Physicochemical Properties of Gossypin (11S Protein) and Congossypin (7S Protein) of Glanded Cottonseed

I. Mohan Reddy[1] and M. S. Narasinga Rao*

Gossypin and congossypin, major storage proteins of cottonseed, are characterized by sedimentation constants ($S_{20,w}$) of 11.0 and 7.6, molecular weights of 240 000 and 143 000, $E_{1cm}^{1\%}$ at 280 nm of 7.6 and 6.0, and UV absorption maxima of 279 and 277 nm, respectively. Both proteins have a fluorescence emission maximum at 325 nm. They have a globular shape, an oligomeric structure with subunits, and a secondary structure consisting mostly of β-pleated and aperiodic structures. The proteins are rich in glutamic acid, arginine, and aspartic acid and relatively low in sulfur amino acids.

The physical and chemical properties of a protein exert major effects on its functional properties, which in turn determine its end use in foods. Therefore, a detailed knowledge of the structure and properties of proteins is essential for their better utilization. Gossypin, the 11S protein, and congossypin, the 7S protein (Mohan Reddy and Narasinga Rao, 1988), are the storage proteins and constitute the major fractions of cottonseed proteins. Because of the potential of cottonseed proteins as food supplements, gossypin and congossypin have been the subject of numerous investigations (Cherry and Leffler, 1984). Several methods for the isolation of these proteins, and some of their physicochemical properties have been reported (Rossi-Fanelli et al., 1964; Ibragimov et al., 1969; Yuldasheva et al., 1975; Youle and Huang, 1979; Zarins and Cherry, 1981; Mohan Reddy et al., 1982; Zarins et al., 1984). A recent review of the physicochemical properties of gossypin and congossypin by Cherry and Leffler (1984) reveals that no information is available on the conformational and spectral aspects of these proteins.

In a previous paper, we reported an integrated method for the isolation of gossypin and congossypin (Mohan Reddy and Narasinga Rao, 1988). In this paper the amino acid composition, spectral and conformational properties, and subunit composition of these proteins are reported.

EXPERIMENTAL SECTION

Materials. Glanded cottonseeds (*Gossypium herbaceum* var. Jaydhar) were obtained from Karnataka State Seeds Corp. Ltd., Mysore, India, and stored cold.

The sources of chemicals used were as follows: tris-(hydroxymethyl)aminomethane (Tris), *N*-bromosuccinimide (NBS), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), dithiothreitol, bovine serum albumin (BSA), ovalbumin, pepsin, trypsinogen, β-lactoglobulin, lysozyme, ribonuclease A, and Coomassie brilliant blue were from Sigma Chemical Co.; ammonium persulfate and bis(acrylamide) were from Koch-Light Laboratories; TEMED and 2-mercaptoethanol (2-ME) were from Fluka; sodium dodecyl sulfate (SDS) and bromophenol blue were from BDH. SDS was recrystallized twice from aqueous ethanol. All other chemicals used were of reagent grade.

Isolation of Gossypin and Congossypin. The proteins were isolated from low-gossypol cottonseed flour by the method described previously (Mohan Reddy and Narasinga Rao, 1988). The proteins were found to be homogeneous by sedimentation velocity, gel electrophoresis, gel filtration, and ion-exchange chromatography.

Protein Concentration. Absorption values of 7.6 and 6.0 at 280 nm for 1% solution were used for determining the concentration of gossypin and congossypin, respectively (Mohan Reddy and Narasinga Rao, 1988).

Amino Acid Analysis. Protein samples (10 mg) were hydrolyzed in vacuuo in 6 N HCl (electronic grade) containing 0.1% phenol and 0.01% 2-mercaptoethanol for 24 h at 110 ± 1 °C according to the method of Moore and Stein (1963). Amino acid analysis was performed with an LKB 4150 α-amino acid analyzer equipped with a programmer and integrator. An LKB standard amino acids kit was used as an internal standard to calibrate the integrator.

Tryptophan Estimation. Since tryptophan is destroyed during acid hydrolysis (Blackburn, 1968), it was estimated by the NBS method of Spande and Witkop (1967).

