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Note

Iodine-induced aggregation of amylose chains by proteins

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Amylose is unique in forming, with iodine in aqueous systems, a blue helical complex having iodine molecules packed inside the helix[1]. In solution, amylose exists essentially as a random coil with short regions of extended, loose helical conformation.

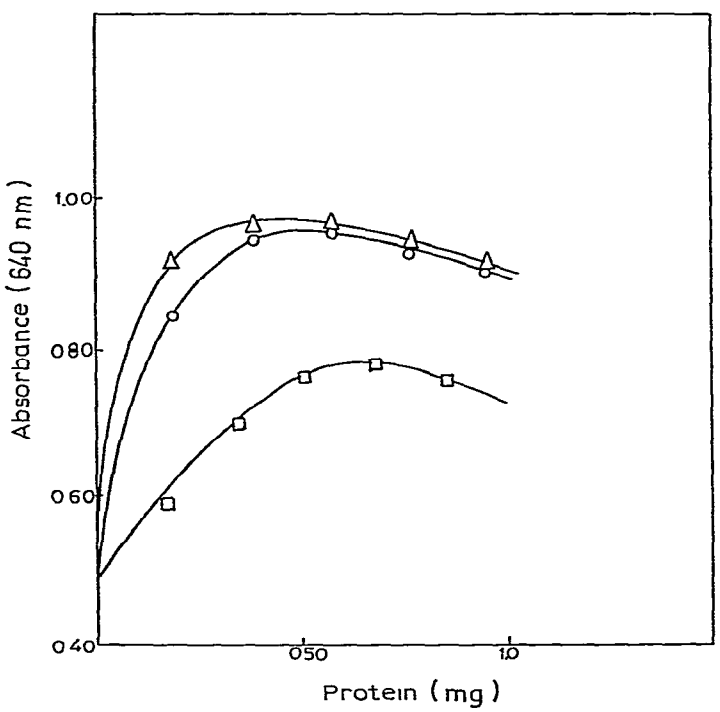

Fig. 1. Influence of protein concentration· amylose (0 54 mg), 0 1M Tris–HCl buffer (0 5 ml, pH 7.2), 6 7 × 10^{-4}M iodine (0.5 ml), and protein (0 1–1 mg) made up to 5 ml with water, incubated at 30° for 1 h; egg albumin (—○—), bovine serum albumin (—△—), Con A (—□—).

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In the presence of a suitable complexing agent, these loose helical regions undergo contraction, forming a compact V-type helix with ligand molecules trapped inside the helix[2-6].

We now report a novel, reversible interaction of the amylose–iodine complex with proteins, which is closely associated with the coil→helix transition of the amylose chain, and the high adsorptive capacity of this complex appears to be due to hydrophobic interactions.

Concanavalin A (Con A) gives a precipitin reaction with polysaccharides and glycoproteins containing α-D-gluco- or α-D-manno-pyranosyl residues. Interaction of Con A with amylose does not produce a precipitate, although amylose contains[7] D-glucose residues which can interact with Con A.

We have found that the blue amylose–iodine complex interacts with Con A, resulting in an increase in absorbance at 640 nm followed by the precipitation of the protein–amylose–iodine complex (Fig. 1); the supernatant solution was colourless, indicating that all of the iodine was in a complexed state. The blue, flocculent precipi-

