Carbohydrate Research, 73 (1979) 219–226

PROTECTION OF SODIUM DODECYL SULFATE-INDUCED AGGREGATION OF CONCANAVALIN A BY SACCHARIDE LIGANDS

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(Received November 24th, 1978; accepted for publication, December 12th, 1978)

ABSTRACT

Concanavalin A is visibly aggregated by low concentrations of sodium dodecyl sulfate, maximum aggregation being obtained at pH 4.6. Other denaturants, such as urea, guanidine hydrochloride, Triton X-100, cetyltrimethylammonium bromide, Tween 80, and Brij 35 are ineffective in promoting visible aggregation. The sodium dodecyl sulfate-induced aggregation of concanavalin A requires the presence of an intact, saccharide-ligand binding-site. Rapid and complete reversal of the detergent effect was achieved by use of saccharides which bind to the lectin. Such compounds as tryptophan and *o*-nitrophenyl β-D-galactopyranoside did not inhibit the aggregation of concanavalin A by sodium dodecyl sulfate, suggesting that the detergent does not bind the hydrophobic pocket on the surface of the protein. The results suggest that concanavalin A may have an additional, ligand-binding site which is metal-dependent and which can be modified by the addition of a saccharide ligand.

INTRODUCTION

Refinements in the crystal structure of concanavalin A (con A) show that each subunit of the lectin has separate binding sites for hydrophobic ligands, transition metals, Ca^{2+}, and saccharides[1,2]. Additional, distinct, but not well-characterized, sites, such as those for lanthanides[3] and other metals[4], as well as a site[4a] for Rose Bengal, exist in the con A molecule. Occupancy of the various ligand sites of con A confers altered properties on the protein. Saccharides, Mn^{2+}, and Ca^{2+} all induce characteristic difference-spectra when compared with the ligand-deficient lectin[5,6]. Furthermore, heat resistance has been equated with the presence of Mn^{2+}; Ca^{2+} enhances resistance to denaturants, and carbohydrate tends to prevent proteolysis and inhibition by (ethylenedinitrilo)tetraacetic acid[7,8], The foregoing changes in the solution properties of con A have been interpreted[7] in terms of ligand-induced, conformational changes in the protein. Indeed, several lines of evidence point to structural modifications in the lectin upon interaction with ligands. Reeke *et al.*[2] have recently

shown, by use of X-ray crystallographic measurements, that metal-free con A contains regions of disorder around the metal-binding sites and the presumed carbohydrate-binding site. Earlier, Jack *et al.*[9] and Weinzierl and Kalb[10] noted that apo-con A possesses a unique, crystal structure which can be changed by transition metals and Ca^{2+}. Goldstein and his colleagues[11,12] have defined a unique kind of hydrophobic site associated with saccharide binding by con A; they showed that several hydrophobic glycosides bind to the lectin with higher affinities than the unsubstituted carbohydrates. Hardman and Goldstein[13] and Goldstein and Hayes[14] have reviewed the chemical and biological properties of con A.

In a previous study, we noticed[7] that such denaturants as guanidine hydrochloride increased the sedimentation rates of Ca^{2+}-deficient con A. Earlier, Olson and Liener[15] had observed that concentrated urea causes an apparent polymerization of con A. In the present study, we show that another denaturant, namely, sodium dodecyl sulfate (SDS) precipitates with intact con A, and that the precipitation can be inhibited by specific saccharides. The results may be interpreted in terms of a new ligand-site for con A, and afford additional evidence for saccharide-induced, structural changes in the protein.

EXPERIMENTAL

SDS, urea, and guanidine hydrochloride were of the highest quality available from Pierce Chemical Co., Rockford, IL. Manganous chloride and calcium chloride (ACS grade) were purchased from Fisher Chemicals, Pittsburgh, PA. Tryptophan (Trp), cetyltrimethylammonium bromide, Brij 35, Tween 80, D-mannose, methyl α-D-mannopyranoside (Me α-D-Man*p*), D-galactose, *o*-nitrophenyl β-D-galactopyranoside (ONPG), D-glucose, and methyl α- and β-D-glucopyranoside were products of Sigma Chemical Co., St. Louis, MO.