Sulfhydryl Group Estimation. The –SH and SS group contents of the protein were estimated by the method of Beveridge et al. (1974) using Ellman's reagent.

Sedimentation Velocity. Sedimentation velocity experiments were carried out at 27 °C in a Spinco Model E analytical ultracentrifuge equipped with phase-plate Schleiren optics and rotor temperature indicator and control (RTIC) unit, using a standard 12-mm single-sector Kel F cell centerpiece and 1% protein solution at a speed of 59 780 rpm. The plates were read in a Abbe comparator adopted to read the ultracentrifuge plates and $S_{20,w}$ values calculated by the standard procedure (Schachman, 1959).

Viscosity. Apparent viscosity measurements were made on 0.5–2.5% protein solutions in 0.12 M phosphate buffer of pH 8.0 with an Ostwald viscometer having a flow time of 174 s with distilled water at 30 ± 0.1 °C. The protein solutions were passed through a Millipore filter (0.45 μm) before introduction into the viscometer. After temperature equilibration for 30 min, the flow time was measured to within ±0.1 s. The intrinsic viscosity was determined by extrapolation to zero protein concentration of reduced viscosities determined at five different protein concentration.

Molecular Weight Determination. Molecular weight (M_r) was determined from $S_{20,w}$ values and viscosity data by the equation (Schachman, 1959)

$$M_r = \frac{4690(S_{20,w})^{3/2}[\eta]^{1/2}}{(1 - \bar{v}\rho)^{3/2}}$$

where $S_{20,w}$ is the sedimentation constant in water at 20 °C (Svedberg units), $[\eta]$ is the intrinsic viscosity (dL/g), $\bar{v}$ is the partial specific volume, assumed as 0.73 g/mL (Tanford, 1969), and ρ is the density of the solute.

Determination of Subunits and Their Molecular Weights. The number of subunits and their molecular weights were determined by analytical sodium dodecyl

---

Protein Technology Discipline, Central Food Technological Research Institute, Mysore 570 013, India.
[1] Present address: Institute of Food Science, Cornell University, Ithaca, NY 14853.

Table I. Amino Acid Composition of Gossypin and Congossypin

amino acid	gossypin g/100 g protein[a]	gossypin res/M_r 240 000 protein[b]	congossypin g/100 g protein[a]	congossypin res/M_r 143 000 protein[b]
aspartic acid[d]	10.70	223	10.39	129
threonine	2.84	78	3.01	49
serine	4.00	95	4.85	69
glutamic acid[d]	22.46	418	22.41	248
proline	3.30	82	3.50	52
glycine	5.15	217	3.96	89
alanine	4.45	150	3.98	80
half-cystine	0.81	19	nd	
valine	5.27	128	6.28	91
methionine	0.58	11	nd	
isoleucine	4.64	98	3.33	42
leucine	6.57	139	6.00	76
tryptophan[c]	1.78	23	0.62	5
tryrosine	1.53	22	4.10	36
phenylalanine	6.32	103	8.37	81
histidine	2.73	48	3.74	39
lysine	2.67	50	3.17	35
arginine	14.20	218	11.70	107

[a] Values are averages of two, independent determinations. [b] To the nearest integer values. [c] Estimated by the method of Spanda and Witkop (1967). [d] Includes asparagine and glutamine. nd = not detected.

sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), using BSA (68 000), ovalbumin (43 000), pepsin (35 000), trypsinogen (24 000), β-lactoglobulin (18 400), and lysozyme (14 300) as molecular weight markers. Using 10% separating gel and 5% stacking gel, the sample (80–100 μg) containing 2% SDS and 0.77% dithiothreitol was loaded and electrophoresis carried out in 0.025 M Tris–glycine buffer of pH 8.3 containing 0.1% SDS for 2 h at 4 mA/gel. The gels were fixed in methanol–acetic acid–water (400:70:530) for 10 h, stained in 0.1% Coomassie brilliant blue R-250 for 10–12 h, and diffusion destained in methanol–acetic acid–water (50:75:875). Relative mobilities of the bands were plotted on a semilog paper, from which the molecular weight of the subunits of gossypin and congossypin was determined.

