concerns, here we reported ^{1}H NMR evidences of a relatively stable sugar-NaCl-water complexes observed on different mono/disaccharides in concentrated NaCl solution, which is not yet reported before. Combining with observed ^{1}H NMR shifts correlated with a concentration of NaCl solution, a more detailed mechanism on role of chloride anion is proposed here for a better elucidation of "NaCl Effect".[21]

It was known that solid state NMR and IR spectra both showed no obvious change, especially in spectral regions characteristic of the ordered and disordered regions of cellulose in the presence of NaCl[22,23], probably because of cellulose's poor solubility in water. Inspired by a sharp difference of ^{1}H NMR shifts of glucosamine in saturated NaCl solution, we envisaged an NMR angle by measuring ^{1}H NMR shifts of monosaccharides and disaccharides might deliver more experimental evidences to understand the mechanism of "NaCl Effect". Hence, water soluble monosaccharides including D-glucose, D-glucosamine hydrogen chloride, N-acetyl-D-glucosamine and D-fructose were initially chosen in order to gain some insights on "NaCl effect" on the intramolecular/intermolecular hydrogen bonding in water via regular ^{1}H NMR measurement. Variant concentration and saturated NaCl solutions were prepared for comparison study.

2.0 Experimental Section

General information

D(+)-Glucosamine hydrochloride was purchased from Shanghai Macklin Biochemical Co., Ltd. Ethanol was purchased from Guangdong Test Agent Technology Co., Ltd. N-Acetyl-D-glucosamine, glucose, D_2O and NaCl were purchased from Aladdin Industrial Corporation. Fructose was purchased from Shanghai TCI Chemical Industry Development Co., Ltd. Sucrose and trehalose were both purchased from Guangzhou Asegene Co., Ltd. Stachyose（80%） was purchased from Macklin Co., Ltd. All reagents were used without further purification. Saline solution (medical, 0.9%) was purchased from Hebei Tiancheng Pharmaceutical Co. Ltd. Deionized water was used in all experiments. All reagents were used without further purification. ^{1}H NMR spectra was recorded on Bruker AV-300 (300 MHz) instrument at room temperature.

2.1 Preparation for different concentrations of NaCl solutions (wt%)

1. 0.5 % NaCl solution: 29.2 mg NaCl was dissolved in 6 mL deionized water.
2. 1.9% NaCl solution: 116.8 mg NaCl was dissolved in 6 mL deionized water.
3. 2.4% NaCl solution: 146.1 mg NaCl was dissolved in 6 mL deionized water.
4. 4.8% NaCl solution: 1 g NaCl was dissolved in 20 mL deionized water.
5. 9.1% NaCl solution: 2 g NaCl was dissolved in 20 mL deionized water.
6. 13.0% NaCl solution: 3 g NaCl was dissolved in 20 mL deionized water.

7. 16.7% NaCl solution: 4 g NaCl was dissolved in 20 mL deionized water.

2.2 General procedure for ^{1}H NMR investigation on monosaccharides

Monosaccharide (0.5 mmol) was added into different concentration of NaCl solution (6 mL) and the mixture was stirred for 6 h at room temperature. After then, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for ^{1}H NMR to determine chemical shift.

2.3 General procedure for ^{1}H NMR investigation on oligosaccharides

Sucrose (107.0 mg, 0.31 mmol, Mw: 342.3 g/mol, 8 OHs/molecular) or trehalose (107.0 mg, 0.31 mmol, Mw: 342.3 g/mol, 8 OHs/molecular) or stachyose (148.8 mg, 0.18 mmol, purity: 80%, Mw: 666.6 g/mol, 14 OHs/molecular) was added into different concentration of NaCl solution (6 mL) and the mixture was stirred for 6 h at room temperature or 60℃; After then, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure below 50℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for ^{1}H NMR to determine chemical shift.

2.4 General procedure for ^{1}H NMR investigation volume impact (9.1% NaCl solution) of "NaCl effect" on N-acetyl-D-glucosamine

N-Acetyl-D-glucosamine (0.5 mmol) was added into 9.1 wt% NaCl solution (1.5 mL, or 3 mL or 6 mL or 12 mL) and the mixture was stirred for 6 h at room temperature. After then, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for ^{1}H NMR to determine chemical shift.

Note: sample concentration means the concentration of NaCl in NMR tube. In procedures 2.1 -2.4, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was dissolved into 0.4 mL D_2O and it led to a concentration increase in NMR tube (2.5 times of prepared solution).

