Intrinsic Fluorescence and Quenching Studies of Gluten Proteins[1,2]

S. G. STEVENSON and K. R. PRESTON

Cereal Chem. 71(2):155-159

ABSTRACT

Intrinsic fluorescence and quenching studies of gluten protein extracts and their gel-filtration fractions were used to examine the location and environment of tryptophan residues in wheat gluten. The gluten protein was extracted with either 2*M* sodium thiocyanate (NaSCN), 0.05*M* acetic acid (HAC), 3*M* guanidine hydrochloride (GuHCl), or 3*M* urea. Samples for gel filtration were extracted with 2*M* NaSCN. Stern-Volmer quenching constants (K_{SV} ~8) and accessibilities (74–124%) obtained for acrylamide quenching of gluten protein extracts indicated that the majority of the tryptophan residues are located on the surface of these proteins or in easily accessible (polar) regions. Emission maxima near 350 nm (EX = 295 nm) and a lack of change in the magnitude of Stokes' shift under denaturing conditions (8*M* GuHCl or 6*M* urea) supported this conclusion. Tryptophan residues from both high (glutenin) and lower (gliadin) molecular weight gel-filtration fractions showed similar results. Accessibilities to acrylamide ranged from 101–114%. Acrylamide and iodide Stern-Volmer quenching constants for the high molecular weight fraction ($K_{SVacryl} = 5.9$, $K_{SVI^-} = 1.1$) showed values that were lower than those obtained for the low molecular weight fractions ($K_{SVacryl} = 10.1$, $K_{SVI^-} = 2.1$), suggesting differences in the topography of these proteins in the regions in which these residues are located.

The use of intrinsic and extrinsic fluorescent probes has proven effective in the study of protein structure (Eftink and Ghiron 1981, Eftink 1991a). The intrinsic fluorescence of proteins is normally dominated by tryptophan residues. Tyrosine and, to a much lesser extent, phenylalanine can also contribute.

Tryptophan fluorescence is very sensitive to solvent polarity. Thus, the emission spectra of tryptophan residues in proteins is strongly influenced by their environment. This information can be useful in providing an indication of the location of these residues in proteins, as well as affording methods to study association reactions and denaturation (Teale 1960). However, other factors such as poor resolution of individual residues and specific solvent effects may complicate the interpretation of spectral data.

[1]Contribution 699 of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg, MB. R3C 3G8

[2]Presented at the AACC 77th Annual Meeting, Minneapolis, MN, 1992.

Fluorescence quenching techniques can be used to contribute more specific information on the location of tryptophan residues in proteins and the topography of the macromolecule itself. Quenching results can also be used to monitor structural changes which may be caused by altering ambient conditions. The most commonly used quenchers include oxygen, acrylamide, and iodide (Eftink 1991a). All three are highly efficient collisional quenchers. Analysis of the effect of quencher concentration upon protein fluorescence (Stern-Volmer plots) can supply information on quenching constants and an estimation of tryptophan accessibility (Lakowicz 1983).

Genot et al (1984) showed that tryptophan residues in 0.05*M* acetic acid soluble extracts of flour and gluten proteins have an emission maximum of approximately 350 nm. These results indicate that these residues occur mainly in a polar environment. In the present study, emission spectra and fluorescence quenching characteristics of gluten protein extracts and gel-filtration fractions were used to study the properties of tryptophan residues.

MATERIALS AND METHODS

Chemicals

The chemicals used were commercially obtained and of ACS purity unless otherwise noted; they were made up to desired concentrations using deionized, distilled water: 2.0*M* sodium thiocyanate, 0.05*M* acetic acid, 5.0*M* potassium iodide (KI) with $10^{-4}M$ sodium thiosulphate added to prevent I_3^- formation, and 0.2% w/v electrophoretic-grade dithiothreitol (DTT) from Fisher Scientific (Winnipeg, MB); 3.0*M* Grade 1 guanidine hydrochloride and 3.0*M* urea from Sigma Chemical Co. (St Louis, MO); 5.0*M* electrophoresis grade acrylamide from BioRad Laboratories (Mississauga, ON).