Absorption Spectra. The absorption spectrum of the two proteins in 0.05 M Tris-HCl buffer, pH 7.0, containing 1.0 M NaCl was recorded in a Beckman DU-8B spectrophotometer in the range 240–500 nm, in a 1-cm quartz cell at 27 °C.

Fluorescence Spectra. Fluorescence emission spectra were measured in a Perkin-Elmer Hitachi fluorescence spectrophotometer, Model 203, at room temperature (∼27 °C), in the range 300–400 nm after excitation at 280 nm. Protein solutions having an absorbance of 0.1 at 280 nm in 1-cm cell were used.

Circular Dichroism (CD) Spectra. The measurements were made with a Jasco-J 20C automatic recording spectropolarimeter equipped with xenon arc lamp. The instrument was calibrated with camphor-d_{10}-sulfonic acid. Slits were programmed to yield a 10-Å (1-nm) bandwidth at each wavelength. All measurements were made in 0.12 M phosphate buffer, pH 8.0, at 29 °C unless otherwise mentioned. The measurements were made in the far-ultraviolet region (200–260 nm) and near-ultraviolet region (250–350 nm) using quartz cells of path length 0.1 and 1.0 cm and protein concentrations ranging from 0.6–0.8 and 2.0–2.2 mg/ml, respectively. Mean residue ellipticity, $[\theta]_{MRW}$ (deg·cm²·dmol⁻¹), was calculated by the standard procedure (Adler et al., 1973). A value of 115 for the mean residue weight (MRW) was used based on the amino acid composition of the proteins.

Table II. Chemical and Physicochemical Constants of Gossypin and Congossypin

	gossypin	congossypin
sedimentn const $(S_{20,w})$[a,b]	11.0	7.6
intrins viscosity $[\eta]$,[b] dL·g⁻¹	0.034	0.037
M_r (by sedimentn velocity and viscosity method)	240 000	143 000
abs coeff $(E_{1cm}^{1\%})$ at 280 nm[a]	7.6	6.0
abs max,[a,b] nm	279	277
fluorescence emission max,[b] nm	325	325
phosphorus content,[a] %	nil	nil
gossypol content,[a] %		
(a) free	nil	nil
(b) bound	nil	nil
no. of subunits (SDS-PAGE)[e]	5 (nonidentical; 3 major; 2 minor)	8 (nonidentical)

[a] Mohan Reddy and Narasinga Rao (1986). [b] Values are in 0.05 M Tris-HCl buffer, pH 7.0, containing 1.00 M NaCl. [c] Values are in 1.0 M NaCl. [d] Values are in 0.12 M phosphate buffer, pH 8.0. [e] In 0.025 M Tris–glycine buffer, pH 8.3, containing 0.1% SDS.

RESULTS AND DISCUSSION

Amino Acid Composition. The amino acid compositions of gossypin and congossypin are shown in Table I. The -SH and SS groups estimated by Ellman's reagent gave values of 2 and 32 μg/g of gossypin and 8 and 4 μg/g of congossypin, respectively. Amino acid composition indicates that there are some differences in glycine, valine, isoleucine, tryptophan, tyrosine, phenylalanine, histidine, and arginine contents of the proteins. However, the proteins are rich in glutamic acid, arginine, and aspartic acid and relatively low in cystine and methionine. This relationship seems to be common to globulins of seed proteins in general (Altschul et al., 1966) and oilseed proteins in particular (Prakash and Narasinga Rao, 1984).

Elmore and King (1978) reported that arginine, glutamic acid, and aspartic acid play an important role during embryogenesis and germination of cottonseed. They further reported the following: (i) Arginine was the major source of reduced nitrogen for the germinating cottonseed and was used in preference to some other amino acids. (ii) Glutamine is the principal metabolic form of reduced nitrogen during cottonseed germination. (iii) Asparagine is the chief nitrogen transport compound in the process of cottonseed germination. Similar results were reported for lupin seed (Atkins et al., 1975). Therefore, the predominance of glutamic acid, arginine, and aspartic acid in gossypin and congossypin is related to their role in cottonseed metabolism.

