Biochem. J. (1986) 240, 227–231 (Printed in Great Britain)

Structure and stability of *Ricinus communis* haemagglutinin

Jean-Pierre FRÉNOY,* Anh-Tuan TRAN† and Roland BOURRILLON†

*I.N.S.E.R.M. U. 180 and †Faculté de Médecine Lariboisière Saint-Louis, U.E.R. Biomédicale des Saints-Pères, 45 Rue des Saints-Pères, 75006 Paris, France

The molecular properties of the haemagglutinin of *Ricinus communis* (RCA I or RCA 120) were evaluated by analytical ultracentrifugation, light-scattering, c.d. and fluorescence. The native molecule had a fairly expanded structure ($f/f_0 = 1.43$) and dissociated into two subunits of equal size in 6 M-guanidinium chloride. This native structure was stable in alkali (up to pH 11) and resistant to thermal denaturation at neutrality. A pH-triggered change in the haemagglutinin conformation was observed and characterized by analytical ultracentrifugation, c.d. and fluorescence between pH 7 and 4.5, the range in which its affinity for galactosides decreased [Yamasaki, Absar & Funatsu (1985) Biochim. Biophys. Acta **828**, 155–161]. These results are discussed in relation to those reported in the literature for other lectins and more especially ricin, for which a pH-dependent conformation transition has been observed in the same range of low pH.

INTRODUCTION

The haemagglutinin of *Ricinus communis* (RCA I or RCA 120) has aroused considerable interest because of its high affinity and particular specificity for glycoproteins and glycolipids containing galactose (Nicolson & Blaustein, 1972; Olsnes *et al.*, 1974; Baenziger & Fiete, 1979; Debray *et al.*, 1981; Turpin *et al.*, 1984*a*). It has therefore been extensively used to characterize these components on cell surfaces.

Polymerized *Ricinus* haemagglutinin is a potent mitogenic lectin (Turpin *et al.*, 1984*b*), and several reports indicate that it is also toxic, although less so than ricin (Kornfeld *et al.*, 1974; Bernard *et al.*, 1979; Dodeur *et al.*, 1980; Turpin *et al.*, 1984*b*).

Compared with ricin, this haemagglutinin (M_r 120000) is composed of two A-type and two B-type chains, and its toxicity indicates that it penetrates the cells and is then exposed to different pH inside the endocytotic vesicles. Several findings suggest that under certain conditions this lectin undergoes conformational changes (Curatolo *et al.*, 1978). Thus below pH 5.5 a marked decrease was observed in its affinity for galactosides (Yamasaki *et al.*, 1985). Apparently contradictory results were reported for the effect of temperatures upon agglutination induced by *Ricinus* haemagglutinin: some authors reported an optimal temperature for the agglutination of glycolipid-containing vesicles and complex polysaccharides (Curatolo *et al.*, 1978; Curatolo, 1982), whereas others found that low temperatures inhibited agglutination induced by this lectin (Kaneko *et al.*, 1973); others again observed a characteristic transition of the adsorption coefficient on gels at 18 °C (Hsu *et al.*, 1976). Moreover, with respect to conformational changes, comparison of the susceptibility of the haemagglutinin molecule to various physical conditions with that of ricin (see the preceding paper, Frénoy, 1986) is interesting because it concerns two closely related proteins of the same origin (Cawley *et al.*, 1978; Butterworth & Lord, 1983; Lord, 1985) but different valence, and because it should facilitate our understanding of the mechanisms of their biological effects.

In the present investigation the molecular structure and stability of *Ricinus* haemagglutinin were evaluated by analytical ultracentrifugation and several spectroscopic methods, and the results are discussed in relation to those obtained for ricin.

MATERIALS AND METHODS

A homogeneous haemagglutin preparation was obtained from *Ricinus communis* (var. *sanguineus*) seeds (Vilmorin-Andrieux, Paris, France), at the same time as ricin preparation, by the procedure of Nicolson & Blaustein (1972). The haemagglutinin preparation was observed to be homogeneous by polyacrylamide-gel electrophoresis at pH 7.5, ultracentrifugation and immunoelectrophoresis. Its haemagglutinating activity was checked against human O^- erythrocytes and was 1.7 μg (minimum haemagglutinating dose). The haemagglutinin concentration was determined by absorbance measurements at 280 nm, at pH 7, by using $a = 1.4$ litre $\cdot g^{-1} \cdot cm^{-1}$ (Podder *et al.*, 1974).

