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# Macromolecular Complexes of Chitosan

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## I. INTRODUCTION

### A. Macromolecular Complexes

Macromolecular complexes are molecular aggregates of two or more complementary polymers, which have definite composition and characteristics and arise from intermolecular interactions, such as Coulomb forces, hydrogen-bonding forces, charge-transfer forces, and van der Waals forces. The complexes can be divided into the following four classes according to the nature of their interactions [1]: polyelectrolyte complexes, hydrogen-bonding complexes, charge-transfer complexes, and stereocomplexes.

It is rare that these intermolecular interactions take place singly, and hydrophobic interactions are also significant factors in aqueous media. Although these secondary binding forces, among which Coulomb forces are strongest, are weaker than primary binding forces, they play very important roles in the biological systems (e.g., molecular recognition, accumulation and interpretation of genetic information, and formation of higher-order structure of proteins).

Biological systems comprise different kinds of macromolecules, such as nucleic acids, enzymes, proteins, and polysaccharides, and most of them have definite structure and specific functions. Antigen–antibody reactions and enzymic reactions, for example, are very specific. In these cases, intermacromolecular interactions play important roles. Many bioreactions proceed via formation of macromolecular complexes in the beginning of the reaction, and the concerted interactions are included. As a simplified model of these bioreactions, it is rational to investigate the macromolecular complexes. The majority of researches on macromolecular complexes have been directed toward a better understanding of biological systems and of structure and properties of functional units. However, macromolecular complexes have recently found their way into practical applications, in particular, as biomaterials.

### B. Structure and Properties of Chitosan

Chitin, poly[β(1→4)-2-acetoamido-2-deoxy-D-glucopyranose], is one of the most abundant natural polysaccharides and is present in crustacea, insects, fungi, and yeasts. The total annual global estimates of accessible chitin amount to $1 \times 10^9$ tons [2]. It is obtained primarily as a by-product of the seafood industry. Deacetylation of chitin by alkali readily affords chitosan (Ch), poly[β(1→4)-2-amino-2-deoxy-D-glucopyranose]. Ch is also found in various fungi. However, the molecular structure of Ch is believed to be a copolymer of *N*-acetyl-glucosamine and glucosamine; usually the glucosamine content is more than 90%. It is known that 50% *N*-deacetylated chitin (50% *N*-acetylated Ch) is water soluble [3]. Kubota and Eguchi showed that the water solubility of half *N*-acetylated Ch increased with decreasing the molecular weight, as shown in Fig. 1 [4]. In addition, the solubility of half *N*-acetylated Ch with the molecular weight lower than 10,000 is rather high even in aqueous dimethylacetamide and aqueous dimethyl sulfoxide (DMSO) [5].

Because Ch has an amino group in the repeating unit, it affords ammonium group in aqueous acidic media. Owing to its cationic nature, Ch spontaneously forms water-insoluble complexes with anionic polyelectrolytes. Therefore, Ch has been used mainly as a flocculant for the treatment of wastewater. However, it has recently been used in biomedical and pharmaceutical fields because of its favorable properties of good biocompatibility, low toxicity, and biodegradability.

