*J. Biosci.*, Vol. 5, Number 4, December 1983, pp. 301–309 

# Effect of sodium dodecyl sulphate on the high molecular weight protein fraction of mustard (*Brassica juncea*) and rapeseed (*Brassica campestris*)

A. GURURAJ RAO « and M. S. NARASINGA RAO
Protein Technology Discipline, Central Food Technological Research Institute, Mysore 570 013

« Present address: Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland 44106, Ohio, USA.

MS received 23 April 1983

**Abstract.** The effect of sodium dodecyl sulphate on mustard and rapeseed 12S protein has been monitored by the techniques of ultracentrifugation, viscosity, difference spectra and fluorescence spectrophotometry. At low concentration of sodium dodecyl sulphate (<3.47 mM) mustard protein undergoes aggregation and at higher concentrations it dissociates to 1.8 S protein, the dissociation being complete at 17.3 mM sodium dodecyl sulphate. The rapeseed protein, on the other hand, undergoes dissociation at all the concentrations of sodium dodecyl sulphate. The reduced viscosity values of mustard protein in the presence of the denaturant are higher than those of rapeseed protein. Similarly in difference spectra change in absorbance values of mustard protein are higher.' The relative fluorescence intensity of the mustard protein increases with sodium dodecyl sulphate concentration, upto 0.87 mM and this is followed by fluorescence quneching at higher denaturant concentrations. However, with the rapeseed protein fluorescence quenching was observed at all concentrations of sodium dodecyl sulphate.

**Keywords.** Mustard protein; rapeseed protein;. denaturation; sodium dodecyl sulphate.

## Introduction

We have reported that the high molecular weight (mol. wt.) protein fraction, the 12 S protein, from mustard *(Brassica juncea)* and rapeseed (*Brassica campestris*) differ in amino acid composition, subunit composition, susceptibility to proteolysis and secondary structure (Gururaj Rao and Narasinga Rao, 1981). They also differ in their susceptibility to denaturation by urea and guanidinium hydrochloride (GndHCl); rapeseed protein is more easily denatured (Gururaj Rao and Narasinga Rao, 1983). In this communication we report the results of the effect of sodium dodecyl sulphate (SDS).on the mustard and rapeseed 12S protein.

## Materials and methods

### *Materials*

The source of mustard seed and rapeseed was the same as described previously (Gururaj Rao and Narasinga Rao, 1983). SDS from British Drug House, India was recrystallised from 50% ethanol.

Abbreviations used: SDS, Sodium dodecyl sulphate; GndHCl, guanidinium hydrochloride; RTIC, rotor temperature indicator and control; mol. wt., molecular weight.

*Isolation of the 12 S protein*

It was isolated by the procedure reported earlier (Gururaj Rao and Narasinga Rao, 1981).

*Protein concentration*

This was determined by absorbance measurements at 280 nm, using a value of $E_{1cm}^{1\%}$: 9.9 for mustard protein and 10.8 for rapeseed protein, (Gururaj Rao, 1980).

Ultracentrifugation was performed at room temperature (~27°C) in a Spinco Model E analytical ultracentrifuge equipped with rotor temperature indicator and control (RTIC) unit and phase plate schlieren optics, using 1% protein solution in the appropriate solvent. The solution was left at room temperature for 3h after the addition of SDS. The runs were made at 59,780 rpm. From the photographs taken at different intervals of centrifugation, $S_{20\ w}$ was calculated by the standard procedure (Schachman, 1959). Weight-average sedimentation coefficient, $S_{20,w,}$ was calculated from the second moment of the entire gradient curve (Goldberg, 1953).

*Gel filtration*

Sephadex G-200 (Pharmacia, Sweden), which had been equilibrated with.the buffer, was packed into a column 2.5 × 50 cm. Protein solution (5 ml) containing about 50 mg of protein was loaded on the column. When the experiment was performed in the presence of SDS, the column was equilibrated with the buffer containing the appropriate concentration of SDS and the eluting buffer also contained SDS. Fractions (4.0 ml) were collected and absorbance measured at 280 nm.