Its M_r values were determined under various conditions in a Beckman model E ultracentrifuge equipped with interference optics and temperature control by an R.T.I.C. unit. Proteins were dialysed in the buffer solution for 40 h before experimentation, and equilibrium measurements were made until the readings did not change with time; the results reported in this paper were obtained after 48 h or 72 h of centrifugation. Data were plotted as $\ln c$ versus r^2 and M_r values calculated according to the equation:

$$M_r = [d(\ln c)/dr^2] \times [2RT/(1-\bar{v}\rho)\omega^2]$$

A partial specific volume, $\bar{v}$, of 0.714 was calculated from the chemical composition of the haemagglutinin and also used in 6M-guanidinium chloride (Lee & Timasheff, 1974).

For the c.d. spectra, cells with pathlengths of 0.2 and

* To whom correspondence should be addressed.

0.5 cm were used for the near-u.v. readings (haemagglutinin concentrations 6.14 and 1.98 mg/ml respectively) and cells with pathlengths of 0.1 and 1 cm for far-u.v. readings (haemagglutinin concentrations 0.25 and 0.025 mg/ml respectively). The mean residue weight of *Ricinus* haemagglutinin, calculated from its amino acid composition, was 109.9.

At low ionic strength, the M_r of haemagglutinin was measured by low-angle laser-light-scattering with a Chromatix KMX-6 apparatus. Solutions of *Ricinus* haemagglutinin at pH 7.25 (0.21–0.52 mg/ml), pH 3.87 (0.22–0.55 mg/ml) and pH 11.85 (0.35–0.70 mg/ml) were injected into the light-scattering apparatus through a 0.22 μm Millipore filter. Apparent M_r ($M_{app.}$) values were obtained from the equation:

$$Kc/\Delta R_\theta = 1/M_{app.} + 2A_2c$$

where ΔR_θ is the Rayleigh factor measured, c is the concentration in g/ml and K is the polymer optical constant defined as:

$$K = (2\pi^2 n^2/\lambda^4 N)\,(dn/dc)^2(1+\cos^2\theta)$$

where n is the refractive index of the solution at the incident wavelength λ, N is Avogadro's number and θ is the angle of scattered light collection. The refractive-index increment dn/dc was taken as 0.19 for the haemagglutinin.

All other materials and methods were as described and used for the study of ricin in the preceding paper (Frénoy, 1986).

RESULTS

The M_r of native *Ricinus* haemagglutinin has been reported to be 120000±3000 (Nicolson & Blaustein, 1972; Olsnes *et al.*, 1974; Zentz *et al.*, 1979). At pH 6.75 in 0.01 M-sodium phosphate buffer containing 0.15 M-NaCl, a single sedimenting boundary was observed with our preparation of haemagglutinin, with an $s^0_{20,w}$ of 6.51 S (Fig. 1*a*). The sedimentation coefficient was independent of the haemagglutinin concentration when the latter was between 1.0 and 3.0 mg/ml. A frictional ratio (f/f_0) of 1.43 was calculated from the M_r and $s^0_{20,w}$.

The fluorescence emission peak of the haemagglutinin reached its maximum at 332 nm when the excitation wavelength was 280 nm, and the spectrum did not change significantly when it was 295 nm. In 6 M-guanidinium chloride the tryptophan peak was about 30 nm and there was only a discrete shoulder emission at 305 nm (Fig. 2*a* inset).

As in the case of ricin, an excess of lactose (0.1 M), used as a ligand, had little effect on the fluorescence of *Ricinus* haemagglutinin at neutral pH ($\leqslant 2\%$ intensity enhancement, < 1 nm blue-shift). Lactose has also been shown not to affect the M_r of this haemagglutinin (Zentz *et al.*, 1979).

After reduction of the disulphide bonds, two polypeptide chains were observed by SDS/polyacrylamide-gel electrophoresis of *Ricinus* haemagglutinin, with M_r values between 30000 and 35000 (Olsnes *et al.*, 1974; Nicolson *et al.*, 1974). However, in the absence of reduction the subunit size, which corresponds to the half-molecule, was determined by SDS/polyacrylamide-gel electrophoresis only (Cawley & Houston, 1979). Here we evaluated this parameter in 6 M-guanidinium chloride.