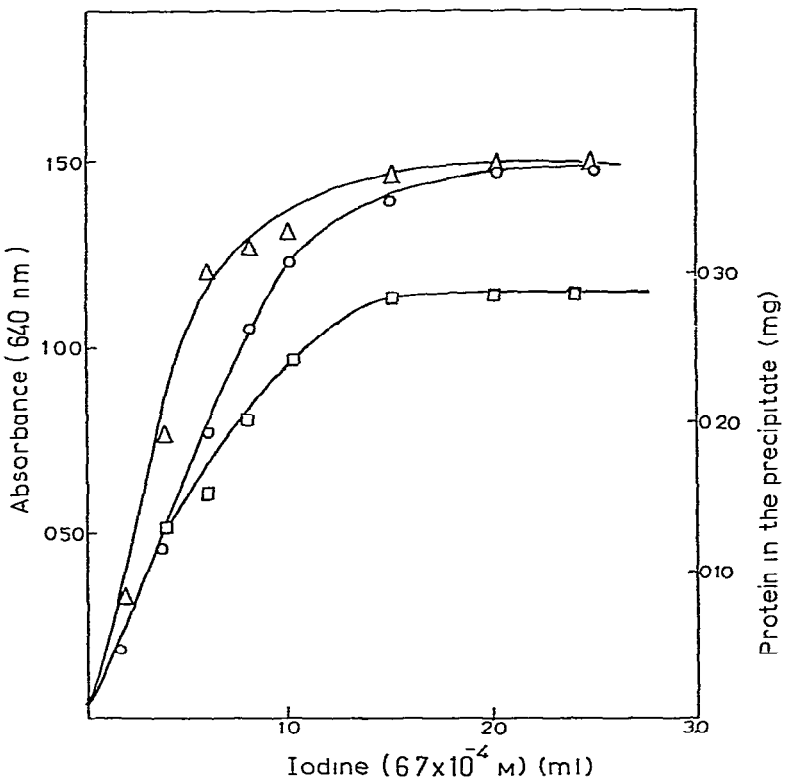


Fig. 2. Influence of iodine concentration. Amylose–iodine (—○—): amylose (0.45 mg), 0.1M Tris–HCl buffer (0.5 ml, pH 7.2), and 6.7×10^{-4}M iodine (0.2–2.5 ml) made up to 5 ml with water, and incubated at 30° for 1 h; amylose–iodine–Con A (—△—): same as above + Con A (0.825 mg). Protein (—□—) in the precipitate was determined by the method of Lowry *et al*[17] after reduction of iodine with sodium thiosulphate.

tate could be readily dispersed by shaking on a vortex mixer, giving a clear blue solution having the same absorbance at 640 nm and no change in the absorption spectrum. This precipitate could not be dissociated by Con A-specific sugars, implying that the interaction was nonspecific. Similar results were observed with other proteins, for example, bovine serum albumin, egg albumin, milk casein, calf-thymus histone, and fibrinogen[8].

The influence of iodine concentration on amylose–iodine–Con A interaction showed that, at a low concentration (10% of the optimum), there was no aggregation. As the concentration of iodine was increased, the amount of precipitate also increased and remained constant after reaching the saturation level. The protein and amylose contents of the precipitate also followed the same pattern (Fig. 2). Similar results were obtained with other proteins.

This reaction was found to be specific for the amylose–iodine complex, but not for the starch–iodine complex; addition of protein to the amylopectin–iodine complex resulted in decoloration, due to the interaction of loosely bound iodine with protein. Small conformational changes in amylose chains induced by interaction with iodine could be detected. Thus, protein at ~ 10 μg/ml is sufficient to detect the coil→helix transition by forming a precipitate of amylose–iodine–protein complex.

The ratio of protein to amylose at optimum concentration of iodine (3.35 mmol of I_2/mg of amylose) indicates the high adsorption capacity of the amylose–iodine complex, and the ratio is characteristic of the protein (Table I). This reaction is very specific for proteins. Addition of such polyionic substances as DNA, RNA, and dextran sulphate to the amylose–iodine complex either caused an increase in the absorbance (640 nm) without precipitation or had no effect. Degradation of amylose by salivary alpha-amylase (EC 3.2.1.1), or protein by papain (EC 3.4.4.10) or trypsin (EC 3.4.4.4), decreased the precipitation of protein–amylose–iodine complex.