*Viscosity*

The measurements were made at 30 ±0.1° C in an Ostwald viscometer having a flow time of 184 swith distilled water. Protein (1%) solution was used. The blanks for these measurements contained the appropriate concentration of SDS.

*Difference spectra*

Difference spectra produced by the addition of different amounts of SDS to the protein were recorded in a Perkin-Elmer double beam recording spectrophotometer, 124, in the range 240 to 300 nm. Protein solutions having an absorbance of 1.8 at 280 nm were used.

*Fluorescence spectra*

A Perkin-Elmer fluorescence spectrophotometer Model 203 was used. The emission was measured in the range 300-400 nm after excitation at 280 nm. Protein solution having an absorbance of 0.05 at 280 nm was used.

*Other measurements*

A Radiometer pH meter TTT2 was used for pH measurements and a Carl-Zeiss ultra-violet spectrophotometer for absorbance measurements.

## Results and discussion

Unless otherwise mentioned all the measurements were made in 0.025 M Tris-glycine buffer of pH 8.3.

The sedimentation velocity pattern of the mustard protein consisted of a single symmetrical peak with an $S_{20,w}$ value of 11.3 (figure 1A). The addition of SDS upto 0.87 mM did not cause any change in either the pattern or the sedimentation coefficient. At 1.21 mM SDS, however, the peak became less symmetrical and broad.

