Salt-induced inhibition of the precipitin reaction of concanavalin A with polysaccharides and glycoprotein

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The time course of the precipitin reactions of concanavalin A with glycogen, dextran and ovalbumin was investigated by a light-scattering method near 30°C in 10 mM-Tris/HCl buffer, pH 7.4, containing neutral salts, i.e. NaCl, KCl, NaBr, KI and NaClO₄. With 0.8 µM-lectin and 0.36 mg of glycogen/ml, the half-life, *t*_{1/2}, of the precipitin reaction was independent of salt concentration between 0.1 M and 1.5 M, and was the same (175 s) in the presence of NaCl, KCl, NaBr and KI but was significantly (27%) higher in NaClO₄. In contrast, the five salts caused significant to marked enhancement in *t*_{1/2} for the reactions of concanavalin A with dextran and ovalbumin. Likewise, whereas the turbidity produced in 1 h as a result of lectin-glycogen precipitation remained unchanged, those measured for the binding of dextran and ovalbumin were decreased in the presence of three salts. The increase in *t*_{1/2} and decrease in turbidity were found to be higher with NaClO₄, followed by KI; NaBr produced moderate and NaCl (or KCl) small but generally significant inhibition of the precipitin reactions with dextran and ovalbumin. The results showed that the lectin-ligand precipitin reactions involve salt-sensitive polar interactions that are less pronounced with compactly folded ligands such as glycogen.

Concanavalin A is composed of four identical protomers held together by, among other things, polar interactions, hydrogen bonds and electrostatic interactions (Recke *et al.*, 1975; Hardman & Ainsworth, 1976; Hardman & Goldstein, 1977); the dimer-dimer contact is stabilized by hydrogen bonds and six inter-dimer salt bridges (Recke *et al.*, 1975). Each protomer contains one saccharide-binding site, which appears to react with only one saccharide residue of di- and tri-saccharides (Landschoot *et al.*, 1980a,b). Most of the amino acid residues near or at the carbohydrate-binding site would interact with the specific sugars through polar interactions. These findings, taken together with the observations that the lectin dimer is substantially less effective in precipitating polysaccharides than is the tetramer (Hardman & Goldstein, 1977; Waseem & Salahuddin, 1983), and that both are identical in their reactions with small sugars (Hardman & Goldstein, 1977; Landschoot *et al.*, 1980a,b), suggest that factors such as

pH and salt, which affect polar interactions, would also influence the interaction of the lectin with carbohydrates, especially polysaccharides.

The effect of pH and salt on the binding of carbohydrates to concanavalin A has yet to be investigated systematically, although the carbohydrate binding has been studied at a few pH values and salt concentrations (Hardman & Goldstein, 1977; Loontjens *et al.*, 1977; Landschoot *et al.*, 1980a,b). Thus concanavalin A has been found to combine with small sugars similarly at both an acid and a neutral pH (So & Goldstein, 1968; Hassing & Goldstein, 1970; Loontjens *et al.*, 1977; Landschoot *et al.*, 1980a,b), but the extent of the concanavalin A-polysaccharide precipitin reaction was considerably smaller at an acid pH than at neutral pH (Hardman & Goldstein, 1977; Waseem & Salahuddin, 1983). This may not be due to the acid-induced dissociation of concanavalin A tetramer into dimer (McKenzie *et al.*, 1972), since the pH-dependence of the acid dissociation of the lectin (McKenzie *et al.*, 1972) is apparently not the same as that of the lectin-polysaccharide precipitin reaction (Waseem, 1982). Further, although the binding of methyl α-D-glucopyranoside to the

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lectin was dependent on ionic strength (Hassing & Goldstein, 1970), the binding of another specific sugar, *p*-nitrophenyl α -D-mannopyranoside, was independent of ionic strength (So & Goldstein, 1968). The data on the effect of salts on the precipitin reaction of concanavalin A with complex and large ligands, e.g. polysaccharides, glycoproteins and cells (Hardman & Goldstein, 1977), are too sparse to lead to any definite conclusion. These considerations prompted us to investigate systematically the effect of five different neutral salts, namely NaCl, KCl, NaBr, KI and NaClO₄, on the precipitin reaction of concanavalin A with three specific large ligands, namely glycogen, dextran and ovalbumin. As the five salts are likely to have different effects on polar interactions, their influence on the precipitin reactions should be different. The results presented below not only underscore the importance of polar interactions in the precipitin reaction, but also show that the sensitivity of a precipitin reaction to salt may depend on the structure of a specific macromolecular ligand.