2.5 Procedure for ^{1}H NMR comparison study (glucose)

2.4.1 Preparation of 9.1% NaCl solution

2 g NaCl was dissolved in 20 mL deionized water.

2.5.2 Procedure

D-glucose (0.5 mmol) was added into a NaCl solution (9.1 wt%, 6 mL) and the mixture was stirred for 6 h at room temperature. After then, 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for ^{1}H NMR to determine chemical shift. Meanwhile

another 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 1 mL D_2O), part of them for ^1H NMR to determine chemical shift for comparison.

2.53 Procedure for ^1H NMR study of NaCl solution directed preparation in D_2O.

Preparation of 9.1% NaCl solution: 0. 2 g NaCl was dissolved in 2 mL D_2O.

D-glucose (0.1 mmol) was added into above NaCl solution (1.2 mL) and the mixture was stirred for 6 h at room temperature. After then, 0.5 mL of the reaction mixture was taken out (2 times of concentration in preparation) for ^1H NMR to determine chemical shift.

2.5.4 Procedure for preparation a control (D-glucose in D_2O).

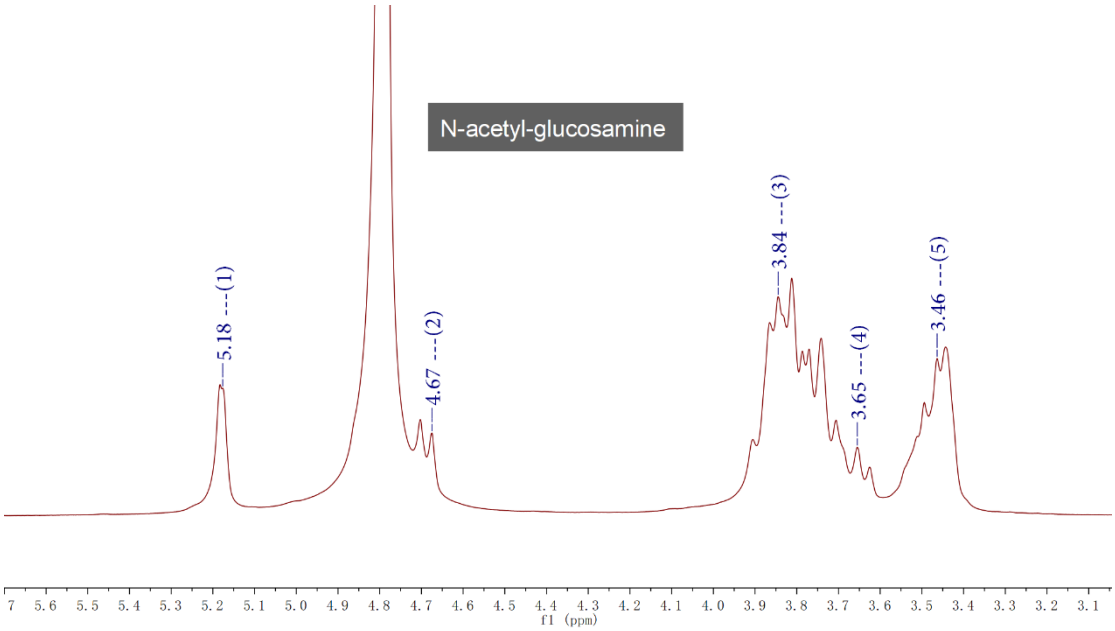
D-glucose (0.05 mmol) was added into D_2O (0.6 mL); and the reaction mixture was taken out for ^1H NMR to determine chemical shift.

3.0 Results and Discussion

3.1 ^1H NMR Study of monosaccharides in NaCl solution (1 wt% to saturated solution)

Initially, 0.5 mmol of N-acetyl-D-glucosamine was dissolved into 6 mL of NaCl solution in a different concentration and the mixtures continued to stir for 6 hours at room temperature before one portion was taken out for ^1H NMR measurement. Five easily identified peaks, including hydrogens on 1-position carbon of both α-anomer and β-anomer[24], were marked (**Figure 2**) in order to track their changes in different concentrations of NaCl solutions.