The precipitated protein–amylose–iodine complex could not be dissociated by increasing the ionic strength (0.2→2.0M NaCl) or by variation in pH (2.0–8.0), implying that ionic interactions do not significantly contribute to the stability of

TABLE I

ADSORPTION OF PROTEIN ON AMYLOSE–IODINE COMPLEX

Protein	*Protein/Amylose*[a]
Bovine serum albumin	0.76
Egg albumin	1 38
Milk casein	0 66
Calf thymus histone	0 56
Concanavalin A	0.79

[a]The protein and amylose contents were determined by the methods of Lowry *et al*[17] and Dubois *et al.*[18], respectively, after the reduction of iodine with sodium thiosulphate.

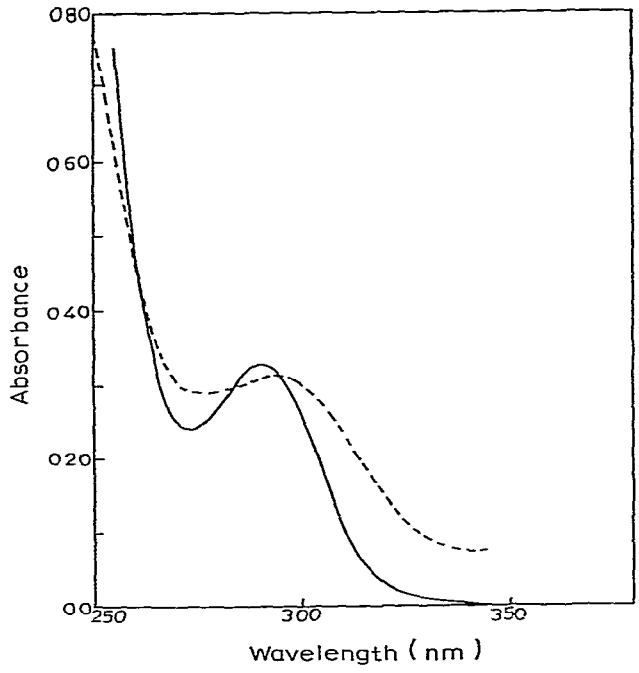
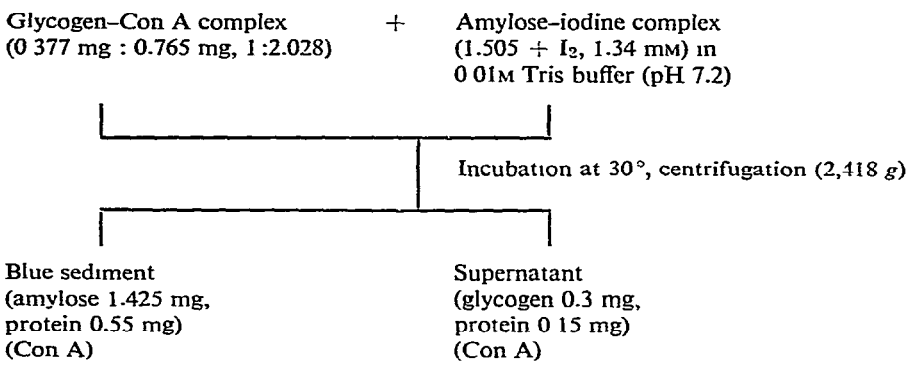


Fig. 3. Absorption spectra of bovine serum albumin· ——, 0·4 mg/ml, pH 10 (adjusted with NaOH); – – – –, 0.45 mg/ml desorbed from amylose–iodine complex and dissolved in 0.145M NaCl adjusted to pH 10.

