Synthetic Hydrogen-Bonding Receptors for Biologically Essential Monosaccharides

Masahiko Inouye*

PRESTO, Japan Science and Technology Corporation
Department of Applied Materials Science
Osaka Prefecture University

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Abstract: Novel macrocyclic saccharide receptors that possess terpyridine skeletons as a hydrogen-bonding site are presented. On modification of the hydrogen-bonding sites and the macrocyclic bridges, the receptors showed selective bindings for ribofuranosides, deoxyribofuranosides, and glucopyranosides, respectively. The binding affinities of the receptors were very high, so that native monosaccharides were solubilized into non-polar solvents in the presence of the receptors. The hydrogen-bonding interactions and the binding modes between them were characterized by $^1H$ NMR spectroscopy.

1. Introduction

The hydrogen-bonding interaction is quite unique in terms of its vectorial character in solution, so that the hydrogen bonds play a leading role in most biological events. For example, complementary hydrogen bonds arise in a very specific fashion between the purine and pyrimidine bases of the two strands of double-helical DNA in order to define the duplex architecture and to regulate the genetic information. The directed hydrogen-bonding interactions are also seen in enzyme–substrate and in protein–saccharide complexes. In this connection, constructing synthetic models through employing hydrogen-bonding is important in supramolecular chemistry because it may not only serve as a basic concept to understand biological functions but also lead to the development of new pharmaceutical methodologies, new types of biorelevant materials, etc. Among the many artificial hydrogen-bonding models, however, only a few of them have been effective for the recognition of biological molecules with a three-dimensional complexity such as saccharides. This is possibly because of the great difficulty in the molecular design and the synthesis of artificial models having multiple hydrogen-bonding sites that are arranged at one's own will.

One goal of our research is to create “intelligent” supramolecules and supramolecular systems, in which several conjugated functions are regulated by molecular recognition events. In 1990, we introduced conceptually new artificial receptors, crowned spiropyrans. The isomerization of the spiropyrans to the colored merocyanines was induced by recognition of alkali-metal cations (Figure 1). In our continuous studies, we are confronted with difficulties in the design and synthesis of the host architectures capable of recognizing three-dimensionally complexed saccharides via hydrogen bonds. Our project of this saccharide recognition is not yet complete, but is already bearing fruitful results. Thus, the present account deals with the performance of artificial receptors that have been designed and synthesized by us for biologically essential monosaccharides.

2. General Considerations for Hydrogen-Bonding Interactions in Aprotic Solvents

Naturally occurring enzymes have three-dimensional hydrophobic binding pockets, in which various hydrogen bonds between the enzymes and substrates are seen. The pocket is constructed with hydrophobic amino acid residues that prevent water from invading into the cavity, so that specific hydrogen-bonding is possible. Most artificial hydrogen-bonding systems, however, have been investigated in aprotic solvents such as CHCl$_3$ and CH$_2$Cl$_2$ because synthetic molecules must be composed of much simpler skeletons. For amide–amide type hydrogen bonds, the strength was assessed by systematic comparison of experimental -$\Delta$G$_{298}$ for complexation with the number of hydrogen bonds participating in the interaction. The data revealed substantially constant increment, which is 5±1 kJ/mol per hydrogen bond in CHCl$_3$. On the other hand, hydrogen–bonding interactions of OH groups of such as saccharides remain to be studied more thoroughly. Indeed, artificial hydrogen-bonding receptors so far synthesized exhibited rather small binding affinities for saccharides, i.e., stabilization energy of no more than only 3–4 kJ/mol per hydrogen bond. Are the values intrinsic, or simply not tuned up enough?


As a starting point for the design of saccharide receptors, we chose a 2,2':6',2''-terpyridine skeleton with ethynediyl...
spacers as the hydrogen-bonding motif to the saccharide-OH groups. The decision was based on (i) the utilization of stronger O-H···N hydrogen bonds rather than O-H···O ones, (ii) consideration for the arrangement of plural saccharide-OH groups, and (iii) incorporation of moderate rigidity and flexibility into the hydrogen-bonding sites on the receptors. The sp-carbon spacers will allow flexibility for partial rotation about the pyridine-ethynediyl bonds, maintaining linearity along the pyridine-pyridine axis, which would result in optimum for the expected hydrogen bonds to various saccharides (Figure 2). In the terpyridine, the most predominant conformation is anticipated to be the anti form, in which each pyridine nitrogen atom is located on opposite sides of the ethynediyl bonds in order to cancel the dipoles (Figure 3). Indeed, ab-initio calculation displayed that the anti form is more stable than the desired syn form by ca. 10 kJ/mol. The loss in the energy resulting from the rotation about the pyridine-pyridine axis in order to interact with saccharides may successfully be compensated by pre-organization of the syn-terpyridine conformation into macrocyclic structures during the synthesis.