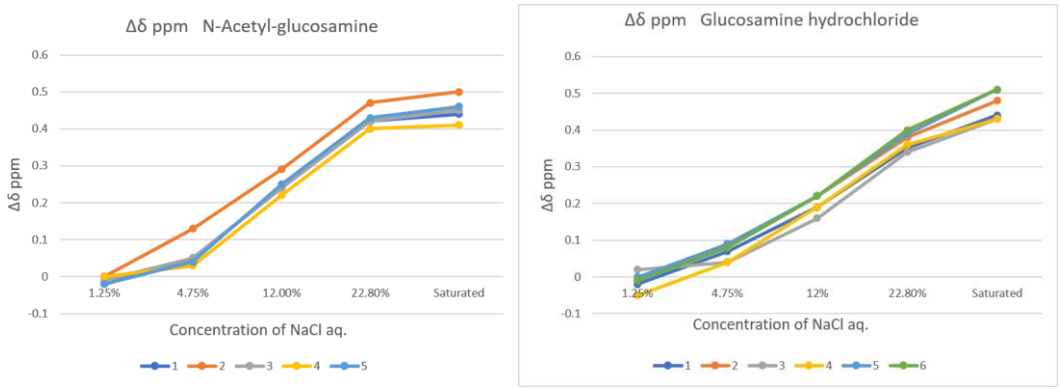
Figure 2. ^1H NMR Spectra of N-Acetyl-D-glucosamine and Five Peaks Marked for Tracking.



For N-acetyl-D-glucosamine, protons on 1-position carbon of α-anomer and β-anomer displayed at δ 5.18 and 4.67 respectively on ^1H NMR spectra in D_2O, the former was at left

side of D_2O peak while the latter was at right side. In 1.25 wt% NaCl solution, influence of NaCl to ^1H NMR shifts of all marked five peaks (△δ <= -0.02 ppm) were almost intangible; meanwhile the shifts became obvious (△δ = 0.03 - 0.13 ppm) in 4.75 wt% NaCl solution (**Figures 3** and supporting information). An interesting observance was that the shift (△δ = 0.13 ppm) of the second marked peak (H_1 of β-anomer) was the bigger than that (△δ = 0.05 ppm) of the first one (H_1 of α-anomer) in 4.75 wt% NaCl solution; indicating the presence of NaCl had a stronger influence on β-anomer of N-acetyl-D-glucosamine. A significant deshielding effect was observed for all protons of N-acetyl-D-glucosamine (△δ = 0.26 ppm) in the presence of 12 wt% NaCl solution (in NMR tube; 4.8 wt% in preparation), which was induced by hydrated NaCl (**Figures 3**). A remarkable downfield shifting (△δ = 0.18 ppm) on ^1H NMR shift for all five peaks was also observed increasing of 12 wt% NaCl solution to 22.8 wt% NaCl solution (in NMR tube; 9.1 wt% in preparation). Such an obvious shift is comparable to what K. R. Enslow *et.al*[16] had observed with 0.75M D-xylose in 6 M NaCl solution. Change (△δ <= 0.02 ppm) became intangible when further increase of concentration to saturated NaCl solution (in NMR tube; 13.0 wt% in preparation). Based on these data (**Figures 2**), it could be concluded that maximized perturbation of intramolecular/intermolecular hydrogen bonding within N-acetyl-D-glucosamine molecular was reached in 22.8 wt% NaCl solution and in which relatively stable sugar-NaCl-water complex formed. Meanwhile, such a stable complex was never observed via ^1H NMR measurement before to our best knowledge.

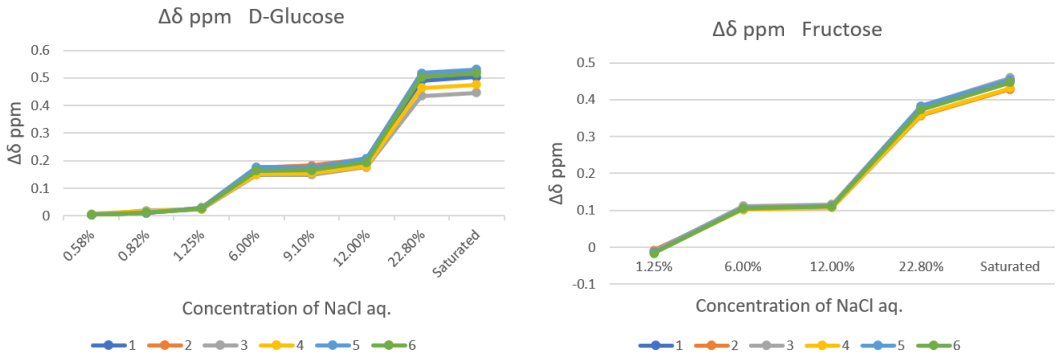
Figure 3. ^1H NMR Shifts of N-Acetyl-D-glucosamine and D-Glucosamine Hydrogen Chloride Correlating with Concentration of NaCl solution by Tracking Marked Five/Six Peaks