The intrinsic viscosity [$\eta$] of Ch depends on the pH and the ionic strength, as shown in Fig. 2 [6]. If the pH of the solution is increased, the intermolecular and intramolecular electrostatic repulsions between cationic charges are reduced. This allows the Ch chains to come closer and thus lowers the hydrodynamic volume of the Ch molecules. This effect may enhance the interchain and intrachain hydrogen bonding. Similarly, as the ionic strength increases, [$\eta$]
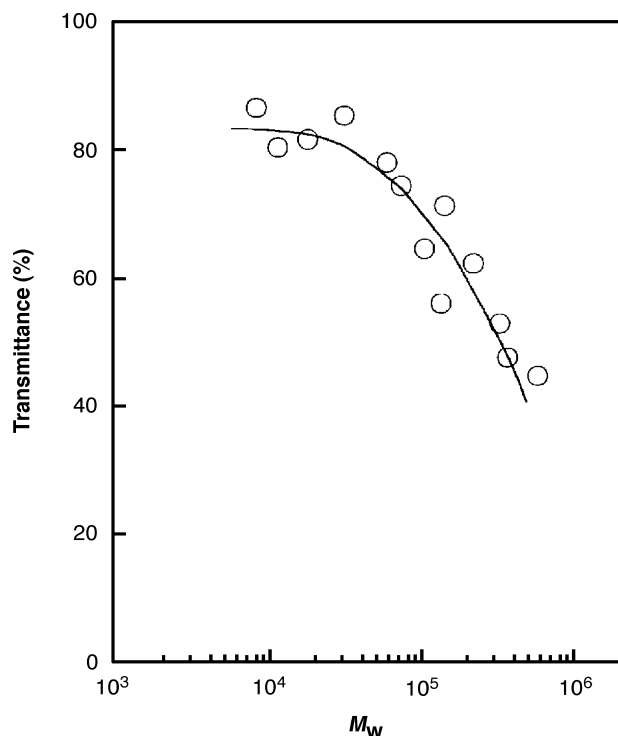
**Figure 1** Dependence of water solubility of half *N*-acetylated Ch on the molecular weight. (From Ref. 4.)

decreases due to the shielding effect of the counterions. Although Cölfen et al. suggested that the hydrodynamic behavior of Ch was consistent with wormlike chain model [7], the Ch molecule is rather stiff. The flexibility of some polysaccharides has been investigated in terms of a "stiffness parameter" *B* (i.e., the dependence of the intrinsic viscosity on the ionic strength). The *B* values for a variety of polymers are compiled in Table 1. The *B* value of Ch was estimated as 0.08 [8]. Terbojevich et al. obtained *B* values from 0.043 to 0.091 for Ch with degrees of acetylation ranging from 52.2% to 12.1% [9]. These values are essentially the same as those for carboxymethyl cellulose and hyaluronic acid, greater than DNA, and less than polyacrylate. These solution properties of Ch greatly affect the formation of macromolecular complexes.

## II. FORMATION OF MACROMOLECULAR COMPLEXES OF CHITOSAN

The formation of macromolecular complexes in solution, depending on the intensity of polymer–polymer and polymer–solvent interactions, leads to the separation of complexes as a solid or liquid phase. Because Ch is a cationic polyelectrolyte, most studies on the macromolecular complexes of Ch are concerned with polyelectrolyte complexes (PECs). However, only a few articles dealt with the polyionic interaction between polysaccharides, until Kikuchi
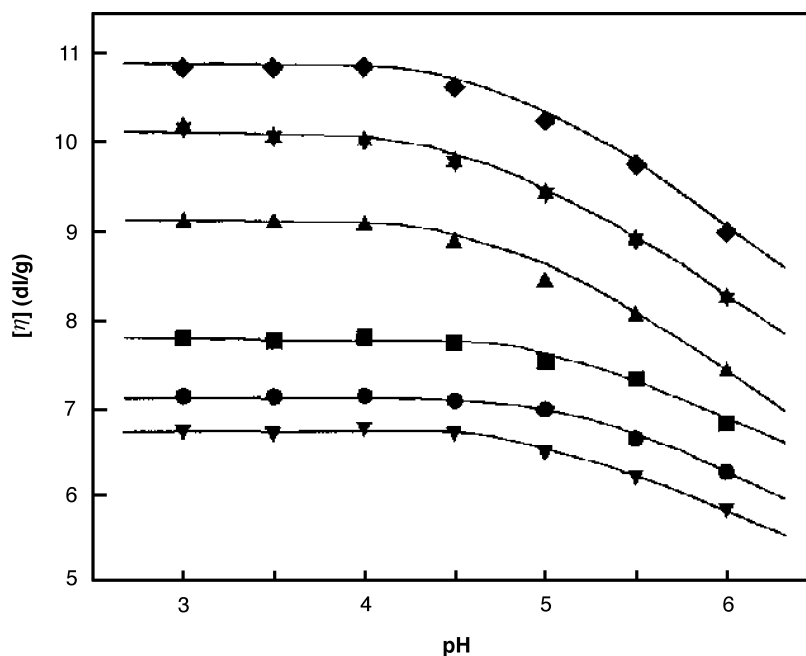