Con A was prepared from defatted, jack-bean meal (Schwarz/Mann, Orangeburg, NY) according to Agrawal and Goldstein[16]. The protein (9 mg/mL) was stored at 4° in 1.0M sodium chloride, or as a freeze-dried powder. Metal-free con A was prepared as described by Doyle and Stroupe[8]. Routine measurements showed that the lectin contained <0.01 mol of Mn^{2+} or Ca^{2+} per mol of protein. The metal-deficient derivative would not precipitate with glycogen unless Mn^{2+} and Ca^{2+} were added to mixtures of the lectin and D-glucan. Concentrations of con A were determined by absorbance measurements at 280 nm, assuming that 1.0 mg of the protein/mL gives an extinction of 1.14 (see ref. 17).

The SDS-induced aggregation of con A was studied turbidimetrically at room temperature. SDS was added to solutions of con A, and the resulting absorbance was determined by use of a Spectronic 20 colorimeter after 30–40 min. Rounded cuvets (1.25 cm) and a wavelength of 500 nm were routinely employed. Buffers, pH values, lectin concentrations, and the amount of SDS present in individual experiments are given in the text. Final reaction-volumes were always 3.0 mL. Hydrogen-ion concen-

trations were determined, by use of a glass electrode, immediately following absorbance readings. All buffer solutions were prepared according to Gomori[18].

RESULTS

When the concentration of con A was varied in the presence of a constant amount of SDS, the results shown in Fig. 1 were obtained. Under these conditions, 70–90% of the protein was precipitated. The pH-dependency of the aggregation phenomenon is shown in Fig. 2. Maximum opacity was observed at pH 4.6. Diminished

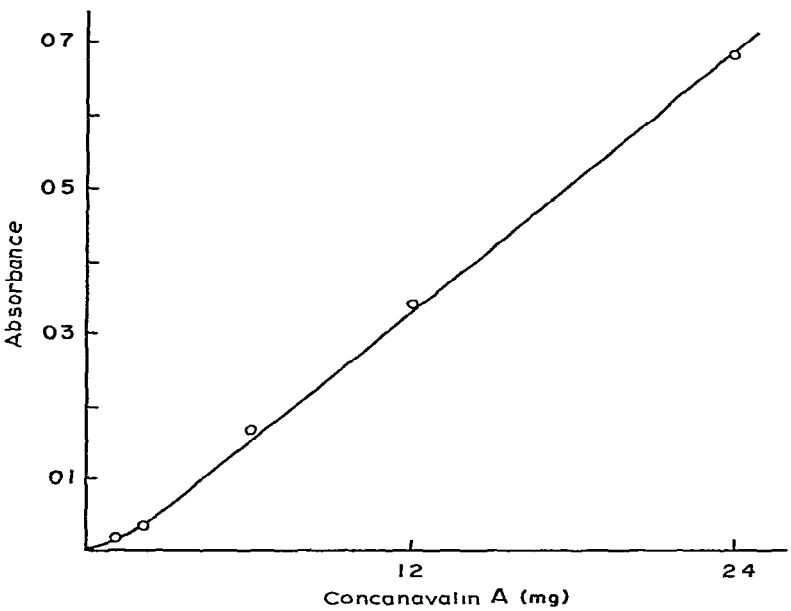

Fig. 1. Dependence of the concentration of protein on the SDS-induced aggregation of concanavalin A. (Native con A was mixed with 5 mg of SDS in 0.1 M sodium phosphate, pH 5 8)

aggregation on the acid side of the pH optimum may reflect a loss of metal from con A, as acidic conditions are known to cause dissociation of metal from the protein[14]. We have observed extensive, yet transient, opacities of SDS–con A solutions at pH 3–4. A marked inhibition of the SDS-induced aggregation was only achieved by Me α-D-Man*p* between pH 5.0 and 6.0 (see Fig. 2). In the presence of Me α-D-Man*p*, the pH of half-maximal turbidity was shifted from 5.7 to 5.2. When inhibition of aggregation is plotted as a function of pH (inset, Fig. 2), the mid-point for inhibition occurs at pH ~5.4.