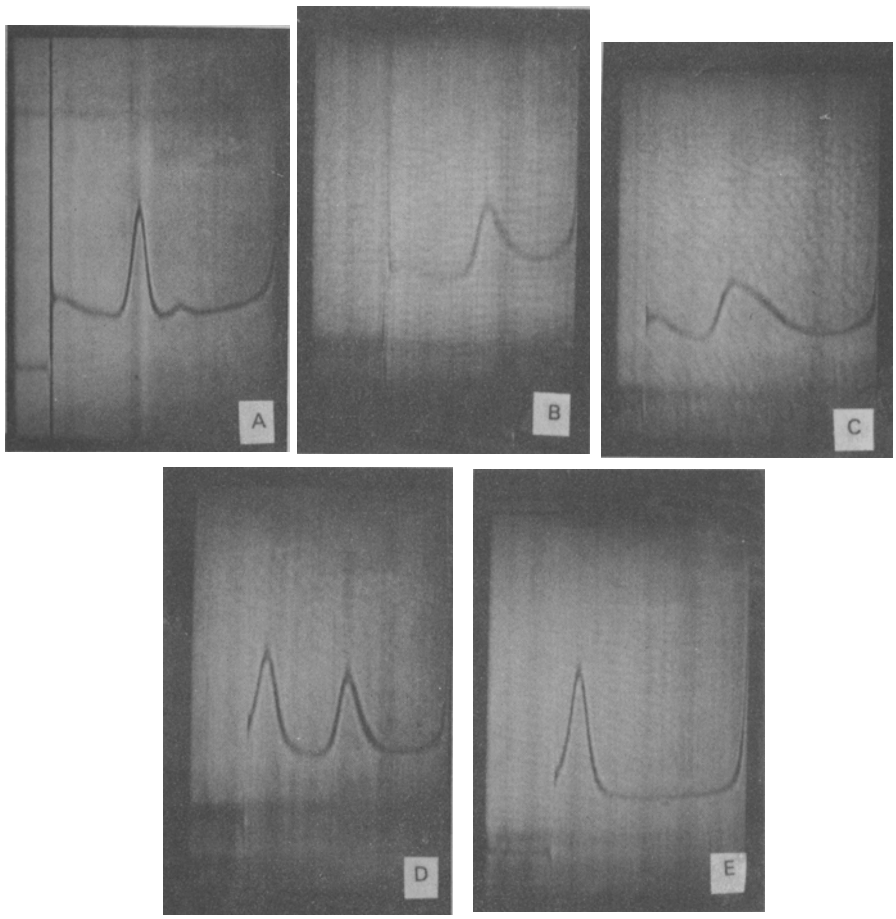


**Figure 1.** Sedimentation velocity pattern of mustard 12 S protein (in 0.025 M Tris-glycine buffer, pH 8.3).

**A.** Protein; **B.** Protein+1.21 mM SDS; **C.** Protein+ 3.47 mM SDS; **D.** Protein + 8.67 mM SDS; E. Protein + 17.34 mM SDS.

This effect was more pronounced at higher concentrations of SDS up to 3.47 mM. The $S_{20,w}$ value increased with SDS concentration; at 3.47 mM it attained a value of 14.0 S (figure 2). At higher concentrations, dissociation into 1.8 S protein occurred (figure 1D) and at 17.34 mM SDS concentration, 1.8 S protein alone was present (figure 1E).

The effect of SDS on the rapeseed protein was different. It dissociated the 12 S protein into 1.8 S protein even at low concentration and dissociation was complete at 17.34 mM SDS (figure 3). No evidence for any assymetrical peak nor increase in $S_{20,W}$ was observed. $S_{20,w}$ value decreased continuously with increase in SDS concentration (figure 2).

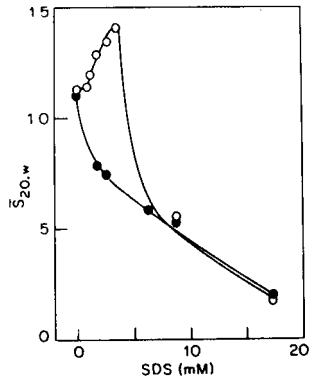


**Figure 2.** Weight-average sedimentation coefficient, $S_{20,w}$ as a function of SDS concentration.

(O), Mustard 12 S protein;(●)rapeseed 12 S protein.

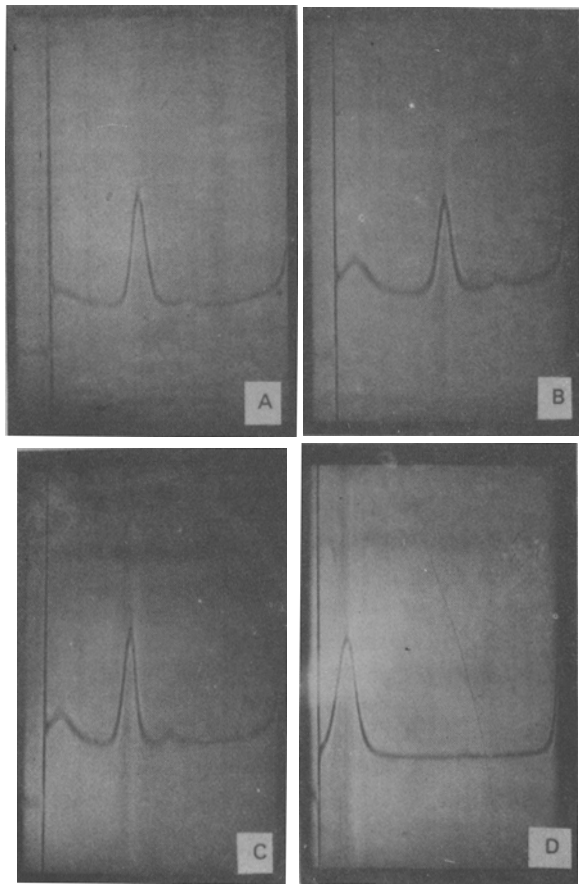


**Figure 3.** Sedimentation velocity pattern of rapeseed 12 S protein (in 0.025 M Tris-glycine buffer pH 8.3).

**A.** Protein; **B.** Protein+2.60 mM SDS; **C.** Protein+ 3.47 mM SDS; **D.** Protein +

In gel filtration experiments mustard 12 S protein gave a major peak eluting at 230 ml (figure 4A). The latter is a non-protein fraction (MacKenzie and Blakely, 1972). With the addition of SDS, the elution volume of the major peak shifted to lower value, and at 2.6 mM SDS, it was 88 ml. In our experiments, the accuracy of determination of elution volume *($V_e$)* is expected to be ± 4 ml. Shift of $V_e$ to lower value would suggest aggregation of the protein. At higher SDS concentrations, there were fractions eluting at 110 and 140 ml suggesting dissociation of the protein (figure 4C).