**Figure 2** Effects of pH and ionic strength on intrinsic viscosity [$\eta$] of Ch solution: ♦, 0.050 mol/L; ✦, 0.075 mol/L; ▲, 0.100 mol/L; ■, 0.200 mol/L; ●, 0.300 mol/L; ▼, 0.500 mol/L NaCl. (From Ref. 6.)

**Table 1** Stiffness Parameter, *B*, for Ch and Some Polymers

| Polymer | Degree of acetylation (%) | *B* |
|---|---|---|
| Ch | 12.1 | 0.091 |
| | 20.1 | 0.060 |
| | 42.1 | 0.061 |
| | 52.2 | 0.043 |
| Polyphosphate | — | 0.44 |
| Polyacrylate | — | 0.23 |
| Amylose xanthate | — | 0.22 |
| Carboxymethylamylose | — | 0.20 |
| Carboxymethylcellulose | — | 0.065 |
| Hyaluronic acid | — | 0.07 |
| Sodium pectinate | — | 0.044 |
| DNA | — | 0.006 |

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first reported the PEC containing Ch as a component [10]. Several reviews on PEC have been published so far [11,12]. The formation of PEC is governed not only by the nature of the individual polyelectrolyte components, such as characteristics of polyions (strong or weak), position of ionic sites, charge density, molecular weight, flexibility, functional group structure, hydrophilicity and hydrophobicity, and stereoregularity, but also by the reaction conditions, such as pH, ionic strength, polymer concentration, mixing ratio, and temperature. This, therefore, may lead to a diversity of physical and chemical properties of the complexes. PECs arise due to interactions between oppositely charged polymers and can be additionally stabilized through short-range interactions such as hydrophobic interactions and hydrogen bonds.

## A. Thermodynamics and Stoichiometry of Complex Formation

The complexation reaction of macromolecules is significantly different from that of low-molecular weight molecules due to the polymer effects by which a relatively stable complex occurs, although the force of each bound pair is small. This implies that the apparent equilibrium constant of the polymer–polymer complex is extremely large and the reaction is apparently irreversible. The free-energy change $\Delta G^o$ for macromolecular complexation is a function of the degree of polymerization, and the equilibrium constant abruptly increases at the critical chain length [13]. Pérez-Gramatges et al. revealed that complexation of Ch with poly(acrylic acid) (PAA) proceeded cooperatively and the larger degree of conversion, $\theta$, was obtained for the higher-molecular weight Ch [14]. The relationship between the stability constant, *K*, and $\theta$ (Fig. 3) indicates that this complex consists of a long sequence of bound pairs of repeating units. The slope of this curve shows the influence of neighboring functional groups on their reactivity.