Preparation of Defatted Gluten

Gluten was prepared from chloroform-defatted (MacRitchie and Gras 1973) straight-grade No. 1 Canada Western Red Spring wheat flour following the method of Doguchi and Hlynka (1967). Protein content of the defatted gluten was 81.2% (N × 5.7, as is basis) as determined by the procedure of Mitcheson and Stowell (1970). Moisture content was 8.8% as determined by AACC method 44-15A (AACC 1983).

Sample Extraction and Gel Filtration

Defatted gluten (500 mg) was dispersed in 25 ml of the selected solvent by vortexing for approximately 1 min in a 40-ml capped centrifuge tube. The sample was rotated at room temperature overnight at 50 rpm on a rotator (Roto-Torque, Cole Parmer, Niles, IL), then centrifuged at 27,500 × *g* for 15 min at 20°C. The supernatant was saved, and an additional 25 ml of solvent was added to the residue in the test tube, dispersed with vortexing, and rotated for an additional 3 hr at room temperature. The sample was centrifuged at 27,500 × *g* at 20°C for 15 min, and the supernatant was pooled with that from the first extraction. A third extraction used the same conditions as the second. The resulting supernatant was combined with those from the other extractions. For gel filtration, the supernatant from the first 2*M* NaSCN extraction of a 1.0-g sample was used to obtain a high concentration of protein for column loading. Protein (N × 5.7) extraction rates were estimated by dialyzing supernatants and residues against 0.05*M* acetic acid, freeze-drying, then measuring nitrogen content by micro-Kjeldahl according to the procedure of Mitcheson and Stowell (1970).

Sephacryl S-300 Superfine (Pharmacia, Baie d'Urfé, PQ) columns were packed according to manufacturer's instructions, as outlined by Preston (1982). Final gel dimensions of approximately 2.5 × 100 cm were used. An 8-ml aliquot of the supernatant (10 mg of protein/ml) was loaded onto the column and eluted with 2*M* NaSCN at 40 ml/hr with upward flow. Protein concentration of fractions in the collection tubes was measured at 280 nm using an LKB 2138 Uvicord S monitor (LKB, Bromma, Sweden) with a 0.1-ml flow-through cell. Fractions of 8 ml per tube were collected, and contents of tubes from each of the four major peaks were used for further study. The four peaks had average molecular weights (MW) of >300,000 (fraction 1), 38,000 (fraction 2), 18,000 (fraction 3), and 14,500 (fraction 4) and were classified as glutenins, higher MW gliadins, and two lower MW gliadins, respectively (Preston 1982).