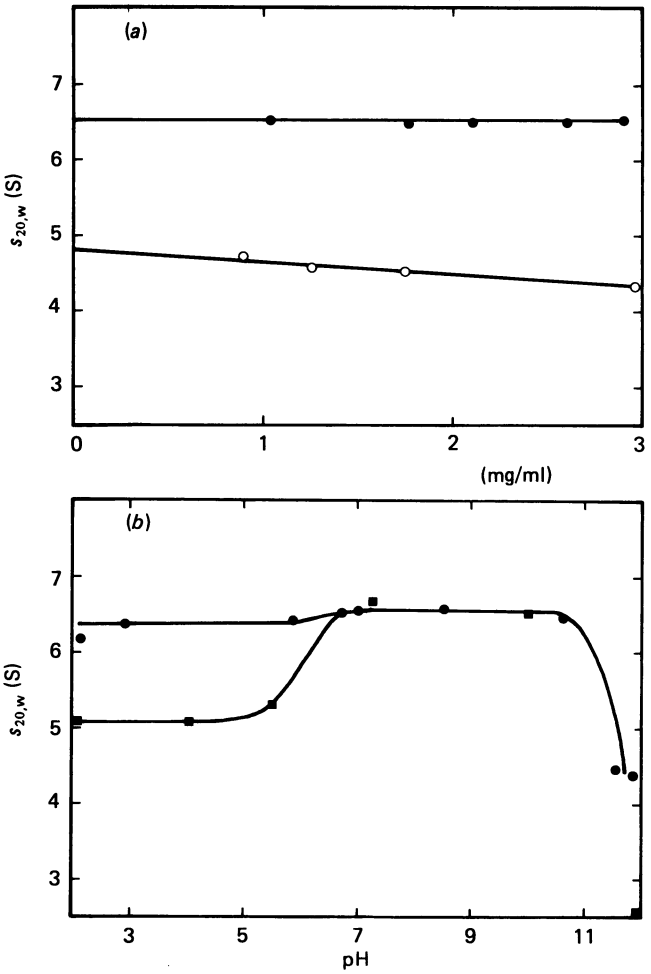

Fig. 1. Sedimentation of *Ricinus* haemagglutinin at 20 °C

(*a*) Sedimentation coefficient of the haemagglutinin as a function of initial protein concentration at pH 6.75 (●) and 11.85 (○). Experiments were performed in 0.01 M-sodium phosphate buffer containing 0.15 M-NaCl. (*b*) Effect of pH on the sedimentation coefficient of the haemagglutinin. Experiments were performed at haemagglutinin concentrations 2.65–2.96 mg/ml in 0.01 M-sodium phosphate buffer in the absence (■) or in the presence (●) of 0.15 M-NaCl. Values are corrected for density and viscosity of the solvent.

A linear dependence of $\ln c$ versus r^2 was found in this solvent (results not shown), which is known to rupture most of the non-covalent bonds in proteins, and two experiments at 20 °C, performed at protein concentrations of 0.54 and 0.88 mg/ml, gave an average M_r of 62000±4000.

Thermal stability

A sedimentation coefficient of 4.17 S was measured at 4 °C for *Ricinus* haemagglutinin (1.91 mg/ml), which corresponds to 6.62 S at 20 °C after correction for solvent density and viscosity.

The fluorescence intensity of *Ricinus* haemagglutinin tryptophan residues, measured at 340 nm, gradually declined as the temperature was raised from 10 to 70 °C (Fig. 2*a*) Above 50 °C this decrease in intensity was accompanied by a very small red-shift from 332 to 334 nm in the emission maximum (corrected spectra).