In order to gain more clues, similar ^1H NMR tracking investigation in different concentrations of NaCl solution was also performed with other three abundant monosaccharides (D-glucose, D-fructose and D-glucosamine hydrogen chloride) in nature (**Figure 3 and 4**). Above three monosaccharides were known to interact with sodium cation in aqueous solution differently.[7] Similarly, in 1.25 wt% NaCl solution, influence of NaCl to ^1H NMR shifts of all marked six peaks (△δ <= -0.05 ppm) of D-glucosamine hydrogen chloride was almost intangible; meanwhile the shifts became obvious (△δ = 0.04 - 0.09 ppm) in 4.75 wt% NaCl solution (**Figures 3** and supporting information). A different observance was that gap of the shift (△δ = 0.09 ppm) of the second marked peak (H_1 of β-anomer) and that (△δ = 0.07 ppm) of the first one (H_1 of α-anomer) was narrow in 4.75 wt% NaCl solution; indicating the presence of acetyl group had

an obvious influence and the anomeric effect became not obvious. Interestingly, with D-glucosamine hydrogen chloride stable complex could only be obtained till NaCl concentration was increased to saturated solution (in NMR tube; 16.7 wt% in preparation). The significant delay to a stable sugar-NaCl-water complex was likely due to free amine moiety of D-glucosamine hydrogen chloride (stronger inter/intramolecular bonding).

Figure 4. ^{1}H NMR Shifts of D-Glucose and D-Fructose Correlating with Concentration of NaCl solution by Tracking Marked Six Peaks.



In 1.25 wt% NaCl solution, a downfield effect (△δ = 0.02 - 0.03 ppm) to all marked six peaks of D-glucose was observed (**Figures 4** and supporting information), unlike the existence of both upfield effect and downfield effect to the marked five/six peaks of N-acetyl-D-glucosamine/D-glucosamine hydrogen chloride. Significant downfield changes (△δ = 0.15 - 0.18 ppm) were observed upon increase of concentration of NaCl solution from 1.25 wt% to 6 wt% (in NMR tube; 2.4 wt% in preparation). No change at all or little change (△δ = 0 - 0.03 ppm) for all marked peaks was found upon further increase of concentration of NaCl solution from 6 wt% to 9.1 wt% or from 9.1 wt% to 12 wt% or from 22.8 wt% to saturated solution); meanwhile significant changes (△δ = 0.26 - 0.32 ppm) were observed upon increase of concentration of NaCl solution from 12 wt% to 22.8 wt%. A possible stable sugar-water-NaCl complex was formed in 22.8 wt% NaCl solution. Trends of ^{1}H NMR shifts of D-glucose in marked six peaks were pretty like a staircase, not a line; indicating a stepwise formation of hydrogen (H_2O) -chloride bonding.

For D-fructose, an upfield effect (△δ <= -0.02 ppm) to all marked six peaks was observed in 1.25 wt% NaCl solution, probably because of its rigid furanose structure. Similar to the observance for D-glucose, obvious ^{1}H NMR shifts (△δ = 0.10 -0.11 ppm) could still be observed for all six marked peaks when further increase of concentration to 6.0 wt% from 1.25 wt%; meanwhile little change (△δ <= 0.01 ppm) was shown upon further increase of concentration from 6.0 wt% to 12 wt%. Surprisingly, anomeric effect was intangible for D-frutose. Continued increase to saturated NaCl concentration led to small changes (△δ <= 0.08 ppm) for all six marked peaks on ^{1}H NMR spectra. Such observed staircase-like trend indicated that correlation of ^{1}H NMR shift of other monosaccharides (without amine moiety) to concentration of NaCl solution might also be similar, which is an important insight for understanding of “NaCl effect” at atom level.