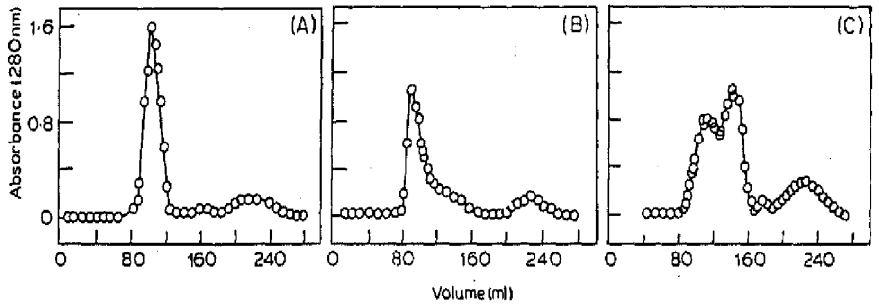


**Figure 4.** Gel filtration pattern of mustard 12 S protein on Sephadex G-200 (in 0.025 M Tris-glycine buffer, pH 8.3).
**A.** Protein; **B.** Protein+2.60 mM SDS; **C.** Protein+8.67 mM SDS.

In the case of the rapeseed protein association of the protein was not indicated at any SDS concentration. At 2.6 mM SDS concentration, $V_e$ of the major peak was the same as that of the protein in the absence of SDS (figure 5 A, B). However the peak was less sharp and a shoulder at 125 ml was observed. At 8.7 mM SDS the major peak had shifted to 110 ml and another peak at 132 ml was observed.

Thus both ultracentrifugation and gel filtration experiments indicated that at low concentrations of SDS mustard protein aggregated to a high mol. wt species and at high concentrations it dissociated the protein to low mol. wt. (1.8 S) protein. On the other hand rapeseed protein showed only dissociation.

Reduced viscosity of the mustard protein was 5.3 cc/g. Addition of SDS increased it (figure 6). The increase was in two steps;upto 5,20mM SDS it increased and attained a constant value between 5.20 and 10.40 mM SDS, then increased and attained a steady value of 11.5 cc/g. Thus even at the highest SDS concentration, the protein did not attain random-coil conformation (Tanford, 1970). Rapeseed protein had a value of 4.4 cc/g for reduced viscosity. Addition of SDS increased the value. There was no clear indication of two steps. The highest value obtained at 17.34 mM SDS was 10.4 cc/g, slightly lower than with mustard protein.

The addition of SDS produced difference spectra with troughs at 295 nm, 287 nm and 278 nm. The intensity of the troughs increased with SDS concentration (figure 7). This was true of both the proteins. The Δ A values of the mustard protein (figure 7A) at 287 nm or 295 nm at any SDS concentration, were higher than those of rapeseed protein (figure 7B) at the same SDS concentration, suggesting greater perturbation of the tyrosine residues of the mustard protein.