Fluorescence Measurements

Fluorescence measurements were made with a microcomputer-controlled SLM Aminco SPF-500C spectrofluorometer (SLM Instruments, Urbana, IL) using 5-nm bandwidths for both excitation and emission, as well as ratio mode and right-angle sample presentation. An excitation wavelength of 295 nm was used to isolate tryptophan fluorescence (from tyrosine and phenylalanine), and emission was measured over a range of 310–410 nm. To minimize inner cell effects, gluten extracts were diluted with buffer to maintain an absorbance (A) of 0.1 or less at 280 nm. When necessary, corrections for inner cell effects were applied to the fluorescence results as described by Bolen and Holloway (1990), that is, $F_{corr} = F_{obs}\ \text{antilog}[(A_{EX} + A_{EM})/2]$. Quenching studies were conducted at 25°C with a thermostated cell holder. Absorbances of the gluten solutions with and without added quencher were measured on a Milton Roy Spectronic 1001+ (Fisher Scientific, Winnipeg, MB). For quenching studies, small aliquots of concentrated (5.0*M*) quencher, acrylamide or KI with sodium thiosulphate ($10^{-4}M$), were added to diluted gluten extracts to produce a series of samples containing 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.1, 0.15, and 0.2*M* quencher. A volume of buffer was also added to the diluted gluten extracts to maintain the same protein concentration; the total amount of added quencher solution plus buffer was kept constant. Due to turbidity, iodate quenching could not be performed on acetic acid or urea extracts. Solvent spectra were subtracted from the sample spectral data to correct primarily for Raman scatter at approximately 328 nm (Eftink 1991b). Corrected sample spectral data were plotted to determine the maximum emission wavelength. Classical Stern-Volmer plots were used to determine Stern-Volmer collisional quenching constants (K_{SV}). For these plots, F_0/F is plotted against $[Q]$ where F_0 and F represent fluorescence intensity in the absence and presence of quencher, respectively; Q represents quencher concentration; and K_{SV} is the slope of the plot. Modified Stern-Volmer plots ($F_0/(F_0-F)$ versus $1/[Q]$) were used to estimate tryptophan accessibility to quencher. Accessibility was determined by calculating the reciprocal value of the intercept as a percentage (1/intercept × 100) (Lakowicz 1983). Stern-Volmer plots were analyzed using linear regression. Lotus 1-2-3 V2.4 (Lotus Dev. Canada, Toronto, ON) software was used for all statistical data analysis. All spectral manipulation was done with Spectrum Processor V2.4 software supplied by SLM Instruments for use with the SPF-500C. Experiments were replicated four times unless otherwise indicated.

TABLE I
Maximum Emission Wavelengths[a] and Stern-Volmer Constants[b] (K_{SV}) for Acrylamide and KI Quenching of Defatted Gluten Protein Extracted with Various Solvents at 25°C

		Acrylamide			KI	
	EM_{max} (nm)	K_{SV}[c] (M^{-1})	r[d]	Acc[e] (%)	K_{SV} (M^{-1})	r
0.05*M* HAC	350	8.2 ± 0.4	0.999	93 ± 8	...	...
2*M* NaSCN	351	8.1 ± 0.4	0.999	127 ± 43	1.6 ± 0.1	0.991
2*M* NaSCN with 0.2% DDT[f]	350	6.6 ± 0.4	0.999	111 ± 7	1.2 ± 0.1	0.992
3*M* GuHCl	353	8.3 ± 1.2	0.996	74 ± 5	5.0 ± 0.5	0.972
8*M* GuHCl	354	7.3 ± 0.3	0.999	97 ± 9	3.5 ± 0.5	0.990
3*M* Urea	352	8.5 ± 0.4	0.992	90 ± 7	...	...
6*M* Urea	352	9.4 ± 0.5	0.997	94 ± 21	...	...

[a] λ_{EX} = 295 nm, λ_{EM} range = 310–410 nm.
[b] Fluorescence values adjusted for absorbance. Mean of three to five values.
[c] Values ± standard deviation $n = 4$.
[d] Correlation coefficients.
[e] Accessibilities.
[f] $n = 2$. DTT = dithiothreitol.

TABLE II
Maximum Emission Wavelengths[a] and Stern-Volmer Constants[b] (K_{SV}) for Acrylamide and KI Quenching of Gel-Filtration Fractions from 2.0*M* NaSCN Gluten Protein Extracts at 25°C

Gel-Filtration Fractions[c]	EM_{max} (nm)	Acrylamide K_{SV}[d] (M^{-1})	Acrylamide r[e]	Acc[f] (%)	KI K_{SV} (M^{-1})	KI r
1	350	5.9 ± 0.4	0.999	104 ± 21	1.1 ± 0.2	0.979
2	353	10.1 ± 0.5	0.998	101 ± 7	2.1 ± 0.2	0.997
3	352	9.1 ± 1.5	0.998	113 ± 21	1.6 ± 0.4	0.993
4	350	8.6 ± 0.8	0.997	114 ± 56	1.5 ± 0.2	0.994

[a] λ_{EX} = 295 nm, λ_{EM} range = 310–410 nm.
[b] Fluorescence values adjusted for absorbance. Mean of three to five values.
[c] Average molecular weights: fraction 1 = >300,000 (glutenins); fraction 2 = 38,000 (higher molecular weight gliadins); fraction 3 = 18,000 (gliadins); fraction 4 = 14,500 (gliadins) (Preston 1982, 1984).
[d] Values ± standard deviation (n = 4).
[e] Correlation coefficients.
[f] Accessibilities.