It worth being noted that shifting trends of all marked peaks were similar in all four

monosaccharides which indicated that pKa value has no observable effect on perturbation of hydrogen bonding, and generation of new hydrogen bonding networks was non-selective. However, anomeric difference did exist for some monosaccharides in relatively low concentration of NaCl solution. Another key insight is that chloride anion likely plays major role on induction of ^{1}H NMR shift because of two reasons: 1) sodium cation is known to have less specific locations of binding due to its univalent and readily hydrated property in water.[25] Typically, two or three hydroxyl groups of monosaccharides are necessary to bind with each sodium cation and it is not so surprising that only one or two hydrated sodium cations can interact with each monosaccharide weakly. 2) Sodium cation is known to be buried inside water shells in its hydrated form meanwhile chloride anion is on the top of water cluster in its hydrated form;[26] in addition, strong perturbation of anions by exerting on adjacent H atoms was reported[27] through experimental Raman spectral measurements with classical MC simulations.

3.2 ^{1}H NMR Study of disaccharides/oligosaccharide in NaCl solution (12 wt% to saturated solution) at room temperature and 60℃.

Disaccharides and oligosaccharides have stronger hydrogen bonds with water because of more chelating bonds involved, and self-associate to form cluster is also involved in aqueous solution. Next, two disaccharides (sucrose and trehalose) and one tetrasaccharide (stachyose) were selected as targets for ^{1}H NMR comparison study in higher concentrations of NaCl solutions at room temperature and 60℃ (supporting information) in order to provide more viable references for NaCl promoted perturbation of hydrogen bonding of polysaccharides such as cellulose. As a disaccharide formed by a 1,1-glycosidic bond between two α-glucose units, trehalose has a stronger hydrogen bonding network than D-glucose. Disaccharide containing fructose moiety and fructose need a higher concentration of NaCl solution to generate the stable sugar-NaCl-water complexes since the ring of fructose hydrate is more rigid than that of glucose hydrate, leading to less mobility of H-bonded water.[28]

At room temperature in 12 wt% NaCl solution, ^{1}H NMR shifts ($\triangle\delta$ = 0.27 -0.30 ppm) for all four marked peaks of trehalose (in NMR tube; 4.8 wt% in preparation) were much bigger than those observed ($\triangle\delta$ = 0.15 -0.16 ppm) with sucrose (supporting information). It was easy to be understood because one molecule sucrose composed of two monosaccharides (glucose and fructose) and the moiety (fructose) in slow rate determined the rate of sucrose in perturbation of hydrogen bonding. Such difference between shifts of all four peaks of trehalose ($\triangle\delta$ = 0.34 -0.40 ppm) and those of sucrose ($\triangle\delta$ = 0.39 -0.43 ppm) became very little in case that concentration of NaCl solution was over 22.8 wt% (in NMR tube; 9.1 wt% in preparation).

Replacement of sucrose to tetrasaccharide (stachyose) led to little change on the correlation between ^{1}H NMR shift and concentrations of NaCl solution (supporting information). Such similar change indicates that NaCl promoted stable sugar (moiety)-NaCl-water complexes are likely present as well for oligosaccharides in water.

An increase of mixing temperature to 60℃ had positive effect ($\triangle\delta$ = 0.11 ppm) on perturbation of hydrogen bonding of sucrose (**Table 1** and supporting information) in relatively low concentration of NaCl solution (12 wt% in NMR tube, 4.8 wt% in preparation);

smaller shifts (△δ = 0.02 ppm) for all four marked peaks of stachyose was also observed upon the increase to 60℃ (see supporting information). However, influence became intangible in relatively high concentration of NaCl solution (22.8 wt%) for both disaccharides and stachyose (**Table 1** and supporting information). Such observance indicated the formed sugar-NaCl-water complexes are relatively stable and could be prepared in relatively low concentration of NaCl solution under elevated temperature or in higher concentration at room temperature. For trehalose, a remarkable upfield effect (△δ = -0.13 -0.14 ppm) was observed in 12 wt% NaCl solution, probably because self-associate of trehalose was strongly accelerated at 60℃.

Table 1. ^{1}H NMR shifts (Four marked peaks of sucrose) under different concentrations resulted from increase of temperature (60 °C).

Concentration (NMR sample)	1 (△δ)	2 (△δ)	3 (△δ)	4 (△δ)
0	-0.011	-0.010	-0.010	-0.011
12.0%	0.109	0.107	0.106	0.111
22.8%	-0.032	-0.030	-0.029	-0.032
Saturated solution	0	0	0.001	0.001

The significant shifts for all marked peaks of sucrose in the presence of 12 wt% NaCl solution at room temperature and 60℃ indicated the importance of temperature; particularly the temperature might play a more important role for polysaccharides that have poor solubility in water due to large molecular weight. NaCl concentration was well known to have strong effect on hydrogen bonding in water due to solvation[29-31]. The higher concentration of NaCl solution, the better effect on perturbation of hydrogen bonding network based on ^{1}H NMR shifts.