this complex. Similarly, treatment of the complex with such polarity-reducing agents as ethylene glycol (10–50%, v/v), amino acids with hydrophobic side-chains (DL-alanine, L-leucine, DL-isoleucine, L-phenylalanine; 0.01–0.05M, pH 4.8 and 7.2), or glycine (0.1–1.0M, pH 7.2), or such denaturating agents as urea (1–6M, pH 4.8 and 7.2) and sodium dodecyl sulphate (1%, w/v) or Triton X-100 (0.2–1%, v/v) failed to effect dissociation. The precipitate suspended in 0.145M sodium chloride was not dissociated on incubation at 20–50°. However, sodium thiosulphate or any reducing substance could readily dissociate the complex by reducing the iodine. The absorption spectrum of the desorbed protein differs from that of the native protein (Fig. 3). Similar changes in the absorption spectrum of the protein were observed on treatment with iodine. These changes are probably due to the partial iodination of the protein. The strong adsorption of the protein on the helical amylose–iodine complex is expected to cause some changes in the tertiary structure of the protein. This effect is evident from the observation that the Con A–glycogen precipitate was readily dissociated by the amylose–iodine complex, with the formation of an amylose–iodine–Con A precipitate and release of glycogen in the supernatant (Scheme 1). Some labile proteins and enzymes are likely to be denatured in this process.

Amylose also forms helical complexes[9,10] with a variety of ligands, for example, 1-butanol, sodium dodecyl sulphate (SDS), 1-pentanol, cyclohexanol, thymol, fatty



Recovery: protein, 91%; glycogen, 79%

Scheme 1 Dissociation of con A–glycogen complex by amylose–iodine complex

acids, and methyl sulphoxide. It was expected that these helical complexes would interact with protein in a similar fashion. However, amylose complexes involving 1-butanol, 1-pentanol, and cyclohexanol in solution were not precipitated on addition of protein. Preliminary experiments indicate that an amylose–SDS–protein complex is precipitated on addition of protein to the amylose–SDS complex.

The amylose–iodine complex is precipitated in phosphate buffer (0.01M, pH 7.0) or sodium chloride (0.05M, pH 7.2), but the precipitate is dissociated on dilution, implying that its formation is due to increase in ionic strength. The precipitate of amylose–iodine–protein complex cannot be dissociated on dilution.

The dissociation of the complex on reduction of iodine indicates that it is closely linked with the helix→coil transition of amylose chains and that non-covalent interactions are chiefly responsible for its formation. It appears that the coil→helix transition induced by iodine probably generates some hydrophobic regions on the surface of the complex. Interaction of these hydrophobic regions with the hydrophobic pockets on the surface of protein molecules[11, 12] results in the aggregation of amylose chains complexed with iodine.

Hydrophobic chromatography is widely employed for the separation and purification of proteins and enzymes[13, 14]. It is based on the interaction between the accessible hydrophobic crevices in the protein and the hydrocarbon chains of the substituted alkylamines on agarose[12]. Recently, the reversible immobilization of beta-amylase in active form on a hydrophobic gel has been reported[15].

The characteristic feature of hydrophobic interactions is that they diminish upon decreasing the ionic strength of the medium, and this effect has been used[13, 16] to desorb proteins from a hydrophobic bed. Attempts to desorb protein from the amylose–iodine–protein complex by extraction with dilute phosphate (pH 7.0) and Tris–HCl (pH 7.2) buffers (0.004–0.03M) were unsuccessful. Hydrophobic interactions diminish with decrease in temperature[16], but lowering of the temperature from 20° to 0° failed to dissociate the complex.

These observations indicate that protein is strongly adsorbed on the amylose–

iodine complex and can be released only after removal of the hydrophobic pockets generated by the interaction of iodine with amylose. This effect is achieved by reduction of iodine, causing a helix→coil transition in the amylose chain.

Hydrophobic interactions play an important role in a variety of biological systems, for example, the binding of metabolites and drugs to serum albumin as a transport protein, the binding of cofactors and inhibitors to enzymes, antigen–antibody interactions, the immobilization of enzymes *in vivo*, and the structure of cell membranes. In this respect, the nonspecific interaction of proteins with the helical amylose–iodine complex may serve as a model for understanding biological processes involving hydrophobic interactions.