Materials and methods

Concanavalin A prepared from jack-bean meal by affinity chromatography was pure (Agrawal & Goldstein, 1967; Waseem & Salahuddin, 1983) by the criteria of polyacrylamide-gel electrophoresis in 10% gel in 20mM-Tris/glycine buffer, pH 8.2 (Davis, 1964), as well as by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis in 10% gel in 40mM-sodium phosphate buffer, pH 7.2 (Laemmli, 1970). Dextran (M_r 5×10^6 – 40×10^6), methyl α -D-mannopyranoside and oyster glycogen (type II) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Reagents used in polyacrylamide-gel electrophoresis were the same as described before (Ansari *et al.*, 1975). Other chemicals were of reagent grade. Concentrations of NaBr, KI and NaClO₄ solutions were determined by the dry-weight method. Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Concentrations of dextran and glycogen were determined in terms of hexose as recommended by Dubois *et al.* (1956).

The time course of the interaction of concanavalin A with polysaccharide and ovalbumin was studied in TM buffer (i.e. 10mM-Tris/HCl buffer, pH 7.4, containing 1mM each of calcium acetate, magnesium acetate and MnCl₂) containing the requisite concentration of neutral salt at 29.5°C by measuring light-scattering at 480nm either on an EC Spectrophotometer equipped with a locally fabricated thermostatically controlled cell-holder or on a Carl Zeiss Jena Spekol Spectrocolorimeter.

Results

Concanavalin A formed specific precipitates with glycogen, dextran and ovalbumin, as evidenced by the complete dissolution of the precipitate with 0.1M-methyl α -D-mannopyranoside. The time course of the precipitin reaction with the three macromolecular ligands was studied at 29.5°C in TM buffer, pH 7.4, containing the neutral salts. The results obtained with the three ligands were qualitatively similar. Therefore only the data for concanavalin A–glycogen precipitation are shown in Fig. 1, where the inset delineates the first-order kinetic plot obtained by the method of least squares as described previously (Waseem & Salahuddin, 1983). It should be noted that the precipitin reaction could not be studied with precision in TM buffer, pH 7.4, alone, since the stability of concanavalin A was very low in the absence of neutral salt. The observed rate constant computed from the linear plot (see inset of Fig. 1) was converted into the half-life, t_1 . At a given salt concentration, t_1 values for the three concanavalin A–ligand systems were determined in triplicate with a precision of 14% in the present study. Results on the effect of the neutral salts, i.e. NaCl, KCl, NaBr, KI and NaClO₄, on t_1 of the precipitin reaction of concanavalin A with glycogen, dextran and ovalbumin are summarized in Tables 1–3. At a given salt concentration, t_1 for concanavalin A–glycogen precipitation was the same, in the presence of NaCl, KCl, NaBr and KI; t_1 was significantly smaller in the presence of NaClO₄. For example, at a salt concentration of 0.1M the mean value of t_1 was 179.0 ± 9.8 s, which at a 5% level of significance according to the *t*-distribution became 179.0 ± 17.5 s. The mean values ($\pm$ S.D.) of t_1 found at other four salt concentrations, namely 0.2M, 0.5M, 1.0M and 1.5M, were respectively 177 ± 13 s, 163 ± 8 s, 186 ± 10 s and 179 ± 13 s. Evidently the average of t_1 values listed in the last column of Table 1 is 177s, which is lower

Table 1. Effect of neutral salts on the interaction of concanavalin A with glycogen in TM buffer, pH 7.4, at 29.5°C

The concentration of concanavalin A was 0.8 μ M and that of glycogen was 0.36mg/ml.

Concn. of salt (M)	t_1 (s)	
	NaClO ₄	Other salts*
0.1	257	179
0.2	204	167
0.5	193	163
1.0	267	186
1.5	193	179

* The t_1 values represent the averages of the values found in the presence of KCl, NaCl, NaBr and KI.

Fig. 1. Time course of concanavalin A–glycogen precipitin reaction in TM buffer, pH7.4, containing 1.0M-NaCl at 29.5°C. Solutions of concanavalin A (90.8 μM) and glycogen (0.36mg/ml) were incubated and the turbidity was recorded at 480nm as a function of time. The inset shows the first-order kinetic plot of the precipitin reaction.

Table 2. Effect of neutral salts on the interaction of concanavalin A with dextran in TM buffer, pH7.4, at 29.5°C. The concentration of concanavalin A was 17 μM and that of dextran was 2.23mg/ml.

Salt	A_{60}	A'_{60}	t_1 (s)
NaCl (0.5M)	0.66	0.48	179
NaBr (0.5M)	0.56	0.43	174
KI (0.5M)	0.50	0.35	238
NaClO ₄ (0.5M)	0.45	0.15	594

Table 3. Effect of neutral salts on the interaction of concanavalin A with ovalbumin in TM buffer, pH7.4, at 29.5°C. The concentration of concanavalin A was 7.9 μM and that of ovalbumin was 18 μM.