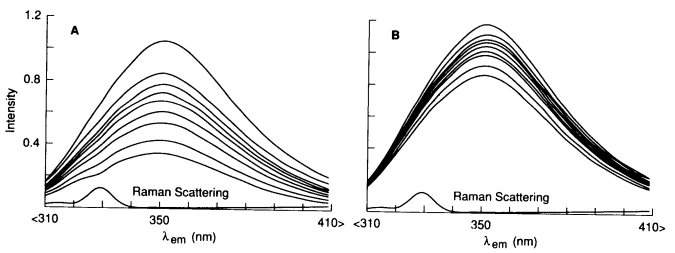

Fig. 1. Effect of quencher concentration on the fluorescence emission spectra (λ_{EX} = 295 nm) of a 2*M* NaSCN gluten extract at 25°C. Intensity of curves in descending order shown for 0, 0.03, 0.04, 0.05, 0.06, 0.08, 0.1, 0.15, and 0.2*M* quencher. Solvent curve subtracted. **A**, acrylamide; **B**, KI.

RESULTS AND DISCUSSION

Fluorescence Emission Maxima

Gluten protein extraction rates of approximately 75% were obtained with 0.05*M* acetic acid, 2.0*M* NaSCN, 3.0*M* GuHCl, and 3.0*M* urea (data not shown). Emission maxima (λ_{EX} = 295 nm) for these extracts (Table I) showed a narrow range (350–353 nm), indicating minimal solvent effects. The large red Stokes' shift for the extracts suggests that the vast majority of the gluten protein tryptophan residues reside in a polar (aqueous) environment (Konev 1967, Lehrer 1971b). Extraction under reducing conditions (2.0*M* NaSCN with 0.2% DTT, 95% protein extracted) did not alter the maximum emission wavelength (Table I). In addition, maximum emission wavelengths did not change when the proteins were subjected to denaturing conditions effected by increasing the concentration of GuHCl to 8.0*M* from 3.0*M* and urea to 6.0*M* from 3.0*M* by addition of solid reagent (Table I). These results suggest that neither reducing nor denaturing conditions alter the exposure of tryptophan residues to the solvent.

The four gel-filtration fractions, representing 2.0*M* NaSCN-soluble glutenins (fraction 1), higher MW gliadins (fraction 2), and lower MW gliadins (fractions 3 and 4), as shown previously (Preston 1982, 1984), had emission maxima similar to those of the gluten protein extracts (Table II). These maxima, ranging from 350 to 353 nm, suggest that the majority of the tryptophan residues in each fraction are located in polar environments.

Emission spectra were also obtained for gluten protein extracts and gel-filtration fractions using an excitation wavelength of 280 nm. This lower excitation wavelength, where both tryptophan and tyrosine absorb, had no detectable effect upon emission maxima. This result indicates that tryptophan still dominates the emission spectrum.

Quencher Effects

Examples of the effect of increasing concentrations of the neutral (acrylamide) and ionic (I^-) collisional quenchers upon the fluorescence emission spectra (λ_{EX} = 295 nm) of a diluted

