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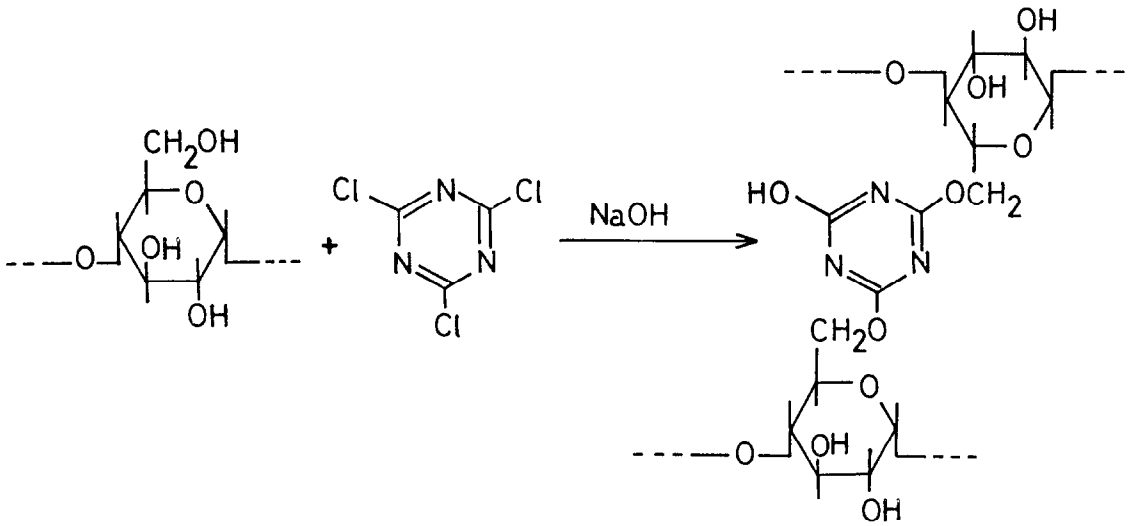
TEMPLATE SYNTHESIS FROM STARCH AS AN APPROACH TO TAILOR-MADE "CYCLODEXTRIN"

Seiji SHINKAI,* Mitsukuni YAMADA, Takaaki SONE, and Osamu MANABE
Department of Industrial Chemistry, Faculty of
Engineering, Nagasaki University, Nagasaki 852, Japan

The crosslink of water-soluble starch with cyanuric chloride in a two phase system gave a template resin with memory for its origin. This is a novel approach to tailor-made "cyclodextrin".

The cyclodextrins are cyclic oligomers made up of glucose units with a simple doughnut shape and presenting a hydrophobic cavity. It is well-known that in the past decade the model studies of enzymic functions by use of cyclodextrins have been continued extensively and successfully.[1-3] The extensive interests in cyclodextrins stem from their excellent abilities not only to recognize the molecular shape but also to catalyze the organic reactions according to enzyme-like saturation kinetics. One may say, therefore, that cyclodextrins are well-constructed miniatures of enzymes[2] and frequently provide clues to understand the enzymic reaction processes in more simplified systems. Very unfortunately, however, there exist only three cyclodextrins, α, β, and γ made up, respectively, of 6, 7, and 8 glucose units. It occurred to us that if one can freely synthesize recognizable host cavities fitting to the shape of guest molecules, it would lead to a new extension of cyclodetrixn chemistry. We consider that the concept recently developed as "memory polymers" synthesized for their origins[4-9] may be useful. The principle is based on the crosslink of synthetic linear polymers in the presence of adsorbed templates. We here report a template synthesis from water-soluble starch and cyanuric chloride as an approach to tailor-made "cyclodextrin". We have found that the crosslinked starch exhibits remarkably high memory for the dye appended as template.