3.1 Ribofuranoside Receptors

One of the most important monosaccharides is ribose, of which derivatives are present in all living systems as a component of RNA and ATP. Although ribose can adopt both furanoside (five-membered ring) and pyranoside (six-membered ring) forms, the most important derivatives are furanosides with a β-glycoside linkage (Figure 4). Preliminary studies on the interactions were made by use of amide-substituted terpyridine 1 with CDC13-soluble methyl β-ribofuranoside (2). The 1H NMR spectra in CDC13 revealed that all hydrogen acceptors of the three pyridine nitrogens and at least one donor of the two amide-NH groups in 1 take part in the complexation with the ribofuranoside 2 (Figure 5). The 1:1 stoichiometry of the complexation was confirmed by the continuous variation (Job) plots, and Benesi-Hildebrand analysis gave the association constant of $K_a = 30 \text{ M}^{-1}$. The Van't Hoff equation (-ΔG° = RTln$K_a$) showed a free energy change (-ΔG°) for the complex formation of 8.4 kJ/mol. The value was lower than that predicted by our postulated factor, namely, the complex formation of at least four hydrogen bonds and, therefore, maximum 2.1 kJ/mol per hydrogen bond. This result may be explained by the free rotation about the pyridine–pyridine axis of 1 (vide supra). Thus, we designed diphenylmethane-bridged polypyridine-macroyclic receptors 3. The bridge was selected so that the cavity formed was just fitted for incorporating the furanose structure. This would hopefully result in an additional attractive force such as van der Waals interaction (Figure 6).

The macrocyclic receptors 3 were synthesized from two components, dianinoterpyridines 4 and dicarboxylic acid derivatives 5, by Mukaiyama's macrocyclization by use of 2-chloropyridinium salt in the final stage. The cyclization proceeded in only poor yields (10%), while other condensing agents were completely unsuccessful in the cyclization. The diaminoterpyridine derivatives 4, the hydrogen-bonding moieties, were derived from the central pyridine ring and the two side rings by sequential Sonogashira reactions. The key step in the preparation of the dicarboxylic acid derivatives 5, the bridges for the macrocyclic structure, was Heck C-C bond formation between the triflates of bis-phenols and acrylic esters (Figure 6). The binding affinity of the macrocyclic receptors 3 for the ribofuranoside 2 did increase. Thus, the association constant for 3a to 2 was 2400 M$^{-1}$; -ΔG° = 19.3 kJ/mol (Figure 7). Noteworthy is the fact that the increased binding affinity compared with that for the acyclic 1 (-ΔG° = 10.9 kJ/mol) was in rough agreement with the energy compensation (~10 kJ/mol; vide supra) resulting from the inhibition of the free-rotation about the pyridine–pyridine axis in 1. The binding force was estimated to be 4.8 or 3.9 kJ/mol per hydrogen bond, when we postulated that the complex was formed with...
four or five hydrogen bonds, respectively, and that no other intermolecular interaction was present. Increasing the electron density of the pyridine nitrogen, a definite increase in the binding affinity was anticipated due mainly to enthalpic factors. Indeed, alkoxy-substitution at the 4' position of the central pyridine ring showed further increment of the association constants. Thus, 3b revealed Ka value of 5200 M⁻¹, the highest value recorded for the ribofuranoside receptors thus far synthesized.

The binding mode was speculated on the basis of the complexation-induced shifts for the receptor and the ribofuranoside signals in the ¹H NMR spectra (2 and 3a: 10 mM in CDCl₃) (Figure 7). Large downfield shifts were observed for the 2-C (2.55 ppm) and 3-C (2.50 ppm) OH protons of 2 and the NH proton of 3a (1.75 ppm), reflecting the formation of a multipoint hydrogen-bonded complex. A relatively small downfield shift of the 5-C OH proton (0.42 ppm) suggested that the direction of the primary OH group was not enough to take advantage of the full potential of the hydrogen-bonding site of 3a. On the other hand, the OCH₃ (ca. 0.3 ppm) and CH₂OH (ca. 0.4 and 0.2 ppm) protons of 2 were shifted upfield. The upfield shifts illustrated that the hydrophobic moieties (aglycon, 5-C, and furan-O) of 2 were placed on the diphenylmethane-bridge that is perpendicular to the terpyridine hydrogen-bonding site.