Kanbayashi and Arai determined thermodynamic parameters for PEC formation in the methyl glycol Ch (MGC)–carboxymethyl cellulose (CMC) system [15]. The formation of PEC gained a large negative $\Delta G^o$, but the factors were dependent on the charge density of CMC. The PEC formation using CMC with low charge density showed a marked exothermic tendency and was classified as an enthalpy-dominating reaction. On the other hand, the reaction using CMC with high charge density showed a smaller exothermic tendency and gained large positive
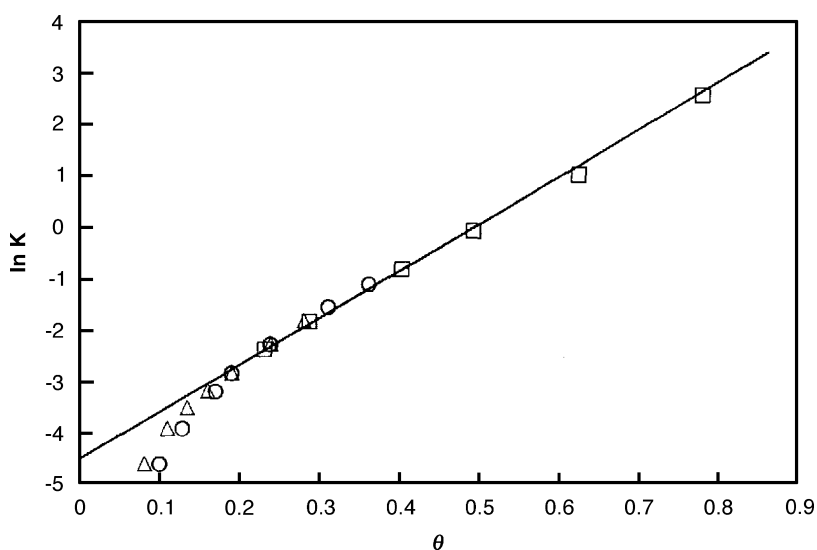


**Figure 3** Linear relationship between the stability constant, *K*, and the degree of conversion $\theta$ for Ch–PAA complexes: $\triangle$, $M_v = 8.5 \times 10^4$; $\bigcirc$, $M_v = 1.1 \times 10^5$; $\square$, $M_v = 2.3 \times 10^5$. (From Ref. 14.) Copyright 1996 Springer-Verlag.

entropy, indicating an entropy-dominating type. They also investigated the dependence of thermodynamic parameters on ionic strength using the MGC–partially hydrolyzed poly(acrylamide) system [16]. The degree of complexation did not change much with the ionic strength (0.001–0.2), and a gain of a large negative $\Delta G^o$ was accompanied by the PEC formation. However, $\Delta H^o$ became a large positive value with increasing ionic strength. Therefore, the higher ionic strength suggested the entropy-dominating-type PEC formation reaction. A much higher ionic strength affects PEC formation; for example, when Ch and $\kappa$-carrageenan (Car) were mixed in the presence of 5.7% NaCl at pH 2.8 on a boiling water bath, such a phase separation did not occur, but a viscous and macroscopically homogeneous PEC mixture was obtained [17]. The presence of $Na^+$ and $Cl^-$ reduces the electrostatic attraction between oppositely charged polyelectrolytes. This mixture gelled as its temperature or ionic strength decreased.

In general, the composition of the reaction mixture, $Z$, is unity at the equivalence point, suggesting a 1:1 stoichiometry for the complex, no matter what order of mixing is chosen. However, $\theta$ varied with $Z$ in the case of Ch–polygalacturonate (PGal) complex [18]. It fell from unity to 0.8 as $Z$ increased from 0.2 to 0.5 and then rose again up to 0.85 for $Z = 1$. If the complexation reaction is terminated before a 1:1 stoichiometry of charge neutralization is reached, water-soluble complexes are possible. In addition, when the PEC involves two polyelectrolytes of different molecular size, soluble nonstoichiometric PEC can be formed. In such a complex, the larger polymer chain behaves as a host macromolecule to the shorter one, and the host polymer should be a strong polyelectrolyte. For weak polyelectrolytes, not all of the repeating units need to have a 1:1 stoichiometry.

## B. Complexes with Polysaccharides

The interaction between oppositely charged polyelectrolytes involving weak polyacid and/or weak polybase is affected by the solution pH because of the change in the degree of dissociation. In this sense, it is of great interest to examine the properties of complexes involving weak polyelectrolytes under various pH conditions. When Ch, a weak polybase, is reacted with a weak polyacid, the insoluble complex formation occurs only in the narrow pH range. Argüelles-Monal et al. reported the complex formation reaction of the Ch–CMC system [19]. At pH 3.6, the PEC was rich in CMC, whereas at pH 4.8 the excess component was Ch. At pH 4, the yield of PEC increased with the addition of one polyelectrolyte solution to the other, and the maximum yield corresponded to the ratio [CMC]/[Ch] = 1. Beyond this point, the yield remained constant, and the composition of the PEC obtained showed the stoichiometry. Similar results were obtained in the case using PAA, a synthetic polymer, as a polyacid [20,21]. The mixing ratio Ch/(Ch + PAA) for maximum insoluble complex formation, $R_{max}$, increased with increasing solution pH. Interestingly, at the initial pH = 6, the supernatant pH of the reaction mixture increased as the complex was formed. At the initial pH = 3, the opposite behavior