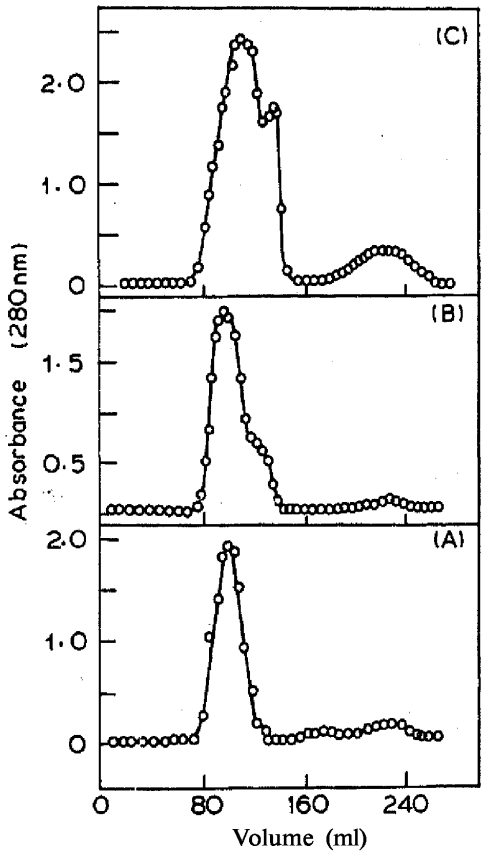


**Figure 5.** Gel filtration pattern of rapeseed 12 S protein on Sephadex G-200 (in 0.025 M Tris-glycine buffer, pH 8.3).
**A.** Protein; **B.** Protein+2.60 mM SDS; C. Protein+8.67 mM SDS.

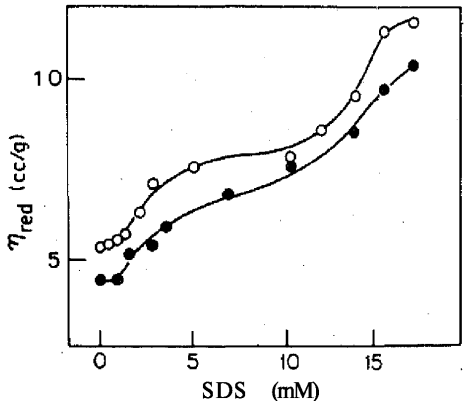


**Figure 6.** Reduced viscosity of the protein as a function of SDS concentration (in 0.025 M Tris-glycine buffer, pH 8.3).
(O), Mustard 12 S protein; (●), rapeseed 12 S protein.

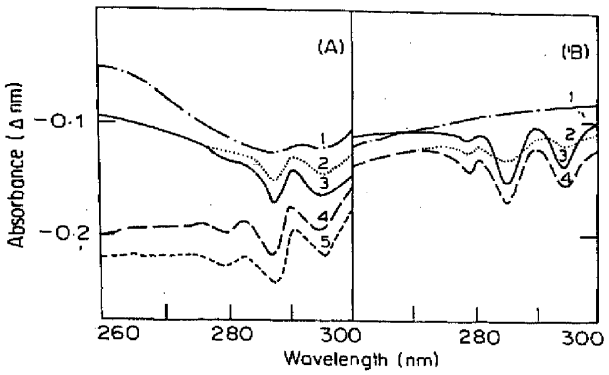


**Figure 7.** Difference spectra of the protein (in 0.025 M Tris-glycine buffer, pH 8.3).
**A**. Mustard 12 S protein.
1, Protein + 1.73 mM SDS; 2, Protein+2.60 mM SDS; 3, Protein+3.47 mM SDS; 4, Protein+6.94 mM SDS; 5, Protein +17.34 mM SDS.
**B**.Rapeseed 12 S protein.
1, Protein + 1.73 mM SDS; *2*, Protein+2.60 mM SDS; 3, Protein+6.94 mM SDS; 4, Protein+ 17.34 mM SDS.

The addition of SDS caused changes in the fluorescence emission spectrum of the proteins. In the case of mustard protein, SDS upto 1.73 mM caused an *increase* in the emission intensity, then decreased it and a plateau value was reached between 5 and 17 mM SDS (figures 8A and 9). Further there was also a red shift in the emission maximum at higher SDS concentration. On the other hand with the rapeseed protein there was fluorescence quenching at all SDS concentrations (figure 9B); plateau value was reached between 5 and 17 mM SDS. Here also a red shift was observed at higher SDS concentration.