Native monosaccharides are sparingly soluble in aprotic solvents. Thus, their extraction into such solvents containing 3b revealed further information regarding the binding ability of the receptors. Selective extraction of ribose into CDCl₃ was observed: the extractability, or affinity to 3b of various monosaccharides decreased in the following order: ribose→deoxyribose→lyxose→xylose→fructose→arabinose→glucose→mannose→galactose. The order disclosed that the extractability of ribose is higher than that of more lipophilic deoxyribose (see also 3.2), and that smaller pentoses generally are better extracted than hexoses. These results indicated that the binding affinities of the receptors for saccharides were governed mainly by hydrogen-bonding interactions and by complementarity between them in size and shape at the molecular level.

3.2 Deoxyribofuranoside Receptors

Another important pentose is deoxyribose, a component monosaccharide of DNA. In the deoxyribofuranoside form, however, only two hydroxyl residues are expected to take part in the hydrogen-bonding interactions. Furthermore, there may be a disadvantageous electrostatic repulsion between the lone electron pairs of the pyridine nitrogens in the ribofuranoside receptors 3 and the 3-OH groups of deoxyribofuranosides (Figure 8). In the case of the ribofuranoside 2, the intramolecular hydrogen bond between 2-OH and 3-OH could offset the repulsion (Figure 7a). We thought that this difficulty could be overcome by replacing the central pyridine ring of the receptor by a pyridone ring: the pyridone–NH protons will change the repulsion into an attractive hydrogen bond. For this modification, the structures of receptors 6 are obvious candidates, in which a novel hydrogen-bonding donor(amide)–acceptor(pyridine)–donor(pyridone)–accept-
The pyridine-donor(amide) arrangement is convergently incorporated in the macrocyclic structures (Figure 9). The new polypyridine-macrocyclic receptors 6 were prepared from 7 and 8 in a manner similar to that described for the synthesis of the ribofuranoside receptors 3. Final deprotection of MOM groups in the central pyridine ring afforded the desired pyridine-pyridone-pyridine type receptors 6 (Figure 9).

To evaluate the recognition abilities of the receptors for deoxyribofuranoside in aprotic solvents, 3-n-octylthymidine (9) was chosen (Figure 10). The n-octyl substituent makes the deoxyfuranoside soluble in such solvents and prevents the nucleobase residue of 9 from interacting with the hydrogen-bonding motif of the receptors. Hence, one can assess a net interaction between the hydroxyl groups of 9 and the receptors. 1H NMR spectra afforded useful information on the hydrogen-bonded complex (6a and 9: 2.5 mM in CDCl3). Large downfield shifts were seen not only for the 3-C OH (3.60 ppm) but also for the primary 5-C OH (2.95 ppm) protons in contrast to that for the combination of the ribofuranoside 2 and the ribofuranoside receptor 3a (see 3.1). The hydrogen-bonding interaction of the newly developed central pyridone was confirmed in the downfield shift for the NH proton (2.50 ppm) as well as the amide-NH protons (1.02 ppm). These shifts of 6a and 9 suggested the recognition mode, depicted in Figure 10.

A surprisingly large association constant of 19000 M⁻¹ was obtained for the receptor 6a to the deoxyribofuranoside 9 and, thus, the free energy change (ΔG°₂⁹₈) was 24.4 kJ/mol. The observed ΔG°₂⁹₈ of the receptor is a remarkably high value for the substrate having only two hydroxyl groups. The value implies the crucial participation of at least four hydrogen bonds of ca. 6 kJ/mol per hydrogen bond. The recognition mode of the receptor 6a may take advantage of the full potential of the two hydroxyl groups of the deoxyribofuranoside 9 for hydrogen-bonding in agreement with the complexation-induced large downfield shifts of their protons. Another notable point is that the deoxyribofuranoside receptor 6b did exhibit selectivity for deoxyribose in the extraction experiment for various native monosaccharides; the extractability of deoxyribose vs ribose is in reverse to that for the ribofuranoside receptor 3b (see 3.1).

The polypyridine-macrocyclic receptors possessing a diphenylmethane derivative as a tight bridge were found to be effective for the recognition of representative pentofuranosides, ribofuranosides and deoxyribofuranosides. The hydrogen-bonding interactions of the receptors are strong enough to solubilize native monosaccharides into nonpolar solvents. Furthermore, the selectivity for each saccharide could be changed by choosing the arrangement of the hydrogen-bonding motifs. 7a-c

### 3.3 Glucopyranoside Receptors

We extended this approach to hexose, especially glucose, which is the most important monosaccharide composing starch and cellulose. 19 In the stable conformation of β-glucose...
copyranosides, the three secondary OH groups (2-C, 3-C, and 4-C) are all equatorial, so pseudo-coplanarity of the hydrogen-bonding site for the terpyridine skeleton will be adequate. The cavity of the diphenylmethane-bridged receptors 3 and 6, however, is just fitted for incorporating the furanose structure, but too small to interact with the pyranose structure. Thus, we designed new polypyridine-macrocyclic receptors 10 possessing a large cavity by replacement of the short diphenylmethane bridges of 3 and 6 by a relatively flexible polyoxyethylene chain (Figure 11).