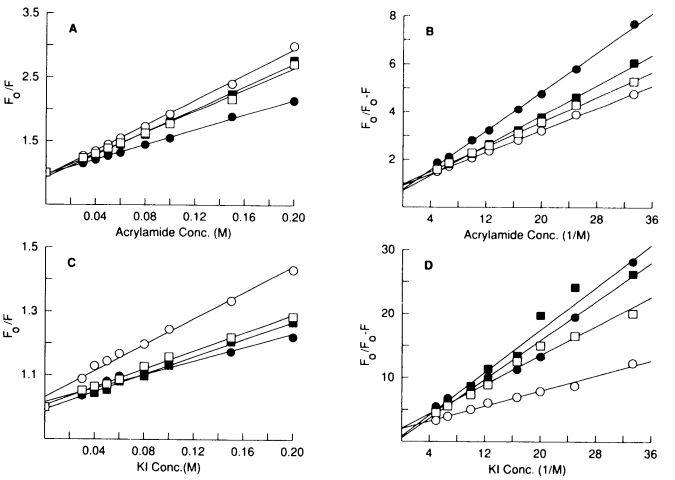

Fig. 2. Classic and modified Stern-Volmer plots for acrylamide (**A** and **B**) and KI (**C** and **D**) quenching of gel-filtration fractions of 2*M* NaSCN extracted gluten. Fractions 1 (○), 2 (●), 3 (□), 4 (■). Solvent curve subtracted.

2.0*M* NaSCN gluten extract are shown in Figure 1. For all extracts and gel-filtration fractions, peak height at maximum emission (about 350 nm) decreased with both quenchers. The effect was much more pronounced with acrylamide than with KI. Examples of both types of plots are given in Figure 2 for acrylamide and KI quenching of the four gel-filtration fractions.

Correlation coefficients for the classical Stern-Volmer plots were very high (>0.99) for extracts and gel-filtration fractions when acrylamide was the quencher (Tables I and II). These highly linear plots are normally indicative of equal accessibility for all fluorophores to quencher (Lakowicz 1983). This evidence is consistent with the effect of quencher concentration on emission spectra. Addition of acrylamide (or iodide), even at higher levels (Fig. 1), did not change the emission maxima (350 nm). The lack of blue shift in emission maxima indicates an absence of buried residues, which would show a much lower value.

When iodide was used as the quencher (Tables I and II), correlation coefficients for classical Stern-Volmer plots were not quite as high as those obtained with acrylamide. The greater degree of variability for iodide quenching, compared with that for the acrylamide, was more evident with modified Stern-Volmer plots (Fig. 2, C vs. D). Because of this variability, accurate intercepts could not be obtained from the plots and, hence, the accuracy of predicting tryptophan accessibility to I^- was compromised.

Values reported in the literature for acrylamide quenching of nongluten proteins range from K_{SV} of 1 for HSA-SDS complex, where the tryptophan is buried, to K_{SV} of 13.0 for ACTH or 10.5 for glucagon, where it is completely exposed (Eftink and Ghiron 1976a). K_{SV} values for acrylamide quenching of the gluten extracts ranged from 6.6 to 9.4M^{-1} (Table I). These values imply

rapid diffusion of the quencher to tryptophan, suggesting that these residues are primarily on or near the surface of the gluten proteins. This view is consistent with the lack of significant change in K_{SV} values of tryptophan residues under denaturing conditions when higher concentrations of GuHCl or urea were used. Reduction of the NaSCN extract with DTT resulted in a lower K_{SV} value, indicating a decrease in tryptophan accessibility. This may indicate that the reduced gluten proteins form a more compact structure, resulting in decreased accessibility by the solvent. Other proteins, for example bovine pancreatic trypsin inhibitor, show a strong tendency to fold into a more compact structure upon reduction (Tidor and Karplus 1993).

Acrylamide K_{SV} values for the gel-filtration fractions (Table II) also indicate rapid diffusion of the quencher to tryptophan. The acrylamide K_{SV} value for the glutenin high MW gel-filtration fraction ($5.9M^{-1}$) was considerably lower than values obtained with the three gliadin fractions (8.6, 9.1, and $10.1M^{-1}$). These differences suggest that tryptophan residues in gliadin proteins are more exposed than those in glutenin proteins.

Calculated tryptophan accessibilities for protein extracts in the various solvents using acrylamide as quencher ranged from 74 to 127%. The magnitude of this range and the high standard deviations associated with most values are most likely due to the wide range of different proteins in each extract and the error normally associated with accurate measurement of intercept value. For gel-filtration fractions, where the proteins in each fraction are more homogeneous, calculated acrylamide accessibility values ranged from 101 to 114%.