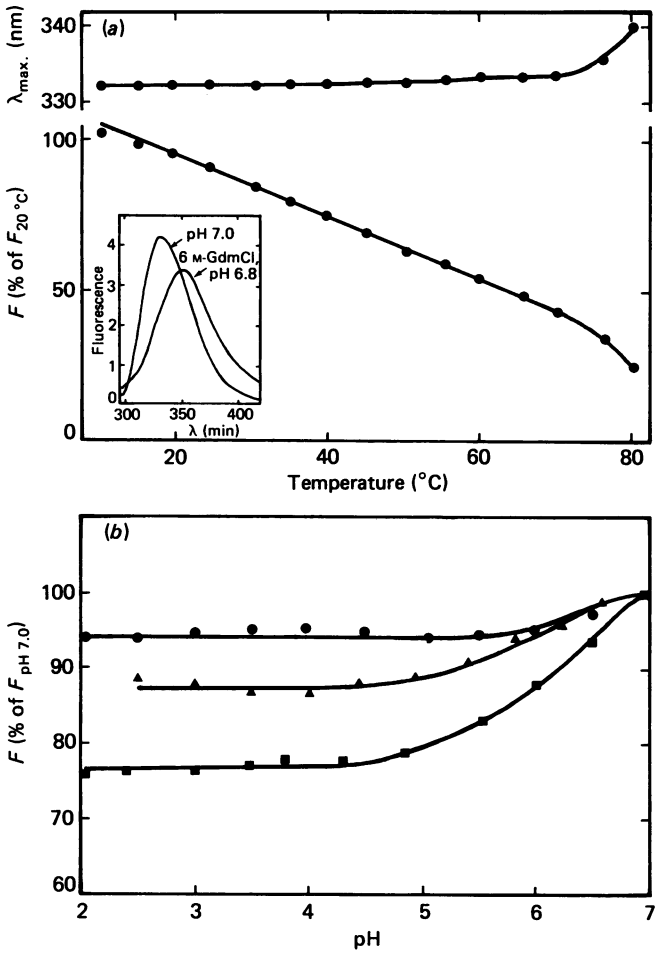

Fig. 2. Fluorescence of *Ricinus* haemagglutinin

(*a*) Temperature-dependence of the fluorescence and wavelength maxima of the tryptophan residues. The haemagglutinin concentration was 0.022 mg/ml in 0.01 M-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl. The inset shows the emission spectra of the haemagglutinin at pH 7.0 and in 6 M-guanidinium chloride (GdmCl) at pH 6.8 (20 °C). (*b*) Effect of pH on the fluorescence of the haemagglutinin. Experiments were performed at 20 °C with haemagglutinin concentrations 0.041–0.067 mg/ml in 0.01 M-sodium phosphate buffer alone (■) and in the presence of 0.15 M-NaCl (●) or 0.1 M-lactose (▲). Excitation was at 280 nm and results are expressed as the percentage of fluorescence intensities measured at 20 °C ($F_{20\,°C}$) or pH 7.0 ($F_{pH\,7.0}$).

These results indicate that no significant structural change occurs between 10 and 50 °C and possibly up to 70 °C. Between 70 and 80 °C the emission maximum shifted from 334 to 341 nm. Such an increase in the wavelength of the emission peak indicates enhanced exposure of the tryptophan residues to the solvent and corresponds to an unfolding of the protein structure. In this connection, loss of haemagglutinating activity was reported after incubation of *Ricinus* haemagglutinin at high temperatures (Nicolson *et al.*, 1974).

Temperature had no noticeable effect on the ratio or intensity of the two peaks observed by c.d. at 285 and 294 nm (Fig. 3*a*). The only effect of temperature was reflected by a widening of these two peaks above 40 °C. By analogy with the results obtained with ricin, the peak at 285 nm corresponds to tyrosine chromophore, and the peak at 294 nm to tryptophan residue(s) in low environmental polarity.

Between 200 and 250 nm we observed a c.d. spectrum that was maximal at 208 nm (Fig. 3*b*) and resembled to that reported by Shimazaki *et al.* (1975). There was no change in the shape of this peak between 5 and 50 °C, and only a small decrease in its intensity ($\leqslant 4\%$) between 30 and 50 °C. Thus in the temperature range studied no gross changes in backbone conformation were detected.

pH stability

A very small decrease in the sedimentation coefficient was observed when the pH of a solution of *Ricinus* haemagglutinin in 0.15 M-NaCl was lowered from neutrality (Fig. 1*b*). In the absence of NaCl, i.e. in 0.01 M-phosphate buffer, a larger decrease was observed between pH 7 and 5, and at pH 4 the sedimentation coefficient of the haemagglutinin had decreased to 5.1 S (Fig. 1*b*). Although no change was observed between 7 and 11, the sedimentation coefficient decreased sharply above pH 11, with or without NaCl ($s^0_{20,w} = 4.82$ S at pH 11.85; Figs. 1*a* and 1*b*).