The effect of the concentration of SDS on the aggregation of con A was also studied (see Fig. 3). Aggregation was first observed at a total concentration of detergent of 0.58mM, and was maximal at ~2.5mM. The strong concentration-dependence is consistent with the concept that detergent-induced aggregation is a

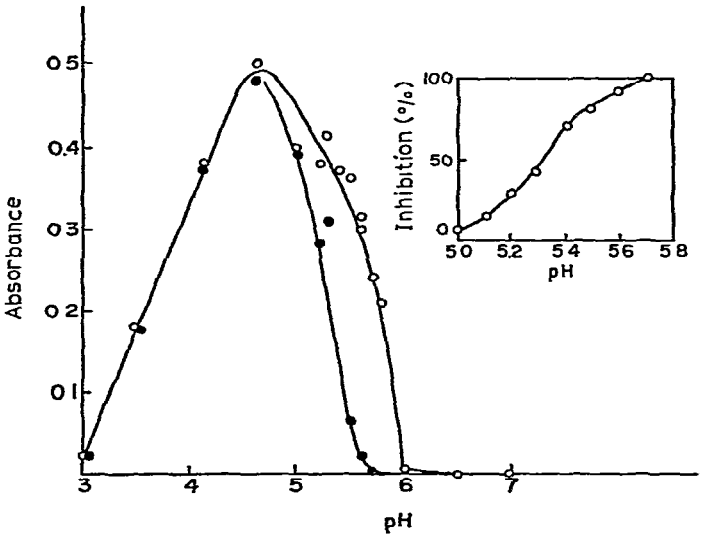

Fig. 2. The effect of pH on the SDS-induced aggregation of concanavalin A. [Incubations were conducted for 10 min in the presence (-●-) and absence (-○-) of methyl α-D-mannopyranoside (0.01 M). The amounts of reactants were: con A, 0.9 mg, and SDS, 5 mg. The buffer was 0.1 M sodium acetate. Inset: Inhibition (percent) of aggregation by methyl α-D-mannopyranoside between pH 5 0 and 5 8]

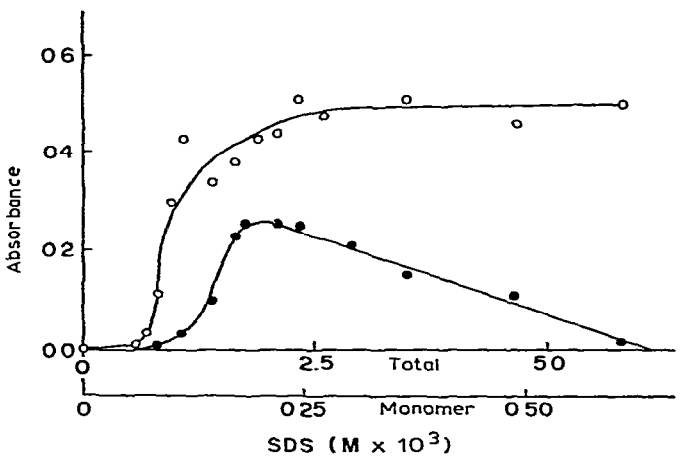

Fig. 3. The effect of concentration of SDS on the aggregation of concanavalin A. [Various amounts of SDS were added to 0.9 mg of con A, and the absorbance was recorded (-○-). The experiment was repeated with D-mannose (0.01 M) present in the reaction mixture (-●-). The concentration of SDS monomer was derived from the data of Reynolds and Tanford[21], based on an ionic strength of 0 58 (see ref. 21). The buffer was 0.1 M sodium phosphate, pH 5 8.]

highly cooperative phenomenon[19]. The SDS–con A precipitate was found to be stable, in view of the fact that absorbances were constant during several hours. At high concentrations of SDS (>35mM), visible aggregation was not observed. Also shown in Fig. 3 are the effects of D-mannose, a high-affinity ligand[14] for con A, on the detergent-