Physicochemical Properties. The different physicochemical properties of gossypin and congossypin are listed in Table II. The intrinsic viscosities of gossypin and congossypin in 0.12 M phosphate buffer, pH 8.0, were 0.034 and 0.037 dL/g, respectively. To the best of our knowledge there are no reports on the intrinsic viscosity of these proteins. Globular proteins are reported to have intrinsic viscosity values in the range 0.03–0.04 dL/g (Tanford, 1969). Thus, gossypin and congossypin appear to have a globular shape and compact structure.

The $S_{20,w}$ value was 11.0 for gossypin and 7.6 for congossypin (Table II). Molecular weights of gossypin and congossypin computed from $S_{20,w}$ and intrinsic viscosity (Schachman, 1959) data were 240 000 and 143 000 respectively. There is some variation in the sedimentation coefficient and molecular weight reported earlier for these proteins (Naismith, 1956; Rossi-Fanelli et al., 1964; Ibra-

gimov et al., 1969; Martinez et al., 1970; Yuldasheva et al., 1975; Yunusov et al., 1979; Martinez, 1979; Youle and Huang, 1979; Zarins and Cherry, 1981; Mohan Reddy et al., 1982; Zarins et al., 1984). The reported value for sedimentation coefficient for gossypin varies from 9 S to 13 S and for congossypin from 5 S to 9.6 S. Molecular weights for gossypin vary from 180 000 to 280 000 and for congossypin from 127 000 to 180 000. Martinez et al. (1970) reported that gossypin and congossypin undergo independently association–dissociation reactions with changes in ionic strength of alkaline buffer. Zarins and Cherry (1981) observed that native acalin A (congossypin) molecule dimerized in dilute NaCl solution in the presence of phosphate ions. Therefore, species variation, differences in the method of isolation of the proteins, and conditions used for ultracentrifugation could have contributed to the reported variations in sedimentation coefficient and molecular weight of these proteins.

The ultraviolet absorption spectrum of gossypin consisted of a maximum at 279 nm and minimum at 250 nm; for congossypin these values were 277 and 248 nm, respectively. The spectra were typical of proteins. The ratio of absorbance at 280 nm to that of 260 nm was 1.53 for gossypin and 1.50 for congossypin. From these values it could be concluded that the proteins were free from nucleic acid impurities (Layne, 1957). This was compatible with our earlier observation that the proteins did not contain any phosphorus (Mohan Reddy and Narasinga Rao, 1988). Absence of phosphorus and thus nucleic acid impurities in the proteins indicates that they are essentially nonconjugated proteins.

Rossi-Fanelli et al. (1964) reported an absorption maximum of 278 nm for acalin A (congossypin), and a similar value was reported for congossypin isolated by DEAE-cellulose chromatography (Mohan Reddy et al., 1982). Absorption maximum for gossypin has not been reported earlier.

Absence of any spectra in the region 380–390 nm where free and bound gossypol absorb (Lyman et al., 1959) was in agreement with our earlier observation that the proteins did not have these impurities (Mohan Reddy and Narasinga Rao, 1988). This observation was confirmed by CD spectral evidence also. When gossypol binds to bovine serum albumin, it induces extrinsic CD bands at 285–390 nm and 300 nm (Maliwal et al., 1985). The CD spectrum of gossypin and congossypin in the region 400–300 nm (not shown in Figure 3) did not indicate peaks at 390 or 300 nm. These are in agreement with our observation on 7s globulin isolated by ion-exchange chromatography (Mohan Reddy et al., 1982). Generally in the published literature, the authors do not state whether the isolated gossypin or congossypin is free from gossypol (free and bound) impurities. This was of particular interest to us since these proteins were used in the study of protein–gossypol interactions.