A possible explanation for the decrease in the sedimentation coefficient at both low pH and high pH was the dissociation of the haemagglutinin into its two half-molecule subunits. A sedimentation-equilibrium experiment in 0.15 M-NaCl gave an M_r value of 115000 ± 7000 at pH 4.08, indicating that under these conditions this haemagglutinin is still a tetramer. Its M_r was also estimated at extreme pH values and low ionic strength by low-angle laser-light-scattering. In 0.01 M-sodium phosphate buffer M_r values of 116000 ± 7000 and 123000 ± 8000 were measured at pH 3.87 and 11.85 respectively. By the same method, an M_r of 121000 ± 3000 was determined at pH 7.25. These results show undoubtedly that this lectin is not dissociated by the changes in the pH.

In the absence of NaCl, the fluorescence intensity of *Ricinus* haemagglutinin was gradually quenched by 25% between pH 7 and 4.5 (Fig. 2*b*), but this was not accompanied by any shift in the emission peak. Below pH 7, the fluorescence changes were independent of the lectin concentration when the latter was between 0.022 and 0.22 mg/ml. Thus, as indicated by the scattering experiments described above, this quenching does not represent a concentration-dependent process such as dissociation. Although less apparent, the quenching of tryptophan-residue fluorescence is always present in 0.15 M-NaCl (Fig. 2*b*). This quenching also occurs in the presence of the ligand lactose (0.1 M), but its degree is between that of the quenchings observed in the absence and presence of NaCl. [On the basis of the association constant, $K_a \simeq 5 \times 10^2$ M^{-1} at pH 4 and 20 °C, reported by Yamasaki *et al.* (1985) for *Ricinus* haemagglutinin and lactose, it can be calculated that with 0.1 M-lactose more than 98% of the lectin-binding sites are occupied at pH 4, under our experimental conditions.] Since in 0.1 M-sucrose, which does not bind to the haemagglutinin, the effect of pH is similar to that observed in 0.15 M-NaCl, the quenching by lactose cannot be attributed to the bulk properties of the saccharide-containing solvent, and is ligand-specific. The presence of a tryptophan residue involved in saccharide binding by *Ricinus* hemagglutinin has been proposed on the basis of u.v. difference-spectroscopy analyses (Yamasaki *et al.*, 1985). Our

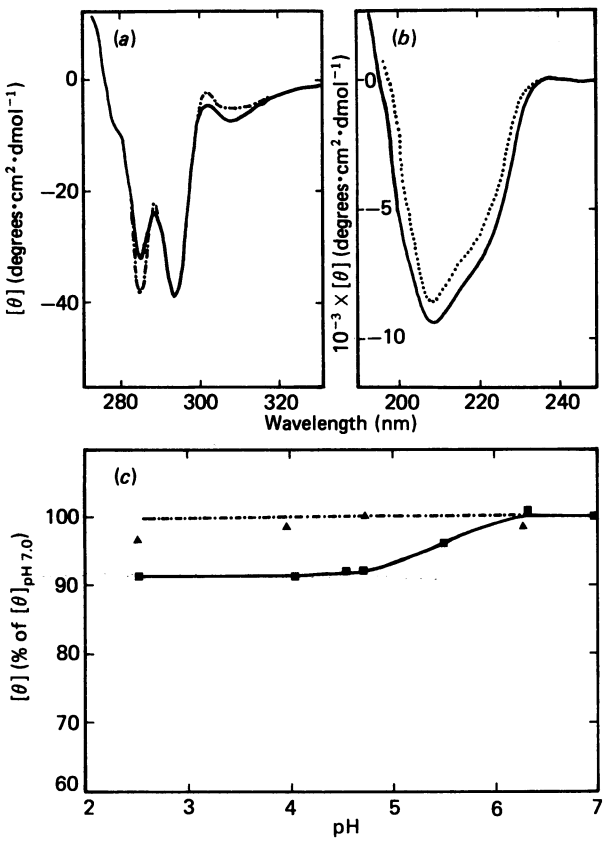

Fig. 3. C.d. of *Ricinus* haemagglutinin at 20 °C

(*a*) Near-u.v. c.d. spectra. The haemagglutinin concentration was 1.98 mg/ml at pH 7.0 in 0.01 M-sodium phosphate buffer in the absence (——) or in the presence (–·–·–) of 0.1 M-lactose. (*b*) Far-u.v. c.d. spectra. The haemagglutinin concentration was 0.25 mg/ml in 0.01 M-sodium phosphate buffer at pH 7.0 (——) and pH 4.7 (·····). (*c*) pH-dependence of the mean-residue ellipticity at 208 nm in 0.01 M sodium phosphate buffer only (■) and in the presence of 0.1 M-lactose (▲). Results are expressed as the percentage of ellipticity measured at pH 7.0 ($[\theta]_{pH\ 7.0}$).