The typical crosslinking method to synthesize "template resin" is as follows. Water-soluble starch (2.0 g, 1.23 x 10^{-2} unit mole) and Methylene Blue (MB)(0.20 g, 6.3 x 10^{-4} mole) as template were dissolved in 5 ml of water. The aqueous solution was mixed with 4 ml of a benzene solution containing 0.40 g (2.2 x 10^{-3} mole) of cyanuric chloride. While vigorously stirring the mixture, aqueous NaOH(20%) solution(1.7 ml) was added at one time. The solidification of the aqueous phase began immediately. After one day, the gel was shattered into small pieces and washed with 50% aqueous pyridine in a Soxhlet extractor until the color of MB disappeared. After washing with methanol the resin was dried in vacuo. Elemental analysis of the template resin indicated that the content of the cyanuryl unit(=cyanuryl unit/(cyanuryl unit + glucose unit)) is 16.7 mol% that is almost equal to the feed ratio(15.2 mol%) and that sulfur atom arising from MB is not detected. The reference resin was prepared according to the same procedure in the absence of MB,[10] the content of the cyanuryl unit being 18.5 mol%. Based on the acid-base titration, we found that the template resin and the reference resin contain 17.0 mol% and 19.3 mol% of the acidic group, respectively. Since these values are in good accord with the contents of the cyanuryl unit, one may consider that the third chloride of cyanuric chloride, which is most unreactive, is remained unreacted and thus hydrolyzed to the acidic hydroxyl group.

50 mg of the resin was swelled and equilibrated in 10 ml of an aqueous solution containing MB(4.73 x 10^{-4} M) at 30°C for one day. The blue filtrate obtained from the reference resin solution contained 1.27 x 10^{-4} M of MB, indicating that 16.8% of MB is not adsorbed. Surprisingly, the filtrate obtained from the template resin solution was almost colorless and contained

Table 1. Binding constant(K, M^{-1}) and number of monomeric units to construct a binding site(1/n)[a]

Resin	Cosolute	Condition	K (M^{-1})	1/n
Template	MB	water(pH~7)	4.66×10^4	9.5
Template	MB	5 mM phosphate(pH 7.04)	3.06×10^3	2.9
Template	MG[b]	5 mM phosphate(pH 7.04)	2.48×10^4	38
Reference	MB	water(pH~7)	1.94×10^4	58
Reference	MB	5 mM phosphate(pH 7.04)	2.00×10^3	13
Reference	MG[b]	5 mM phosphate(pH 7.04)	1.34×10^4	120

[a] 30°C, one day, 10 mg resin in 2 ml of (0.1-10) x 10^{-3} M MB solutions.

[b] Malachite Green

only 4.5×10^{-7} M of MB. The value implies that 99.9% of MB is adsorbed to the template resin! MB in aqueous solution has two absorption maxima at 608 nm and 666 nm with $A_{608}/A_{666} = 0.52$. When MB is included in a hydrophobic cavity of γ-cyclodextrin, A_{608}/A_{666} becomes about 1.3.[11] The wet resins adsorbing MB were subjected to the spectral measurement with a dual-wavelength spectrophotometer. The template resin and the reference resin gave 1.39 and 1.42, respectively, indicating MB to be bound to considerably hydrophobic sites but no difference in the hydrophobicity between two resins.

All the adsorption equilibrium data were evaluated by the equation of the Langmuir isotherm as suggested by Klotz et al.[12]: $1/r = 1/nKa + 1/n$, where r is the number of mole of cosolute bound per base mole(i.e., cyanuryl unit + glucose unit) of polymer, n is the number of binding sites per base mole, a is the molar concentration of free cosolute at equilibrium, and K is the binding constant. The plots of 1/r vs. 1/a gave good linear relationships, in most cases, with correlation coefficients better than 0.99. K and 1/n are summarized in Table 1. The 1/n denotes the number of monomeric units to construct one binding site. It is known that β- and γ-cyclodextrins form the 1:1 and 2:1 complexes with MB, respectively.[11] One may thus presume that seven to eight

glucose units are necessitated to construct a binding cavity. Since the 1/n for the template resin in aqueous solution is 9.5, most of the glucose units are used to construct the binding sites. Under the identical conditions, the reference resin gave 1/n = 58 and the binding constant is smaller by a factor of 2.4 than that for the template resin. Thus, the main difference between two resins is not the binding constant but the number of the binding site. In phosphate buffer, the template resin gave the abnormal 1/n(2.9) suggesting the multiple binding of MB. However, the 1/n is still significantly smaller and the K is greater than those for the reference resin. The similar template effect was observed for the adsorption of Malachite Green(MG). We noticed, however, that the template effect is significantly reduced in 0.005N HCl solution. We thus consider that the glucose cavity and the anionic charge of the cyanuryl unit cooperatively function to bind the cationic cosolute.

In conclusion, the present results indicate that the croslink of water-soluble starch in a two phase system provides a potential method for tailor-made "cyclodextrins". We believe that tailor-made "cyclodextrins" thus synthesized are applicable more precisely and successfully to many novel phenomena attained in past by use of cylodextrins.

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