Salt	A_{60}	A'_{60}	t_1 (s)
NaCl (0.5M)	0.26	0.27	286
NaBr (0.5M)	0.18	0.15	743
KI (0.5M)	0.11	0.15	811
NaClO ₄ (0.5M)	0.08	0.05	1188

than the mean (223 ± 36 s) of t_1 values found at five different concentrations of NaClO₄ (see Table 1). The 90% confidence limit for the mean value (223s) would be 223 ± 38 s. Strikingly, the turbidity

produced due to concanavalin A–glycogen precipitation in 1h, i.e. A_{60} at 480nm under conditions of the kinetic experiments (see Fig. 1), was 0.23 ± 0.02 for all of the five salts, including NaClO₄, regardless of salt concentration.

The precipitation reaction of concanavalin A with dextran is influenced by both the nature and the concentration of the salts (see Table 2); t_1 values in the presence of NaCl and NaBr differ insignificantly (3%), and KCl was indistinguishable from NaCl in its effects on the precipitation reaction. However, t_1 values were substantially higher in the presence of KI and NaClO₄. Results on the turbidities in the presence of 0.5M (A_{60}) and 1.5M salt (A'_{60}) measured at 1h also led to similar conclusions, the turbidity being lowest in the presence of NaClO₄ and next lowest in the presence of KI. Further, A_{60} (or A'_{60}) was significantly lower in 0.5M-NaBr than in 0.5M-NaCl (see Table 2). The differential inhibitory effect of the five salts was more pronounced on the concanavalin A–ovalbumin precipitin reaction (see Table 3); here again the precipitin reaction was found to be the slowest and the turbidity (A_{60} or A'_{60}) lowest in the presence of NaClO₄. At a given salt concentration, e.g. 0.5M-NaCl, t_1 for the precipitin reactions of concanavalin A with glycogen, dextran and ovalbumin was 163s, 179s and 856s respectively (see

Tables 1–3). The observed difference seems to be partly due to the difference in the reactant concentrations used in the three sets of experiments (see Tables 1–3).

Discussion

Results given in the present paper have shown that increasing concentrations of the five salts studied caused significant to marked inhibition of the precipitin reactions of concanavalin A with dextran and ovalbumin and that the inhibition was more pronounced with KI and NaClO₄. Strikingly, the precipitin reaction with glycogen was independent of salt concentrations in the range 0.1–1.5M (see Table 1) and was similarly influenced by NaCl, KCl, NaBr and KI. The reaction in its initial stage, where results yielded t_1 , was significantly slower in the presence of NaClO₄ than in the presence of any of the other four salts, but after 1 h the turbidity, i.e. A_{60} , was the same with all of the five salts, including NaClO₄.

Chemical and structural differences among the three macromolecular ligands may account for the marked differences in the dependence of their reactions with concanavalin A on salt concentrations. Unlike the structures of glycogen and dextran, that of ovalbumin would be sensitive to salts, especially at high ionic strength. Thus the electrostatic free energy of ovalbumin corresponding to 13 negative charges/molecule at pH 7.4 (Cannan *et al.*, 1941) was calculated (Ansari *et al.*, 1975) to be 15.5 kJ/mol (3.7 kcal/mol) at $I=0.15$, but it will be decreased to 8.4 kJ/mol (2.0 kcal/mol) in the presence of 1.5M salt. The decrease in electrostatic repulsion would favour concanavalin A–ligand aggregation. The observed salt-induced inhibition of aggregation or precipitation, taken together with the observation that NaCl and NaClO₄ of equal concentration had appreciably different inhibitory influences on the precipitation reactions (see Tables 1–3), suggest that electrostatic effects alone cannot account for the deleterious effect of salts on the precipitin reaction.

Hydrodynamic data show that glycogen molecule is compact and near-spherical (West *et al.*, 1974), whereas the dextran molecule, with a substantially smaller number of cross-links, is flexible (Tanford, 1961). Therefore the number of potential hydrogen-bonding hydroxy groups that will be freely available for polar interactions with concanavalin A would be appreciably smaller in glycogen than in dextran. Consequently polar interactions between concanavalin A and polysaccharides are likely to be more extensive during the reaction of the lectin with dextran. Since salts would influence non-covalent interactions, including polar interactions, their pronounced inhibitory

effect on concanavalin A–dextran (or concanavalin A–ovalbumin) interaction and lack of such effects on lectin–glycogen reaction are not unexpected. Interestingly, salts such as KI and NaClO₄, which are more effective in disrupting native protein conformation (von Hippel & Schleich, 1969) by weakening non-covalent interactions, including polar interactions, are also better inhibitors of concanavalin A–ligand precipitin reactions (see Tables 1–3).

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