The synthetic strategy for the glucopyranoside receptors 10 was different from those for 3 and 6 because no cyclization proceeded between the diamonoterpyridines 4 with polyoxyethylene-bridged bis(benzoic acid) derivative 11. Success was achieved by base-mediated macrocyclization of the terpyridine-derived bifunctional phenols 12 and tetraethylene glycol di-p-tosylate (13) in the final step of 25% yield (Figure 11). The substantially high yield for the cyclization might result from the template effect of the K⁺ cation of the base to the polyoxyethylene chain. Unfortunately, in all cyclizations described in this account, the presence of the corresponding monosaccharides which are to be recognized by the products, had no influence on the yields.

Treatment of a CDCl₃ solution of 10a (2.5 mM) with 1 equiv of n-octyl β-glucopyranoside (14) resulted in large downfield shifts of the 2-C (2.95 ppm), 3-C (2.25 ppm), and 4-C (2.75 ppm) OH protons, while the shift of the primary 6-C OH proton (0.45 ppm) was rather small. The amide-NH proton of 10a was also shifted downfield (0.90 ppm); on the other hand, the OCH₂CH₂-protons of the alkyl glycoside moieties of 14 were shifted upfield (0.30 and 0.30 ppm) (Figure 12). The downfield shifts reflect the formation of a multipoint hydrogen-bonded complex with a weak participation only for 6-C OH, and the upfield shifts may be attributed to the influence of the diamagnetic anisotropy of the benzene ring of 10a that is perpendicular to the hydrogen-bonding site. On the basis of these observations, a possible recognition mode for the complex is given in Figure 12.

The association constant of 10 to 14 was close to that predicted by the presence of the strong four hydrogen bonds, i.e., 5 kJ/mol × 4 ≈ 20 kJ/mol. Thus, the parent 10a displayed $K_a$ of 5600 M⁻¹ ($\Delta G_{298} = 21.4$ kJ/mol), and the alkoxy-substitution at the 4' position of the central pyridine ring in 10b showed an increment of the association constant: $K_a = 7300$ M⁻¹; $\Delta G_{298} = 22.0$ kJ/mol. On the other hand, rather weak binding was seen between 10a and the epimeric galactopyranoside 15, so 10a showed $K_a$ of 1400 M⁻¹ ($\Delta G_{298} = 17.9$ kJ/mol). The three glucopyranoside-OH groups (2-C, 3-C, and 4-C) of 14 are all equatorial, but not in the case of the three OH groups of 15. Although the macrocyclic structure of 10 can allow flexibility for partial rotation about the pyridine-pyridine axes to some extent, the stable conformation of 10 was found to have the pseudo-coplanarity of the terpyridine moiety. The three OH groups of 14 are suitable for attaining the multipoint hydrogen-bonding to 10 of its relaxed conformation, but not in the case of the three OH groups of 15. This specu...
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4. Conclusion and Future Perspective

Saccharides, which are sometimes called sugars and carbohydrates, have been found to play an important role in the intercellular recognition in connection with carcinogenesis and immune response. A limited number of artificial models, however, has been reported for saccharide recognition compared with those for nucleic acid and amino acid derivatives. We have developed pyrrolidine-macro cyclic receptors and showed the versatility of the terpyridine hydrogen-bonding motif for saccharide recognition. Recent results obtained in other laboratories also demonstrated the successful bindings of particular saccharides with significant recognition strength compared with those for nucleic acid and amino acid derivatives. Easy enough to be similar to those for other biologically important molecules, although many challenging projects, including oligosaccharide recognition and applications to saccharide-reactions, remain to be explored.

References and Notes


19) Recently, Davis and Wareham reported a tricyclic polycyclide receptor that shows a remarkable level of affinity for β-glucopyranoside: Davis, A. P.; Wareham, R. S. Angew. Chem., Int. Ed. 1998, 37, 2270.


Masahiko Inouye was born in Ehime Prefecture, Japan in 1962 and received his B.Sc. (1984) and Ph.D. degree (1990) from Ehime University and Kyoto University, respectively. He joined Osaka Prefecture University, as an Assistant Professor (1989) and became Associate Professor (1995) there. During 1996-1999, he was also a researcher of PRESTO (Precursory Research for Embryonic Science and Technology: a government-sponsored project under the Japan Science and Technology Corporation (JST)). In 1995, he received the Chemical Society of Japan Award for Young Chemists. His research interests are in the areas of synthetic organic chemistry and bioorganic chemistry.