TABLE I

SDS-INDUCED AGGREGATION OF CONCANAVALIN A

Addition[a]		*Absorbance*
Con A (metal-free)	+ SDS	< 0.01
	+ mM Mn^{2+} + SDS	< 0.01
	+ mM Ca^{2+} + SDS	< 0.01
	+ mM Trp + SDS	< 0 01
	+ mM Mn^{2+} + mM Ca^{2+} + SDS	0.49
	+ mM Mn^{2+} + mM Ca^{2+} + mM Trp + SDS	0 50
Con A (unmodified)	+ SDS	0.51
	+ 50 mM sodium sulfate + SDS	0.49
	+ mM Trp + SDS	0 50
	+ urea (10 mg)	< 0 01
	+ guanidine hydrochloride (10 mg)	< 0 01
	+ Tween 80 (20 mg)	< 0 01
	+ Brij 35 (20 mg)	< 0.01
	+ cetyltrimethylammonium bromide (10 mg)	< 0 01
	+ Triton X-100 (20 mg)	< 0.01

[a]The amount of con A present in each experiment was 2.5 mg. The amount of SDS used was 5.0 mg. Final concentrations (or amounts) of other additions are shown. The buffer was 0.1 M sodium acetate, pH 5.2.

induced turbidity. Although D-mannose is capable of partially inhibiting the aggregation reaction over a range of SDS concentrations, complete inhibition of aggregation was observed only at relatively high, total SDS concentrations (6.1 mM).

When other detergents and denaturants (Triton X-100, Brij 35, Tween 80, cetyltrimethylammonium bromide, urea, or guanidine hydrochloride) were substituted for SDS, no turbidity was observed (see Table I). In addition, an intact, carbohydrate-binding site on con A appears to be required, because the metal-free protein was not aggregated by SDS. Furthermore, sulfate anion and such hydrophobic ligands as Trp

TABLE II

SACCHARIDE INHIBITION[a] OF SDS-INDUCED AGGREGATION OF CONCANAVALIN A

Monosaccharide	*Amount required for 50% inhibition (μmol)*
Methyl α-D-mannopyranoside	0.58
Methyl α-D-glucopyranoside	0.78
D-Mannose	1.2
D-Glucose	6.4
Methyl β-D-glucopyranoside	14
D-Galactose	> 100

[a]The reaction mixtures contained 0.9 mg of con A, 5.0 mg of SDS, and varied amounts of the indicated saccharides in a volume of 3.0 mL. The buffer used was 0.1 M sodium phosphate, pH 5.8. The concentration of monosaccharide required for 50% inhibition was derived graphically from a plot of % inhibition *vs.* log saccharide concentration.

and ONPG had no effect on the aggregation process. Manganous ion would not restore the capacity for induced aggregation to the lectin; however, manganous ion plus Ca^{2+} resulted in a preparation which was sensitive to aggregation by SDS.

The effect of specific saccharides on the SDS-induced aggregation of con A is shown in Table II. The sequence of their effectiveness in inhibiting turbidity is of the same order as the binding affinities[14] of these sugars for con A. In addition, the concentrations of ligand required for inhibition of aggregation are comparable to those[14] which prevent precipitation of glycogen by con A.

DISCUSSION

The results of this study demonstrate that relatively low monomer concentrations (0.1mM) of the anionic detergent SDS will induce visible aggregation of con A. This effect is pH-dependent, with maximum turbidity resulting at pH 4.6. Although most proteins are soluble in SDS, aggregation at low concentrations of the detergent has been observed[20]. Furthermore, SDS usually precipitates those proteins maximally on the acid side of their isoelectric point. In contrast, con A, having a pI (ref. 17) of 7.1 (see, however, ref. 14), remains soluble in the presence of SDS at pH 6.0.

Reynolds and Tanford[21] demonstrated that, as the SDS concentration is increased, two classes of detergent binding to protein are observed. At monomer concentrations of 0.5–0.8mM, a wide variety of proteins bind ~0.4 g of SDS per g of protein. Above 0.8mM SDS monomer, a second complex is formed, which is saturated with 1.4 g of SDS per g of protein. We observed detectable aggregation of con A at ~0.06mM SDS monomer, and maximum aggregation at ~0.25mM SDS monomer. It would appear, therefore, that aggregation of con A occurs prior to saturation of the first stage of SDS–protein complex-formation as described by Reynolds and Tanford[21]. On the other hand, quantitative reversal of SDS aggregation by saccharide ligand is obtained only at higher monomer concentrations (0.65mM), and may represent the 0.4 g of SDS/g of protein complex. The soluble SDS–con A complex observed at high concentrations (>35mM) of detergent probably represents the 1.4 g of SDS/g of protein complex.