3.3 NMR instrument impact on ^{1}H NMR shifts of D-glucose in D_2O.

Table 2. ^{1}H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

Entry (E)	Date (brand)	1	2	3	4	5	6
1	Dec. 2021 (JOEL)	5.198	4.607	3.883	3.713	3.460	3.215
2	Sept. 2021 (JOEL)	5.230	4.638	3.909	3.740	3.491	3.246
3	△δ (E2 – E1)	0.032	0.031	0.026	0.027	0.031	0.031
4	2018 (Bruke)	5.143	4.551	3.841	3.665	3.408	3.161
5	△δ (E4 – E1)	-0.045	-0.056	-0.042	-0.048	-0.052	-0.051

As we discussed in the introduction part, many factors might have influence on absolute value of shifts of ^{1}H NMR, here we investigated the impact of NMR instrument on six marked peaks of D-glucose by measuring the sample on different NMR instrument at different date. Both

NMR instruments were in default working conditions at room temperature for users from chemistry laboratories. The error (△δ= 0.03 ppm) between two ^{1}H NMR spectra (in D_2O) recorded on JOEL NMR instrument at Shanghai in Sept.2021 and Dec. 2021 was in acceptable error range (Entries 1 – 3, **Table 2**). The difference (△δ= 0.04 – 0.06 ppm) for ^{1}H NMR spectra of D-glucose in D_2O (Entries 1, 4 and 5, **Table 2**) was slightly bigger when samples were recorded on different NMR instruments (Joel at Shanghai, Dec. 2021 and Bruke at Guangzhou, 2018). Based on these comparison data, a general guideline is that a control sample is strongly suggested to be done at the same time for comparison in case samples in very low concentration of NaCl solution (< 1 wt%) or a change to a new NMR instrument.

3.4 Volume effect

In procedures 2.1 -2.4 in experimental section, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was dissolved into 0.4 mL D_2O and it led to a concentration increase in NMR tube (2.5 times of prepared solution). Sample concentration in **Table 3** means the concentration of NaCl in NMR tube. A recent comparison study (Entries 1 – 3, **Table 3**) on another NMR instrument (JOEL) indicated the difference (△δ= 0.06 – 0.08 ppm) of six marked peaks of D-glucose was very small between the two samples mixed in 22.8 wt% solution (in NMR tube), considering the obvious shifts (△δ= 0.44 – 0.52 ppm) in comparison with D-glucose in the absence of NaCl (supporting information). Not surprisingly, an increase on concentration in NMR tube (2.5 times) led to a sharp change on shifts of all six peaks (Entries 2 and 4, **Table 3**). A control study in NaCl/D_2O solvent (9.1 wt%) was also carried out and part of solvent mixture was directly taken out for ^{1}H NMR measurement (Entries 5, **Table 3**); the shifts of all six marked peaks had little difference (△δ <= 0.02 ppm) with those via evaporation/dissolving process (Entries 4, **Table 3**).

Table 3. ^{1}H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

Entry (E)	Concentration (sample)	1	2	3	4	5	6
1	9.1% (22.8%, 0.4 mL D_2O) (2018, on Bruke)	5.633	5.061	4.276	4.129	3.926	3.664
2	9.1% (22.8%, 0.4 mL D_2O) (Dec. 2021, on JOEL)	5.568	4.992	4.217	4.051	3.850	3.588
3	△δ (E2-E1)	-0.07	-0.07	-0.06	-0.08	-0.08	-0.07
4	9.1% (9.1%, 1.0 mL D_2O) (Dec. 2021, on JOEL)	5.365	4.790	4.032	3.868	3.635	3.380
5	9.1% (D_2O) (Dec. 2021, on JOEL)	5.383	4.799	4.053	3.885	3.654	3.397

Effects of volume of 9.1 wt% NaCl solution (22.8 wt% in NMR tube) was also investigated with