EXPERIMENTAL

Potato amylose of high molecular weight was prepared by the urea dispersion method[19]. Carbohydrate-free Concanavalin A was isolated from *Canavalia ensiformis* (white and red varieties of Abai seeds) by essentially the method of Agrawal and Goldstein[20] with a few modifications. Bovine serum albumin, egg albumin, vitamin-free casein, calf-thymus histone, RNA, DNA, glycogen, and amino acids were commercial materials. All other chemicals were of "AnalaR" grade. Protein solutions (~1 mg/ml) were prepared in 0.145M saline. Reactions were carried out in glass-stoppered test-tubes in Tris–HCl buffer (0.1M, pH 7.2) at 30° ±0.05°. Water saturated with iodine vapour at 26–28° was used[21,22]. A sub-optimum concentration of iodine was chosen (25% of the optimum), so that it was complexed with amylose and its interaction with protein could be avoided.

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REFERENCES

1 R. E. RUNDLE AND F. C. EDWARDS, *J Am. Chem. Soc.*, 68 (1943) 2200–2203.
2 W BANKS AND C T. GREENWOOD, *Carbohydr. Res* , 21 (1972) 229–234.
3 R. M. PURVINAS AND H. F. ZOBEL, *Carbohydr. Res.*, 10 (1969) 129–139.
4 H. BITTIGER, E. HUSEMANN, AND B. PFANNEMUELLER, *Staerke*, 23 (1971) 113–117.
5 E. HAMORI AND M. B. SENIOR, *Ann. N. Y. Acad. Sci.*, 210 (1973) 34–37; M. B. SENIOR AND E. HAMORI, *Biopolymers*, 12 (1973) 65–78
6 J. F. FOSTER, in R. L. WHISTLER AND E. F. PASCHALL (Eds.), *Starch: Chemistry and Technology*, Vol. 1, Academic Press, New York, 1965, pp. 349–389.
7 I. J. GOLDSTEIN, C. E. HOLLERMAN, AND J. M. MERRICK, *Biochim. Biophys. Acta*, 97 (1965) 68–76.
8 S V. ABHYANKAR, S. V. BHIDE, AND N R KALE, *Abstr. Papers, 44th Ann Gen. Meeting Soc. Biol. Chemists (India)*, *Calcutta*, 34 (1975) 9.
9 F. J. GERMINO AND R M. VALLETTA, *J. Polym. Sci., Part A-2*, (1964) 4757–4763.
10 R. L. WHISTLER, in R. L. WHISTLER AND E. F. PASCHALL (Eds.), *Starch· Chemistry and Technology*, Vol 1, Academic Press, New York, 1965, pp. 331–346.

11 C. TANFORD, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York, 1973, pp. 126–142.
12 Z. ER-EL, Y. ZAIDENZAIG, AND S. SHALTELL, *Biochem. Biophys. Res. Commun*, 49 (1972) 383–390
13 J. PORATH, L. SUNDBERG, N. FORNSTEDT, AND I. OLSSON, *Nature (London)*, 245 (1973) 465–466.
14 B. H. J. HOFSTEE, *J. Macromol. Sci, Part A-1*, (1976) 111–147.
15 K. D. CALDWELL, R. AXEN, AND J PORATH, *Biotechnol Bioeng.*, 58 (1976) 433–438
16 S. HJERTEN, *J Chromatogr*, 87 (1973) 325–331.
17 O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
18 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F SMITH, *Anal. Chem*, 28 (1956) 350–356.
19 N. B. PATIL, P. F. GUPTE, AND N. R. KALE, *Makromol. Chem.*, 176 (1974) 1979–1994
20 B. B. L. AGRAWAL AND I. J. GOLDSTEIN, *Biochim. Biophys Acta*, 147 (1967) 262–271.
21 A. D. P. AWTRAY AND R. C. CONNICK, *J. Am. Chem Soc.*, 73 (1951) 1842–1848.
22 S. V. BHIDE AND N. R. KALE, *Biochim. Biophys. Acta*, 444 (1976) 719–726