The fluorescence emission spectrum of the proteins gave a maximum of 325 nm when excited at 280 nm (Figure 1). In aqueous solution free tryptophan gives an emission maximum at 348 nm, tyrosine at 303 nm, and phenylalanine at 278 nm (Lapanje, 1978). Teale (1960) reported that fluorescence emission of proteins having both tryptophan and tyrosine residues is more characteristic of tryptophan, and tyrosyl emission is suppressed. The protein contains both tryptophan and tyrosine (Table I). Thus, the observed maximum at 325 nm indicates that the fluorescence is determined by tryptophan groups embedded in a nonpolar environment of gossypin and congossypin (Shifrin et al., 1971; Mills and Creamer, 1975).
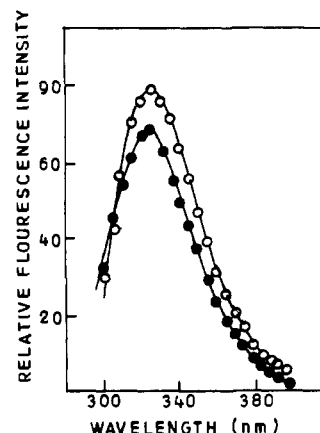

Figure 1. Fluorescence spectra of gossypin and congossypin in 0.05 M Tris-HCl buffer, pH 7.0, containing 1 M NaCl: O, gossypin; ●, congossypin.
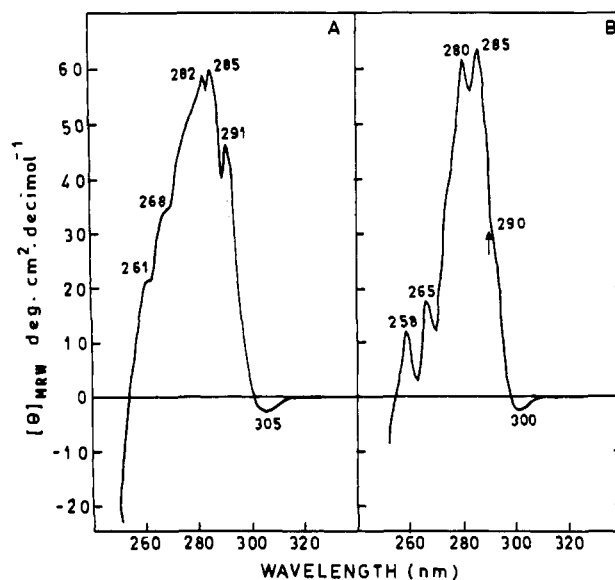

Figure 2. Near-UV CD spectrum of (A) gossypin and (B) congossypin in 0.12 M phosphate buffer, pH 8.0.

The near-UV CD spectrum of gossypin in the region 330–250 nm (Figure 2A) exhibited a trough at 305 nm, positive peaks at 291, 285, and 282 nm, and positive shoulders at 268 and 261 nm; whereas that of congossypin (Figure 2B) exhibited a trough at 300 nm, positive shoulder at 290 nm, and positive peaks 285, 280, 265, and 258 nm.

The CD bands in this region of the spectrum arise from tryptophanyl, tyrosyl, phenylalanyl, and cystinyl side chains and some prosthetic groups (Strickland, 1974). The shoulder at 261 and 268 nm of gossypin and peaks at 258 and 265 nm of congossypin could be assigned to phenylalanine residues; peaks at 280, 282, and 285 nm were possibly due to tyrosine; and the shoulder at 290 nm, peak at 291 nm, and troughs at 300 and 305 nm are due to tryptophan residues (Strickland, 1974). Cystinyl side chains are also known to contribute to the near-ultraviolet CD spectrum, especially in the region 250–270 nm. The amino acid composition of the proteins (Table I) also reveals the presence of these amino acid residues. The minor differences in the near-ultraviolet CD spectrum of the two proteins could be attributed to the differences in their amino acid content.

The far-UV CD spectrum of gossypin in the region 260–200 nm (Figure 3A) showed a trough at 206 nm and a shoulder at 225 nm, whereas that of congossypin (Figure 3B) showed a trough at 210 nm and shoulder at 225 nm.
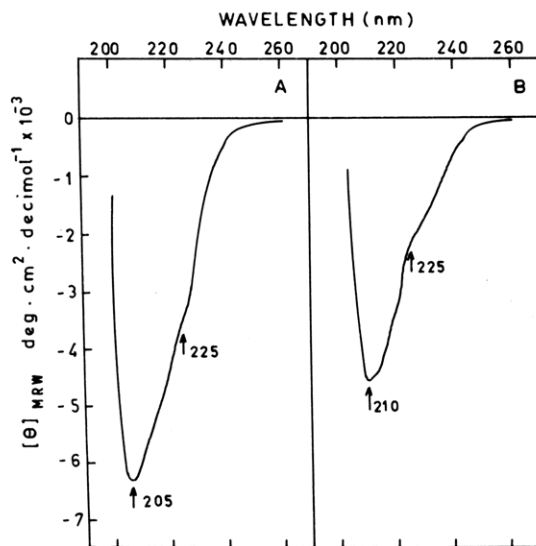
Figure 3. Far-UV CD spectrum of (A) gossypin and (B) congossypin in 0.12 M phosphate buffer, pH 8.0.