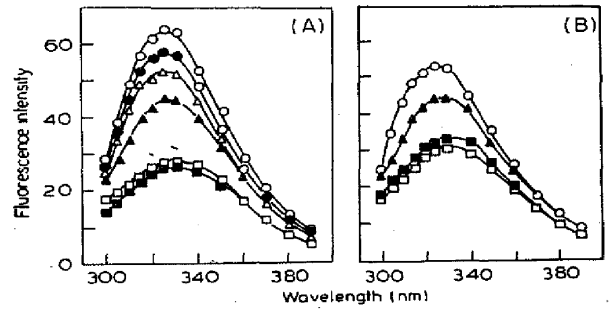


**Figure 8.** Fluorescence spectra of the proteins (in 0.025 M Tris-glycine buffer, pH 8.3).
**A.** Mustard 12 S protein.
(O),Protein; (●) , Protein+0.35 mM SDS; (Δ), Protein+0.87mM SDS;(▲)Protein + 1.73 mM SDS;(□), Protein+8.67 mM SDS;(■), Protein +17.34 mM SDS.
**B**. Rapeseed 12 S protein.
(O),Protein;(▲), Protein+1.73 mM SDS;(■), Protein+8.67mMSDS;(□),Protein + 17.34 mM SDS

Increase in reduced viscosity, appearance of difference spectra and fluorescence quenching (and red shift of emission maximum) can all be taken as evidence of denaturation of the proteins by SDS. Quantitatively speaking, denaturation of the

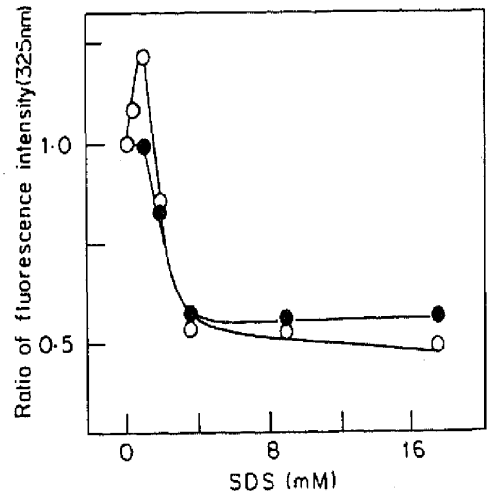


**Figure 9.** Ratio of fluorescence intensity at 325 nm as a function of SDS concentration.

(O), Mustard 12 S protein; (●), rapeseed 12 S protein.

mustard protein occurred to a greater extent than that of the rapeseed protein, as indicated by viscosity and difference spectral data.

These measurements indicate that SDS causes both dissociation and denaturation of the mustard and the rapeseed 12 S protein. However there were some significant differences in the effect of SDS on the two proteins. These were (a) at low concentrations (<3.5 mM) SDS caused aggregation of the mustard 12 S protein and this was followed by dissociation to 1.8 S protein; however with the rapeseed 12 S protein dissociation occurred at all SDS concentrations; (b)the increase in the reduced viscosity of the mustard protein with SDS concentration followed a clearly delienated two-step process, whereas with the rapeseed protein it was not so clear; and (c) the emission fluorescence intensity of the mustard protein increased upto 0.87 mM SDS and then there was fluorescence quenching; with the rapeseed protein quenching occurred at all SDS concentrations.

Aggregation of proteins by SDS has been reported (Ray and Chatterjee, 1967; Jones and Wilkinson 1976;Jones and Rumby, 1978). It is possible that SDS anions first bind to the available cationic sites on the molecule causing an unfolding and exposure of the hydrophobic groups. Aggregation could then occur through hydrophobic interaction. Burkhard and Stolzenberg (1972) report that the interaction of SDS with ferricytochrome C at pH 7.35 occurs through a preliminary electrostatic interaction followed by interaction between the hydrophobic amino acid residues of the protein.