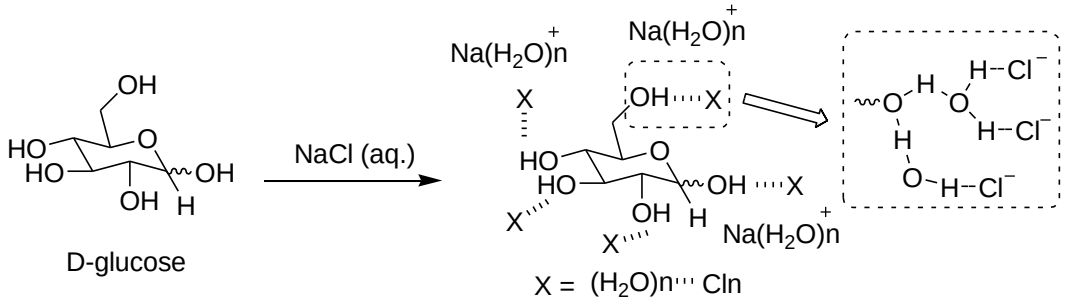
N-acetyl-D-glucosamine (0.5 mmol) (**Table 4**). Downfield shift was observed along with increase of volume till 6 mL, indicated that after then bonding network became steady no change was observed on ^{1}H NMR spectra. The total usage of NaCl was 10 mmol under the turning point condition (6 mL) and molar ratio of NaCl to total hydroxyls (2.5 mmol) of monosaccharide was 4 : 1.

Table 4. ^{1}H NMR Spectra of N-acetyl-D-glucosamine after mixing with different volume of 22.8 wt% NaCl solution (in NMR tube) at room temperature.

Volume	1 (△δ)	2 (△δ)	3 (△δ)	4 (△δ)	5 (△δ)
1.5 mL	0	0	0	0	0
3 mL	0.050	0.054	0.048	0.048	0.058
6 mL	0.236	0.229	0.206	0.227	0.231
12 mL	-0.017	-0.003	-0.003	0	-0.004

3.5 Structure of stable sugar-NaCl-water complexes.

Figure 5. Stable Complex of D-Glucose-NaCl-water in Aqueous Solution.



There are two hydrate forms (pentahydrate and dihydrate) for both D-glucose and D-fructose in aqueous solution depending on the solubility range evidenced by IR Spectroscopy.[28] In the dihydrate form, intramolecular hydrogen bonding between hydroxyls from D-glucose or D-fructose are likely present; the presence of NaCl may perturb these intramolecular hydrogen bonding favoring generation of pentahydrate forms and break the hydrogen bonds between adjacent water to hydroxyls and water from bulk water. All four types of forms including vicinal forms A1, A2 and solvated forms B1 and B2 would be converted into a proposed stable complex as shown in **Figure 5**. In this stable sugar-NaCl-water complex, two chloride anions bind with two water molecules adjacent to each hydroxyl (or acetyl amide) respectively in a non-selective manner; only one or two hydrated sodium cations will bind with sugars and most are very mobile. The three chloride ions per hydroxyl is consistence with our observance that molar ratio of NaCl to total hydroxyls (2.5 mmol) of monosaccharide was 4 : 1 when a stable sugar-water complex formed. And the staircase trend in correlation of ^{1}H NMR shifts (D-glucose/fructose) showed the formation of hydrogen (H_2O)-chloride bond was likely stepwise. Both sodium cations and chloride anions play an important role in perturbing

hydrogen bonds with bulk water in proposed hydrated forms (A1, A2, B1 and B2, **Figure 1**) and intramolecular hydrogen bonding of mono/disaccharides; however, chloride anions may contribute much more to stabilize the sugar-NaCl-water complexes and to the observed ^{1}H NMR shifts. The structure of complexes in NaCl solution is likely the same when they reached a stable status regardless of initial parameters of concentration, volume and temperature, this is of much importance for understanding of the well-known "NaCl effect". This proposed sugar-NaCl-water complexes is based on both J. M. Harvey'[2,3] and T. Suzuki's[4] findings that each hydroxyl group of D-glucose forms two hydrogen bonds with two water molecules (**Figure 1**), and our ^{1}H NMR evidences. Our proposed stable complexes are also consistent with the finding by J. D. Smith *et.al* that[27] changes of hydroxyl (from monosaccharides) vibrational spectrum is induced by the action of halide anion's electric fields on adjacent water molecule near to hydroxyl.

3.6 ^{1}H NMR Study of D-glucose in biological relevant NaCl solution (< 1 wt%).