results indicate the presence of such a tryptophan residue in or near the galactoside-binding site, but, as in the case of peanut agglutinin (Decastel *et al.*, 1985), it was more easily detectable at low pH.

In *Ricinus* haemagglutinin, tryptophan emission was continously quenched by 50% between pH 8 and 11 without any significant change in $\lambda_{max.}$ Above pH 11 this quenching was accompanied by a small red-shift of the maximum from 332 to 336 nm (experiment not shown).

Low pH was not observed to have any clear effect on c.d. in the near-u.v. region, and the relative size of the two peaks at 285 and 294 nm remained the same. However, although these peaks were of similar intensity in the presence of 0.1 M-lactose at neutral pH (Fig. 3*a*), the magnitude of the peak at 285 nm decreased by approx. 10% below pH 6.3. These results may appear somewhat different from those obtained with ricin, but it should be noted that, in the case of ricin at low pH, the ratio of ellipticities at 285 and 294 nm in the presence of 0.1 M-lactose was also lower than in the absence of this ligand (see the preceding paper, Frénoy, 1986).

The intensity of the far-u.v. c.d. spectrum of *Ricinus* haemagglutinin did not change between pH 7 and 11.8 but decreased at pH below 6.3, and at pH 4.7 only 92% of the intensity measured at neutrality was recorded (Figs. 3*b* and 3*c*). This effect of pH on the secondary structure of the haemagglutinin was effectively not observed in the presence of 0.1 M-lactose (Fig. 3*c*).

DISCUSSION

The effects of temperature and pH on lectin conformation have been studied on the few types of lectin for which one or both factors affect their oligomeric equilibrium or solubility, i.e. essentially in concanavalin A and wheat-germ and peanut agglutinins (see Decastel *et al.*, 1985, and references cited therein). The present results show that *Ricinus communis* haemagglutinin, which has many spectroscopic properties in common with ricin, does not dissociate at low pH, although its secondary and more particularly its tertiary structure are sensitive to environmental conditions under which changes are also observed in the conformation of ricin.

The frictional ratio 1.43 calculated for haemagglutinin was definitely higher than the f/f_0 of 1.28 obtained for ricin (see the preceding paper, Frénoy, 1986). Peanut agglutinin is a more globular molecule with a frictional ratio of 1.28–1.39 (Decastel *et al.*, 1981); a ratio of 1.56 was reported for the albumin isolectins of *Phaseolus vulgaris* (Pusztai & Stewart, 1978). The ratio of 1.43 for haemagglutinin is high for a globular protein, and suggests either that the molecule is rigid with a high axial ratio or that it contains globular domains similarly to immunoglobulins (Edelman & Gall, 1969). *Ricinus* haemagglutinin does not have a rod-like structure, since it does not possess the high helical content of fibrous proteins (Shimazaki *et al.*, 1975; Herrmann *et al.*, 1978). Consequently, such an axial ratio would indicate that elements of flexibility related to the existence of molecular domains are present in this haemagglutinin molecule, suggesting that it is more sensitive to proteinases than is ricin.

Of the vegetal lectins, only the tetrameric concanavalin A dissociates into dimers at a low temperature. This dissociation is accompanied by a major decrease in the sedimentation coefficient (Huet, 1975; Huet & Claverie, 1978; Senear & Teller, 1981). *Ricinus* haemagglutinin proved to be thermally stable at neutral pH, and we did not observe the conformational change in this lectin around 25 °C proposed by Curatolo *et al.* (1978). At 4 °C and pH 6.75 the sedimentation coefficient of this haemagglutinin was indistinguishable, after correction to 20 °C for solvent density and viscosity, from the $s^2_{0,w}$ obtained from sedimentation at 20 °C. At neutral pH *Ricinus* haemagglutinin appears to be stable at both high and low temperatures, since when the temperature was raised its fluorescence intensity exhibited only monotonic quenching, and proteins undergoing thermal transitions normally display inflections in their fluorescent profiles (Steiner & Edelhoch, 1963). In addition, only a small decrease of less than 4% was observed in far-u.v. c.d. activity between 30 and 50 °C. Thus dissociation or conformation changes in the haemagglutinin molecule cannot be held responsible for either the inhibitory effect of low temperature on agglutinating activity (Kaneko *et al.*, 1973) or the observation of biphasic Scatchard plots of *Ricinus* haemagglutinin binding to cells at 4 °C (Bernard *et al.*, 1979; Turpin *et al.*, 1984*c*) or, again, the characteristic transition of this lectin's adsorption on agarose gels at 18 °C (Hsu *et al.*, 1976).