Figure 4. SDS-PAGE pattern of (A) gossypin and (B) congossypin in 0.025 M Tris–glycine buffer, pH 8.3, containing 0.1% SDS.

Gossypin has higher ellipticity values at both wavelengths than congossypin. These bands are known to arise from n–π transition of the peptide chromophore and may be due to the presence of antiparallel-β and cross-β structures (Stevens et al., 1968; Raghavendra and Ananthanarayanan, 1981). However, the absence of any fine structure in the spectra possibly indicates the presence of a large proportion of aperiodic strucure in the proteins.

The estimate of secondary structure from the far-UV CD spectral data is unreliable when the content of aperiodic structure is high (Greenfield and Fasman, 1969). Also, meaningful estimates can only be made when spectra are measured down to 184 nm (Manavalan and Johnson, 1985). For these reasons we have refrained from calculating the secondary structure from CD data. Perhaps the only valid conclusion that can be drawn is that the content of ordered structure is more in gossypin than in congossypin.

Since the molecular weights of gossypin and congossypin were high, they were likely to be oligomeric and consist of subunits (Klotz et al., 1970). SDS-PAGE experiments in the presence of dithiothreitol were therefore carried out to determine the number and molecular weight of the subunits. Gossypin consisted of three major bands; two minor bands were also observed (Figure 4A). The molecular weights of the major bands were 25 000, 22 000, and 14 000. Subunits with molecular weights of 28 000, 24 000, ad 17 000 (Yunusov et al., 1978) and 23 000, 20 000, and 15 000 (Cherry and Lefflar, 1984) have been reported for gossypin.

Congossypin consisted of at least eight nonidentical subunits having molecular weights of 66 000, 57 000, 37 000, 34 000, 21 300, 19 800, 18 000, and 16 000 (Figure 4B). Similar numbers of subunits with some variation in molecular weight have been reported for congossypin (Mohan Reddy et al., 1982; Cherry and Leffler, 1984). Thus, the results obtained in the present investigation are compatible with those reported earlier for gossypin and congossypin.

Since all the subunits were not of equal intensity and stoichiometry of the subunits was not established, no attempt was made to calculate the molecular weight of the proteins from SDS-PAGE.

The role of carbohydrates in modifying the physicochemical properties of glycoproteins is well documented (Allen, 1983). Mohan Reddy (1985) reported the carbohydrate content of gossypin as 0.078 and of congossypin as
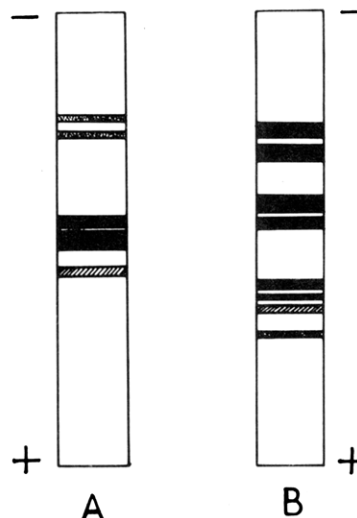
1% expressed in glucose units. Thus, the carbohydrate content of gossypin is very low, and possibly it is not a glycoprotein. Congossypin is reported to contain glucosamine and mannose.

LITERATURE CITED

Adler, A. J.; Greenfield, N. J.; Fasman, G. D. Methods Enzymol. 1973, 27D, 675–735.

Allen, A. Trends Biochem. Sci. 1983, 8, 169.

Altschul, A. M.; Yatsu, L. Y.; Ory, R. L.; Engleman, E. M. Annu. Rev. Plant Physiol. 1966, 17, 113–136.