was observed. As shown in Fig. 4, $pK_a$ of Ch increased as the addition of the polyanion increased, suggesting that the charged carboxylate group induced the ionization of the amino group of Ch [22]. Therefore, the supernatant pH increased at pH 6 as the yield of the complex increased:

$$-NH_2 + {}^-OOC- + H^+ \rightarrow -NH_3^{+\,-}OOC-$$

The supernatant pH decreased at pH 3 as the yield of complex increased:

$$-NH_3^+ + HOOC- \rightarrow -NH_3^+\,{}^-OOC- + H^+$$
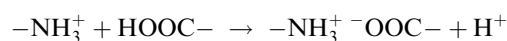
However, changes in the supernatant pH by complexation was dependent on the type of polyanions [23]. For Ch–Car, Ch–alginate (Alg), and Ch–pectin (Pec) complexes, no significant pH change occurred at the initial pH = 3. At pH 4.5, there was a slight increase in pH as a result of complexation of Ch with Alg and Pec, and a more significant increase with Car. At pH 5.4, there was an increase in pH analogous to that at pH 6 for the Ch–PAA system. However, unlike PAA, a maximum pH change was maintained in the wide mixing ratio from 0.2 to 0.4. Differences in polyanion conformation are responsible for the differences in pH changes between Ch–PAA and Ch–Car, Ch–Alg, Ch–Pec complex formation. For example, structural similarity between Ch and Car, Alg, and Pec or flexibility of PAA is possible.

Takahashi et al. observed the difference in basic properties between Ch–Alg and Ch–PAA complexes [24]. In the Ch–Alg system, the insoluble complexes were formed at a constant unit molecular ratio of 1:1.3 (Ch:Alg) at pH 3.7–4.7. However, the unit molecular ratio of the Ch–PAA system was greatly affected by pH, showing a change from 1:2.4 to 1:1.7 (Ch:PAA) with an increase in pH from 3.7 to 4.7. They concluded that this was due to the higher flexibility of the polymer chain of PAA than that of Alg.

On the other hand, owing to the ease of interfacial PEC formation, the fitness of the structures of the backbones of Ch and some polyanions was estimated as follows [25]:

$$\text{heparin (Hep)} > \text{Alg} > \text{carboxymethyl}$$
$$\text{chitin (CMChitin)} > \text{PAA}$$
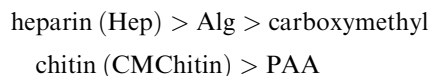
Nakajima and Shinoda showed that the backbone chain conformations of component polymers together with the kind and location of ionizable groups were important factors to discuss the formation and structure of PEC [26]. They used glycol Ch (GC), which is water soluble at all pHs, as a polycation and hyaluronic acid (HA), chondroitin sulfate (CS), Hep, and sulfated cellulose (SCS) as polyanions. In the GC–HA and GC–CS systems, the experimental curves of complex composition, $R$, to pH crossed the theoretical curves at $R = 0.5$ (Fig. 5 shows the GC–HA system as an example.) In both complexes, the neutral complex appears only at $R = 0.5$. The positive and negative charges may remain in the regions $R < 0.5$ and $R > 0.5$, respectively. This result shows that the dominant
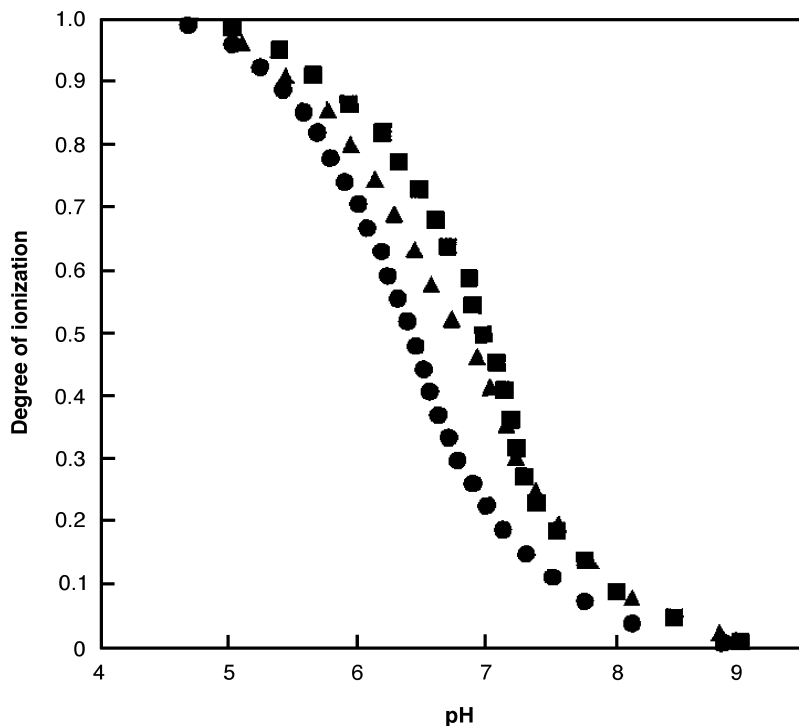
**Figure 4** Degree of ionization for amino groups of Ch in the presence of xanthan. The mole ratio of carboxyl groups to amino groups: ●, 0.30; ▲, 0.60; ■, 1.19. (From Ref. 22.)

than the kind and location of the ionizable groups on the pyranose ring. In the case of the GC–Hep system, the discrepancy between theoretical and experimental curves was rather small, and complex formation seemed to proceed almost stoichiometrically. However, the complex composition was GC/(GC + Hep) = 0.65 at the lower

pH region. They suggested that one of the possible structures having these compositions was the ladder form sandwiching a Hep molecule between two GC molecules. Moreover, the experimental curve of the GC–SCS system was remarkably different from the theoretical curve, as shown in Fig. 6. However, the complex composition at the
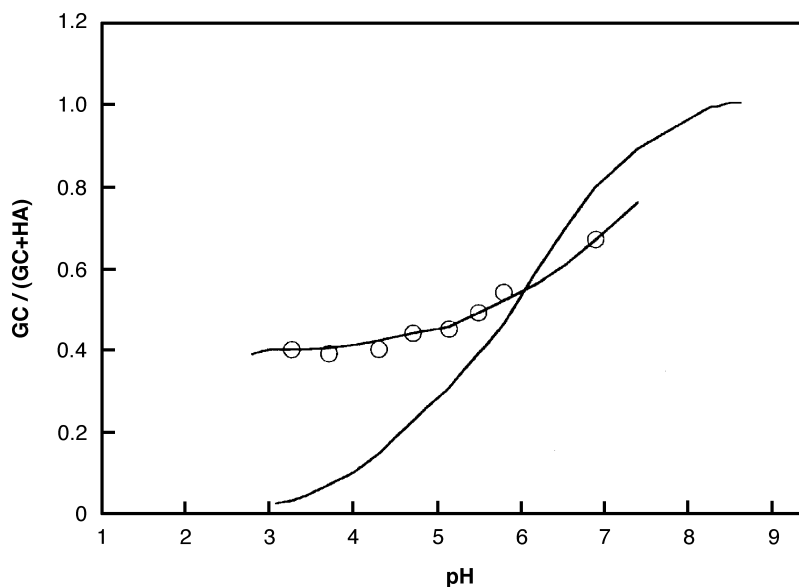


**Figure 5** Composition of complex plotted against pH for GC–HA system. (From Ref. 26.) Copyright 1976, reprinted with

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