At low pH concanavalin A (Huet, 1975; Senear & Teller, 1981; Herskovits *et al.*, 1983) and peanut agglutinin (Fish *et al.*, 1978; Decastel *et al.*, 1985) tetramers dissociate into dimers, and wheat-germ agglutinin dimers into monomers (Nagata & Burger, 1974; Rice & Etzler, 1974; Monsigny *et al.*, 1979). In contrast, the tetrameric forms of phytohaemagglutinin and soya-bean agglutinin remain stable over a wide range of pH (Huet, 1975). Our data show that *Ricinus* haemagglutinin does not dissociate at extreme pH values, but undergoes a molecular transition between pH approx. 7 and 4.5 that is superimposable on that of ricin (see the preceding paper, Frénoy, 1986). Furthermore, its affinity for galactosides diminishes in the same pH range (Yamasaki *et al.*, 1985). At low pH the secondary structure of this haemagglutinin is only damaged slightly, but the tertiary structure is more radically altered. The most pronounced changes were observed at low ionic strength by fluorescence and analytical ultracentrifugation. The latter indicated an 'opening' of the molecule that was not a denaturation, since the quenching of its fluorescence was not accompanied by a change in emission peak. Consequently, either the polarity of the tryptophan environment does not change significantly, or the quenching is due to the interaction in this low pH range of tryptophan and another residue that has acquired a proton in acid (p*K* approx. 5.7–6.0). The combined evidence, however, strongly favours some conformational change that does not involve any further exposure of the tryptophan residues to the solvent.

In conclusion, the *Ricinus* haemagglutinin molecule has a higher axial ratio than the ricin molecule, and is either more flexible or more elongated. However, a pH-dependent conformational transition can be observed for both molecules in the same range of low pH, with more visible changes in the tertiary structure of the haemagglutinin. This may be related to the changes induced by exposure of these toxic lectins to the conditions encountered inside acidic cellular organelles, and it is possible that, as suggested for diphtheria toxin (Blewitt *et al.*, 1985), conformational changes undergone by the two toxic lectins that we have investigated reflect their adaptation to the intracellular media that allows them to express their toxic activity.

We are indebted to (the late) Dr. Harold Edelhoch (National Institutes of Health, Bethesda, MD, U.S.A.) for many helpful discussions. We thank Dr. Guy Muller (C.N.R.S., Faculté des Sciences et Techniques, Mont-Saint-Aignan, France) for access to the low-angle laser-light-scattering apparatus and to Mohammed Anrhourrache for his help with this apparatus. This work was supported in part by grants from the Centre National de la Recherche Scientifique (LA 040293) and the U.E.R. Biomédicale des Saints-Pères (Contract no. 908–134). J.P.F. is Chargé de Recherche at the C.N.R.S.