Atkins, C. A.; Pate, J. S.; Sharkey, P. J. Plant Physiol. 1975, 56, 807–812.

Beveridge, T.; Toma, S. J.; Nakai, J. Food Sci. 1974, 39, 49–54.

Blackburn, S. Amino Acid Determination, Methods and Techniques; Marcel Dekker: New York, 1968; pp 13–22.

Chen, Y. H.; Yang, J. T. Biochem. Biophys. Res. Commun. 1971, 44, 1285–1291.

Cherry, J. R.; Leffler, H. R. In Cotton, Agronomy Monograph No. 24, 1984, 511–569.

Elmore, C. D.; King, E. E. Plant Physiol. 1978, 62, 531–535. Greenfield, N. J.; Fasman, G. D. Biochemistry 1969, 8, 4108–4116.

Ibragimov, A. P.; Yunuskhanov, Sh.; Tuichev, A. V. Biochemistry (Engl. Transl.) 1969, 34, 897–901.

Klotz, I. M.; Langerman, N. L.; Darnail, D. W. Annu. Rev. Biochem. 1970, 39, 25–62.

Laemmli, U. K. Nature (London) 1970, 227, 680–685.

Lapanje, S. Physicochemical Aspects of Protein Denaturation; Wiley: New York, 1978; pp 19–48.

Layne, E. Methods Enzymol. 1957, 3, 447–454.

Lyman, C. M.; Baliga, B. P.; Slay, W. W. Arch. Biochem. Biophys. 1959, 84, 486–497.

Maliwal, B. P.; Appurao, A. G.; Narasinga Rao, M. S. Int. J. Peptide Protein Res. 1985, 25, 382–388.

Manavalan, P.; Johnson, W. C., Jr. J. Biosci, (Suppl) 1985, 8, 141–149.

Martinez, W. H. JAOCS, J. Am. Oil Chem. Soc. 1979, 56, 280–284.

Martinez, W. H.; Berardi, L. C.; Goldblatt, L. A. J. Agric. Food Chem. 1970, 18, 961–968.

Mills, O. E.; Creamer, L. K. Biochim. Biophys. Acta 1975, 379, 618–626.

Mohan Reddy, I. Ph.D. Dissertation, University of Mysore at Mysore, 1985.

Mohan Reddy, I.; Narasinga Rao, M. S. J. Agric. Food Chem. 1988, companion paper in this issue.

Mohan Reddy, I.; Appu Rao, A. G.; Narasinga Rao, M. S. *J. Biosc.* 1982, *4*, 197-208.

Moore, S.; Stein, W. H. *Methods Enzymol.* 1963, *6*, 819-831.

Naismith, W. E. F. *J. Appl. Chem.* 1956, *6*, 283-286.

Prakash, V.; Narasinga Rao, M. S. *Proc. Indian Acad. Sci. (Chem. Sci.)* 1984, *93*, 1205-1214.

Raghavendra, K.; Ananthanarayanan, V. S. *Int. J. Peptide Protein Res.* 1981, *17*, 412-419.

Rossi-Fanelli, A.; Antonini, E.; Brunori, M.; Bruzzeri, M. R.; Caputo, A.; Satriani, F. *Biochem. Biophys. Res. Commun.* 1964, *15*, 110-115.

Sarakar, P. K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* 1966, *55*, 981-989.

Schachman, H. K. *Ultracentrifugation in Biochemistry*; Academic: New York, 1959; p 242.

Shifrin, S.; Luborsky, S. W.; Grochwski, B. J. *J. Biol. Chem.* 1971, *246*, 7708-7714.

Spande, T. F.; Witkop, B. *Methods Enzymol.* 1967, *2*, 498-506.

Stevens, L.; Townend, R.; Timasheff, S. N.; Fasman, G. D.; Potter, J. *Biochemistry* 1968, *7*, 3717-3720.

Strickland, E. H. *CRC Crit. Rev. Biochem.* 1974, *2*, 113-175.

Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, 1969; Chapter 6, p 394.

Teale, F. W. J. *Biochem. J.* 1960, *76*, 381-388.

Youle, R. J.; Huang, A. H. C. *J. Agric. Food Chem.* 1979, *27*, 500-503.