Mattice *et al.*[22] studied the gross conformation of con A in the presence of 17 mM SDS at pH 6.86. Although SDS appeared to induce α-helix formation concomitant with the loss of β structure in the lectin, no evidence was found for the kinds of interactions we now report. Similarly, Jirgensons[23] observed that the secondary structure of con A is altered by SDS, but to a much lesser extent than observed[22] by Mattice *et al.* SDS-induced conformational changes in ribonuclease at low concentrations of monomer detergent (<0.15mM) have been described[24]. Interestingly, Jones and Wilkinson[25] found that, at low concentrations of SDS (30 mol of SDS/mol of protein), precipitation of β-lactoglobulin occurs; they considered the interaction to be of an ionic nature and to be accompanied by a conformational change in the protein. Earlier, Seibles[26] observed that complex-formation between SDS and β-lactoglobulin, resulting in aggregates, is dependent on a functional histidine residue in the protein.

On examining the specificity of saccharide reversal on con A-polysaccharide complex-formation[14], we found a striking parallel in the effects of these same saccharides on the reversal of SDS-induced aggregation of con A. Not only is the order of effectiveness the same, but the magnitude of the concentrations required for 50% inhibition are in close agreement. We consider that these results are consistent with the concept that saccharide binding results in a conformational change in con A. The restricted range of pH over which saccharide reversal is observed (pK $\sim$ 5.4) suggests that a histidine residue may be involved in (*a*) SDS-induced-aggregation, and (*b*) the sugar-induced conformational change. Reactive histidine residues having a pK of 5.5 are known to occur in ribonuclease[27]. One histidine residue (His 24) of con A complexes with Mn^{2+}, as shown by X-ray diffraction[13]. On the basis of titration data and chemical modification studies, Gachelin *et al.*[28] suggested that at least two histidine residues are involved in metal binding by con A, possibly reflecting a difference between the solution structure and the crystal structure of the lectin.

As to the mechanism of the saccharide reversal of the SDS–con A, insoluble aggregate, it is possible that saccharide binding increases the proportion of SDS bound to con A, thereby effecting solubilization of the aggregate; this suggestion is consistent with the fact that increasing the SDS concentration (>35 mM) results in a soluble SDS–con A complex. Alternatively, if saccharide binding results in a conformational change in con A, the SDS-binding site may be masked or altered, resulting in the removal of the detergent from the lectin. It is known that con A will bind saccharides at pH values lower than that pH where we observed SDS- induced lection aggregation[14]. Thus, it is unlikely that saccharide and SDS bind to the same site on the protein. Moreover, because Trp or ONPG failed to inhibit the aggregation, it is unlikely that SDS binds to the hydrophobic cleft in the con A monomer[13,29]. Therefore, SDS probably binds to selected sites on the surface of con A, resulting in the precipitation of the protein. The sites are available only when con A is in the proper conformation, *i.e.* when both metal-binding sites are filled. Hassing *et al.*[30] showed that such saccharides as Me α-D-Man*p* "mask" the titration of carboxyl groups in con A. Akedo *et al.*[31] found that specific saccharides increase the isoelectric point of con A, suggesting that exposed carboxyl groups had become "buried" upon carbohydrate binding. The increased positieve charge on con A as a result of saccharide binding may cause a diminution in the extent of SDS interaction with the protein, consistent with earlier views that the detergent usually precipitates proteins only when the proteins are titrated below their pI values[20]. The Bellos[32] and Pittz and Bello[33] showed that SDS and other charged denaturants can directly complex with oppositely charged amino acids *via* ion-ion interactions, as well as by apolar (hydrophobic) interactions.

ACKNOWLEDGMENTS

This work was supported, in part, by grants from the American Heart Association (70–760) and the American Cancer Society (IN–III).

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