REFERENCES

Baenziger, J. V. & Fiete, D. (1979) J. Biol. Chem. **254**, 9795–9799
Bernard, B., Aubery, M. & Bourrillon, R. (1979) Biochem. J. **182**, 33–38
Blewitt, M. G., Chung, L. A. & London, E. (1985) Biochemistry **24**, 5458–5464
Butterworth, A. G. & Lord, J. M. (1983) Eur. J. Biochem. **137**, 57–65
Cawley, D. B. & Houston, L. L. (1979) Biochim. Biophys. Acta **581**, 51–62
Cawley, D. B., Hedblom, M. L. & Houston, L. L. (1978) Arch. Biochem. Biophys. **190**, 744–755
Curatolo, W. (1982) Biochem. Biophys. Res. Commun. **106**, 1340–1345
Curatolo, W., Yau, A. O., Small, D. M. & Sears, B. (1978) Biochemistry **17**, 5740–5750
Debray, H., Decout, D., Strecker, G., Spik, G. & Montreuil, J. (1981) Eur. J. Biochem. **117**, 41–55
Decastel, M., Bourrillon, R. & Frénoy, J.-P. (1981) J. Biol. Chem. **256**, 9003–9008
Decastel, M., De Boeck, H., Goussault, Y., De Bruyne, C. K., Loontiens, F. G. & Frénoy, J.-P. (1985) Arch. Biochem. Biophys. **240**, 811–819
Dodeur, M., Aubery, M. & Bourrillon, R. (1980) Biochim. Biophys. Acta **628**, 303–313
Edelman, G. M. & Gall, W. E. (1969) Annu. Rev. Biochem. **38**, 415–466
Fish, W. W., Hamlin, L. M. & Miller, R. L. (1978) Arch. Biochem. Biophys. **190**, 693–698
Frénoy, J.-P. (1986) Biochem. J. **240**, 221–226
Herrmann, M. S., Richardson, C. E., Setzler, L. M., Behnke, W. D. & Thompson, R. E. (1978) Biopolymers **17**, 2107–2120
Herskovits, T. T., Jacobs, R. & Nag, K. (1983) Biochim. Biophys. Acta **742**, 142–154
Hsu, H. W., Davis, D. S., Wei, C. H. & Yang, W. K. (1976) Anal. Biochem. **73**, 513–521
Huet, M. (1975) Eur. J. Biochem. **59**, 627–632
Huet, M. & Claverie, J. M. (1978) Biochemistry **17**, 236–241
Kaneko, I., Satoh, H. & Ukita, T. (1973) Biochem. Biophys. Res. Commun. **50**, 1087–1094
Kornfeld, S., Eider, W. & Gregory, W. (1974) in Control of Proliferation in Animal Cells (Clarkson, B. & Baserga, R., eds.), pp. 435–445, Cold Spring Harbor Laboratory, Cold Spring Harbor
Lee, J. C. & Timasheff, S. N. (1974) Biochemistry **13**, 257–268
Lord, J. M. (1985) Eur. J. Biochem. **146**, 403–409
Monsigny, M., Sene, C., Obrenovitch, A., Roche, A. C., Delmotte, F. & Boschetti, E. (1979) Eur. J. Biochem. **98**, 39–45
Nagata, Y. & Burger, M. M. (1974) J. Biol. Chem. **249**, 3116–3122
Nicolson, G. L. & Blaustein, J. (1972) Biochim. Biophys. Acta **266**, 543–547
Nicolson, G. L., Blaustein, J. & Etzler, M. E. (1974) Biochemistry **13**, 196–204
Olsnes, S., Saltvedt, E. & Pihl, A. (1974) J. Biol. Chem. **249**, 803–810
Podder, S. K., Surolia, A. & Bacchawatt, B. K. (1974) Eur. J. Biochem. **44**, 151–160
Pusztai, A. & Stewart, J. C. (1978) Biochim. Biophys. Acta **536**, 38–49
Rice, R. H. & Etzler, M. E. (1974) Biochem. Biophys. Res. Commun. **59**, 414–419
Senear, D. F. & Teller, D. C. (1981) Biochemistry **20**, 3076–3083
Shimazaki, K., Walborg, E. F., Neri, G. & Jirgensons, B. (1975) Arch. Biochem. Biophys. **169**, 731–736
Steiner, R. F. & Edelhoch, H. (1963) Biochim. Biophys. Acta **66**, 341–355
Turpin, E., Goussault, Y., Lis, H. & Sharon, N. (1984*a*) Exp. Cell Res. **152**, 486–492
Turpin, E., Wantyghem, J., Beaudry, P., Néel, D. & Goussault, Y. (1984*b*) Can. J. Biochem. Cell Biol. **62**, 203–208
Turpin, E., Frénoy, J.-P. & Goussault, Y. (1984*c*) FEBS Lett. **175**, 82–86
Yamasaki, N., Absar, N. & Funatsu, G. (1985) Biochim. Biophys. Acta **828**, 155–161
Zentz, C., Frénoy, J.-P. & Bourrillon, R. (1979) Biochimie **61**, 1–6

Received 28 January 1986/12 June 1986; accepted 28 July 1986