Yuldasheva, N. P.; Kuchenkova, M. A.; Yuldashev, P. Kh. *Khim. Prir. Soedin* 1975, *11*, 277-278; *Chem. Abstr.* 1975, *83*, 73855f.

Yunusov, T. S.; Asatov, S. I.; Yadgarov, E. G.; Yuldashev, P. Kh. *Tezisy Dokl.—Sov.-Indiiskii Symp. Khim. Prir. Soedin.*, 5th 1978, 9; *Chem. Abstr.* 1980, *93*, 110604C.

Zarins, Z. M.; Cherry, J. P. *J. Food Sci.* 1981, *46*, 1855-1859, 1862.

Zarins, Z. M.; Phillips, R. D.; Martinez, W. H. *Cereal Chem.* 1984, *61*, 471-474.

Interaction of Gossypol with Gossypin (11S Protein) and Congossypin (7S Protein) of Cottonseed and Glycinin (11S Protein) of Soybean. 1. Reaction Kinetics, Binding Stoichiometry, and Reversibility Studies

I. Mohan Reddy[1] and M. S. Narasinga Rao*

Interaction of gossypol with gossypin, congossypin, and glycinin at pH 9.0, 8.0, and 7.6, respectively, followed by a difference spectral method, indicated that the gossypol–protein complexes were characterized by difference absorption maxima at 430–432 nm (gossypin), 436–437 nm (congossypin), and 430 nm (glycinin). The reaction was time dependent and was complete by 2 h with gossypin, 3 h with congossypin, and 10 h with glycinin. The number of binding sites in protein (n) was 4.0 for both gossypin and congossypin and 5.0 for glycinin. Thus, gossypin and congossypin bind a maximum of 4 gossypol molecules/mol of protein, while glycinin binds 5. Interaction was completely reversible, suggesting that only nonconvalent interactions were involved. The low association constants (K) suggested that the binding was of weak type and involved nonconvalent interactions.

Cottonseed proteins are widely recognized as a potential source of nutrients for human consumption. But their utilization for food uses is limited because of the presence of a toxic polyphenolic pigment, gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-2,2′-binaphthalene-8,8′-dicarboxaldehyde], which is known to interact with proteins during heat processing of the cottonseed kernels (Clark, 1928). Martinez and Frampton (1958), Conkerton and Frampton (1959), Markman and Rzhekhin (1965), and Damaty and Hudson (1975b) have produced evidence to indicate that a major form of binding is the formation of Schiff bases by condensation of the formyl groups of gossypol with ε-amino groups of lysine. However, Bressani et al. (1964) suggested that other factors must be involved in addition to Schiff base formation. Damaty and Hudson (1979) have established that application of heat on flours and isolates containing free gossypol resulted in the formation of insoluble, unhydrolyzable products due to irreversible copolymerization

between gossypol and cottonseed proteins. It is evident from the literature that the exact nature of the interaction has not yet been clearly established, and no systematic study of the interaction of gossypol with cottonseed proteins, under controlled conditions of temperature, pH, etc., has been made. Further, these studies have been made with total proteins, and such studies with total proteins do not enable one to draw conclusions on the nature of binding sites on protein molecule, etc. Therefore, a study of the interaction of gossypol with isolated 11S (gossypin) and 7S (congossypin) proteins of cottonseed (Mohan Reddy and Narasinga Rao, 1988), which constitute about 65% of the total proteins, was initiated. A difference spectral method was used to follow the interaction. A study of the spectral properties of the gossypol–protein complex, reaction kinetics, binding stoichiometry, and reversibility of the interaction is presented. The nature of interaction was predicted from the thermodynamic constants calculated from the difference spectral data. Though the 11S and 7S proteins of cottonseed used in this investigation were free from gossypol impurities (Mohan Reddy and Narasinga Rao, 1988), interaction with another oilseed protein, soybean glycinin, which does not contain any gossypol, was followed for the purpose of comparison. Glycinin resembles 11S or 7S protein of cottonseed in its

---

Protein Technology Discipline, Central Food Technological Research Institute, Mysore 570 013, India.

[1] Present address: Institute of Food Science, Cornell University, Ithaca, NY 14853.