The increase in the fluorescence intensity of the mustard protein at low concentration of SDS could be due to the aggregation of the protein at these SDS concentrations. Fluorescence is due to the imbedded tyrosine and tryptophane residues (Teale, 1960). Aggregation of the protein would make more groups imbedded and this would increase fluorescence intensity. Reference to figures 2 and 9 would show that $S_{20,w}$ reached the maximum value of 14.0 S at 3.7 mM SDS, whereas the maximum increase in fluorescence intensity occurred at 1 mM. This could possibly be due to the effect of protein concentration; for ultracentrifugation 1% protein solution was used and for fluorescence measurements it was 0.005%.

Apparently the denaturation of the mustard 12 S protein by SDS followed a two-step process as indicated by viscosity data. Several proteins follow more than a single-step process in their denaturation (Tanford, 1970).

The reason for the different effect of SDS on the two proteins is not obvious. Proteins bind SDS avidly (Reynolds and Tanford, 1970). The binding would be by ionic and hydrophobic bonds (Steinhardt and Reynolds, 1969). Although there is some difference in the amino acid composition of mustard and rapeseed 12 S protein (Guraraj Rao and Narasinga Rao, 1981) the hydrophobicity value per residue or the fractional charge per residue of the two proteins does not differ signficantly (Gururaj Rao and Narasinga Rao, 1983). In spite of this there was some difference in the binding of SDS by the two proteins, as suggested by the following experiments.

At low concentrations of SDS upto 3.47 mM aggregation of the mustard protein occurred where as dissociation of the rapeseed protein occurred. When the protein solution containing SDS was dialysed extensively against the corresponding concentration of SDS and analysed in the ultracentrifuge, the mustard protein did not show aggregation, contrary to what was observed with the undialysed solution; actually dissociation occurred. In the case of the rapeseed protein identical results were obtained with the dialysed and undialysed solutions. Obviously in the undialysed solution SDS became limiting because of avid binding by the protein. Since dialysis did not make any difference in the case of rapeseed proten, it would imply that it binds SDS less avidly than the mustard protein. The difference in binding of SDS could possibly be due to differences in the sub-unit composition of the two proteins (Gururaj Rao and Narasinga Rao, 1981).

## References


Burkhard, R. K. and Stolzenberg, G. E. (1972) *Biochemistry,* **11,** 1672.

Gururaj Rao, A. (1980) *Chemical and Physicochemical Studies on Mustard Seed* (*Brassicajuncea*) *Protein,* Ph.D. thesis, Mysore University, Mysore.

Gururaj Rao, A. and Narasinga Rao, M. S. (1981) *Int J. Peptide Protein Res.,* **18,** 154.

Gururaj Rao, A. and Narasinga Rao, M. S. (1983) *J., Biosci.,* **5,** 311.

Goldberg, R. J. (1953) *Phys. Chem.,* **57,** 194.

Jones, M. N. and Wilkinson, A. (1976) *Biochem. J.*,**153,** 713.

Jones, A. J. S. and Rumsby, M. C. (1978) *Biochem. J.*,**169,** 281.

MacKenzie, S. L. and Blakely, J. A. (1972) *Can. J., Bot,* **50,** 1825.

Ray, A. and Chatterjee, R. (1967) in *Conformation of Biopolymers,* ed. G. N. Ramachandran, (London: Academic Press) p. 235.

Reynolds, J. A. and Tanford, C. (1970) *Proc. Natl. Acad. Sci. USA,* **66,** 1002.

Schachman, H. K. (1959) *Ultracentrifugation in Biochemistry* (New York: Academic Press).

Steinhardt, J. and Reynolds, J. A. (1969) *Multiple Equilibria in Proteins* (New York: Academic Press).

Tanford, C. (1970) *Adv. Protein Chem.,* **24,** 2.

Teale, F. W. J. (1960) *Biochem. J.,* **76,** 581.