K_{SV} values obtained for iodide quenching (Tables I and II) were considerably lower than those obtained with acrylamide. These lower values are probably related to the inability of I^- to penetrate past the surface of the protein because of its large hydration shell (Lakowicz 1983, Eftink and Ghiron 1976b). I^- quenching constants for $2.0M$ NaSCN (with or without DTT) were lower than values obtained with other extracts (Table I). Although these lower values may be an indication of protein structural changes, it is more likely that these differences are caused by specific and nonspecific ion effects. Lehrer (1971b) demonstrated that charged quenchers, such as I^-, can interact with weak binding sites on protein surfaces near tryptophan residues. This binding is sensitive to ionic strength and to the presence of SCN^-, which is an efficient competitor for these sites (Lehrer 1971a). Lehrer (1971a) also showed that a tryptophan flanked by charged residues might appear to be quenched to a lesser or greater degree than normally expected by an anionic (I^-) quencher. Because the level of quenching was low with KI, as indicated by small K_{SV} values, it is unlikely that tryptophans in gluten proteins are surrounded by positively charged residues. Large differences were not evident among K_{SV} values (range 1.1–2.1) from the gel-filtration fractions (Table II), although the same trend appeared to be evident when compared with the acrylamide results, that is, the K_{SV} for I^- was greatest for the higher MW gliadins (fraction 2) and lowest for the glutenins (fraction 1).

CONCLUSIONS

Evidence presented in this study strongly suggests that tryptophan residues of wheat gluten proteins in aqueous solutions are primarily located on or near the protein surface or in easily accessible (polar) regions.

Gluten protein extracts showed emission maxima of 350 nm when an excitation wavelength of 295 nm was used, regardless of the aqueous solvent used for extraction. This red Stokes' shift indicates that the tryptophan residues are in a polar environment (Konev 1967, Lehrer 1971b). This evidence was supported by the lack of change in maximum emission wavelength when the gluten proteins were subjected to denaturing or reducing conditions. Previous studies by Genot et al (1984) also showed that $0.05M$ acetic acid soluble protein extracts of wheat flour and gluten had emission maxima at about 350 nm when excited at 280 nm.

The addition of quenchers (acrylamide or I^-) reduced, but did not alter, the maximum emission wavelength of the gluten protein extracts, indicating the absence of buried tryptophan residues. This finding is consistent with the highly linear Stern-Volmer plots obtained with both quenchers, which normally indicates that all the tryptophan residues are equally accessible (Lakowicz 1983).

Stern-Volmer quenching constants (K_{SV}), obtained from classic Stern-Volmer plots for acrylamide, demonstrated rapid diffusion of the quencher to tryptophan, indicating that these residues are located on or near the protein surface. High values calculated for tryptophan accessibility using modified Stern-Volmer plots for acrylamide quenching support these conclusions.

The intrinsic fluorescence properties and responses to quenchers of the four $2.0M$ NaSCN soluble gel-filtration fractions also strongly support the conclusion that their tryptophan residues are primarily located on or near the protein surface or in easily accessible (polar) regions. The lower Stern-Volmer quenching constant of the high MW (glutenin) fraction versus the lower MW (gliadin) fractions suggests differences in the topography of these proteins in the region in which these residues are located.

Genot et al (1992) recently used front-face geometry to obtain fluorescence emission and emission spectra of hydrated gluten. Their results showed an emission maxima near 333 nm, suggesting that tryptophan residues in gluten proteins are located in a hydrophobic environment. This is in direct contrast to our findings. However, Genot and coworkers used gluten samples that were much less hydrated than those used in our study. It is quite conceivable that the degree of gluten hydration could have a direct relationship on interactions among gluten proteins. At low water concentration (activity), association among these proteins may be much stronger, thereby reducing tryptophan exposure. This information suggests that tryptophan residues may prove particularly useful as intrinsic fluorescent probes to study surface properties of wheat gluten proteins.