^{1}H NMR shifts of D-glucose in 1.25 wt% showed clear downfield change on spectrum for all six marked peaks (△δ = 0.03 ppm); meanwhile an upfield change (△δ = -0.01 - -0.02 ppm) for all six peaks of fructose was observed under the same condition (**Figure 4** and supporting information). Such observance might be of much value to understanding of sugars in the human body via NMR analysis techniques[32], as well as for understanding of biological mechanism of Na-dependent cotransport of sugars like glucose and fructose. It is known that Na dependent glucose transport has an approximate half maximal Na concentration of around 50-70 mM for the Na dependent glucose cotransporter, whereas fructose is not transported in a Na dependent manner.[33,34] A further exploration on ^{1}H NMR shifts of D-glucose in biological relevant NaCl solution (100 mM and 140 mM) at room temperature was also performed; similar downfield changes were still observable (**Table 5**). This small but clear downfield change indicated glucose-NaCl-water complex might generate in biological system, meanwhile similar fructose-NaCl-water could not form in low concentration of NaCl solution (<1.25 wt%); such difference may explain some of the stereospecificity of this process. Further work with more sensitive ^{13}C NMR to verify such difference would be performed in the future.

Table 5. ^{1}H NMR shifts of D-glucose in biological relevant NaCl solution at room temperature.

Concentration (In NMR tube)	1 (△δ)	2 (△δ)	3 (△δ)	4 (△δ)	5 (△δ)	6 (△δ)
50 mM (100 mM, 0.58%)	0.003	0.003	0.003	0.008	0.005	0.003
70 mM (140 mM, 0.82%)	0.010	0.011	0.019	0.016	0.011	0.011

3.7 A proposed ideal NaCl usage.

Concerning frequent optimization on NaCl usage in literatures, we tentatively propose a calculation model recommending the best usage of NaCl for maximum perturbation of hydrogen bonding network of mono/disaccharides. It is also very relevant for reference in

depolymerization of polysaccharides including starch, chitin and cellulose. Recommended absolute amount of NaCl in solution is 3.5 – 4.0 equivalent to total hydroxyl of saccharides (including protons on amine moiety if glucosamine-based saccharides were used) based on observed maximum ^{1}H NMR shift forming stable sugar-NaCl-water complexes. This recommended ideal usage is consistent with optimized condition in recent reported literature[20]. For temperature, room temperature is usually sufficient for monosaccharides and oligosaccharides, which are soluble in water; however, typical increase of temperature was pretty necessary for cellulose depolymerization according to a recent report[22], stating that "NaCl effect" is only obvious when the temperature is above 210℃.

4.0 Conclusion

In summary, ^{1}H NMR evidences of stable sugar-NaCl-water complexes with monosaccharides and oligosaccharides at room temperature were obtained, and the staircase trend in correlation of ^{1}H NMR shifts (D-glucose/fructose) showed the formation of hydrogen (H_2O)-chloride bond was likely stepwise; this observance is of much importance to further understanding of prevailing "NaCl effect" at atom level. The shifting trends of all marked peaks correlation to concentration of NaCl solutions were very similar in all four monosaccharides; that indicated that pKa value has no observable effect on perturbation of hydrogen bonding and generation of new hydrogen bonding network was non-selective. However, anomeric effect did exist for some monosaccharides at relatively low concentration of NaCl solution. Although both sodium cations and chloride anions promote perturbation of hydrogen bonding network, chloride anions may play a key role in the stabilization of sugar-NaCl-water complexes and induction of ^{1}H NMR shifts. Based on the maximum of induced ^{1}H NMR shifts reaching stable sugar-NaCl-water complexes, a general recommended NaCl usage of 3.5 – 4.0 equivalent mole of hydroxyls (including amines or amides) on saccharides was proposed. We envisage more insights may be achieved when a full characterization of the stable sugar-NaCl-water complexes in aqueous media is available in the future.

Supporting Information.

Completed general information, all reaction procedures, ^{1}H NMR shifts summarized in tables, figures for shift trends of disaccharides/stachyose, reproducing note and all ^{1}H NMR spectra with marked peaks were all included in supporting information.

Author Contributions

Concept was designed by L. Gu; G. Zhu and H. Li carried out experiments and collected related data; L. Gu supervised this project and analyzed the data; Y. Li co-supervised

this project; the manuscript was written by L. Gu and all authors participated in revision. All authors have given approval to the final version of the manuscript.

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