ACKNOWLEDGMENTS

We would like to acknowledge the technical support of Christine Varnes, Maria Lopez-Canadas, and Greg Kawamoto.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 44-15A, approved October 1975, revised October 1981. The Association: St. Paul, MN.

BOLEN, E. J., and HOLLOWAY, P. W. 1990. Quenching of tryptophan fluorescence by brominated phospholipid. Biochem. 29:9638.

DOGUCHI, M., and HLYNKA, I. 1967. Some rheological properties of crude gluten mixed in the farinograph. Cereal Chem. 44:561.

EFTINK, M. R. 1991a. Fluorescence quenching: Theory and applications. Pages 53-126 in: Topics in Fluorescence Spectroscopy, Vol. 2, Principles. J. R. Lakowicz, ed. Plenum Press: New York.

EFTINK, M. R. 1991b. Fluorescence quenching reactions: Probing biological macromolecular structures. Pages 1-41 in: Biophysical and Biochemical Aspects of Fluorescence Spectroscopy. T. G. Dewey, ed. Plenum Press: New York.

EFTINK, M. R., and GHIRON, C. A. 1976a. Exposure of tryptophanyl residues in proteins. Quantitative determination by fluorescence quenching studies. Biochem. 15:672.

EFTINK, M. R., and GHIRON, C. A. 1976b. Fluorescence quenching of indole and model micelle systems. J. Phys. Chem. 80:486.

EFTINK, M. R., and GHIRON, C. A. 1981. Fluorescence quenching studies with proteins. Anal. Biochem. 114:199.

GENOT, C., MONTENAY-GARESTIER, T., and DRAPRON, R. 1984. Intrinsic spectrofluorometry applied to soft wheat (*Triticum aestivum*) flour and gluten to study lipid-protein interactions. Lebensm. Wiss. Technol. 17:129.

GENOT, C., TONETTI, F., MONTENAY-GARESTIER, T., MARION, D., and DRAPRON, R. 1992. Front face fluorescence applied to structural studies of proteins and lipid-protein interactions of viscoelastic food products. 2. Application to wheat gluten. Sci. Alim. 12:687.

KONEV, S. V. 1967. Fluorescence and Phosphorescence of Proteins and Nucleic Acids. S. Udenfriend, translation ed. Plenum Press: New York.

LAKOWICZ, J. R. 1983. Principles of Fluorescence Spectroscopy.

Plenum Press: New York.
LEHRER, S. S. 1971a. Solute perturbation of human serum albumin (HSA) fluorescence by iodide. Biophys. J. 11:72a.
LEHRER, S. S. 1971b. Solute perturbation of protein fluorescence. The quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. Biochem. 10:3254.
MacRITCHIE, F., and GRAS, P. W. 1973. The role of flour lipids in baking. Cereal Chem. 50:292.
MITCHESON, R. C., and STOWELL, K. C. 1970. Application of new analytical techniques to routine malt analysis. I. Determination of barley and malt nitrogen using an auto analyzer technique. J. Inst. Brew. 76:335.
PRESTON, K. R. 1982. Gel filtration of wheat gluten proteins on Sephacryl S-300. Cereal Chem. 59:73.
PRESTON, K. R. 1984. Gel filtration and characterization of neutral salt extracted wheat gluten proteins varying in hydrophobic properties. Cereal Chem. 61:76.
SHEWRY, P. R., and TATHAM, A. S. 1990. The prolamin storage proteins of cereal seeds: Structure and evolution. Biochem. J. 267:1.
TEALE, F. W. J. 1960. The ultraviolet fluorescence of proteins in neutral solution. Biochem. J. 76:381.
TIDOR, B., and KARPLUS, M. 1993. The contribution of cross-links to protein stability: A normal mode analysis of the configurational entropy of the native state. Proteins Struct. Funct. Genet. 15:71.

[Received May 10, 1993. Accepted October 30, 1993.]