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The effect of pH on the conformation and stability of the structure of plant toxin – ricin

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The effect of pH on the conformation of ricin and its A- and B-chains has been studied by measuring their intrinsic fluorescence. At pH 5.0 and 7.5, the structural stability of toxin and subunits was estimated according to the denaturing action of guanidine hydrochloride. It was demonstrated that the fluorescence of native toxin and catalytic A-subunit does not depend significantly on pH in the range pH 3–8, whereas ricin B-chain undergoes a structural transition at pH < 5.0. The structural stability of ricin and isolated chains differs significantly at pH 7.5 and 5.0; the structural stability of ricin and the A-chain increases, whereas that of the B-chain decreases.

Ricin; Protein fluorescence; pH effect; Guanidine hydrochloride

1. INTRODUCTION

The molecules of the plant toxin ricin consist of two polypeptide chains, A-chain (M_r 30625) and B-chain (M_r 31358), which are bound by one disulfide bond. Isolated A-subunit, entering the cytoplasm, catalytically inactivates the 60 S subunit of the ribosomes [1,2]. B-subunit binds to terminal residues of galactose on glycoproteins and glycolipids located on the cell surface [3], and, evidently, promotes transmembrane transfer of the A-chain [4]. It has been demonstrated that toxins penetrate into a cell through receptor-dependent endocytosis [5]. Indirect proof is available that endosomes at pH 5 are the most probable site for transmembrane transfer of the toxin A-chain into the cytoplasm [6]. In spite of much similarity in the general structure, toxins react differently to the elevation of pH by lysosomotropic amines in cellular organelles, which have low pH values under normal conditions. Treatment with NH_4Cl protects cells from the cytotoxic action of diphtheria toxin and modecin [6,7], but does not have any noticeable effect on the activity of abrin and ricin [7,8]. In addition, it has been shown that such treatment leads to enhancement of the action of some immunotoxin preparations containing the ricin A-chain [9]. It has been demonstrated that at pH 5 essential changes occur in the structure of diphtheria toxin [10,11]. The present study is devoted to the investigation of pH effects on the structure of native ricin and its isolated subunits.

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2. EXPERIMENTAL

2.1. *Isolation of ricin and subunits*

Ricin from *Ricinus communis* was obtained according to [12]. A- and B-subunits were isolated as described in [13]. The purity of the obtained preparations was estimated using electrophoresis in polyacrylamide gel in the presence of SDS [14]. The amount of dimers in preparations of isolated chains was measured using liquid chromatography

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under high pressure (Altex, model 100A, USA; TSK-2000 column, 7.5 × 600 mm). B-chain dimers were practically absent in the preparation of ricin, while their content in A-chain preparations was no more than 20%.

Diphtheria toxin, kindly supplied by Yu.A. Khavkin, was tested electrophoretically and used without additional purification.

2.2. *Intrinsic protein fluorescence*

The fluorescence spectra, corrected for the spectral sensitivity of the instrument, were registered using an Aminco SPF-500 spectrofluorimeter (USA) equipped with a standard quartz cuvette (1 × 1 cm). All measurements were performed at 25°C. Fluorescence was excited by light from a xenon lamp at 280 or 295 nm. The intensities of the corrected spectra were proportional to the number of photons emitted per unit wavelength interval. The absolute values of the fluorescence quantum yield were determined by the comparative method [16], assuming that the quantum yield of tryptophan in neutral water solutions is equal to 0.2 at 25°C [17].

Toxin solutions were prepared in 25 mM Na-phosphate or Na-acetate buffer with addition of 100 mM NaCl. During fluorometric titration microadditions of 1 M HCl and 1 M NaOH were performed in the cuvette; the dilution did not exceed 1% and pH was registered using a Radelkis OP-211/1 pH-meter (Hungary). The absorbances of solutions were determined with a Beckman DU-8B spectrophotometer (USA).

3. RESULTS AND DISCUSSION

3.1. *Fluorescence spectra*

Table 1 lists the fluorescence characteristics of ricin, its fragments and diphtheria toxin measured with excitation at 280 and 295 nm. Tyrosine residues in these proteins seem to give a very small contribution to the total emission. The fluorescence of tyrosine residues of the ricin A-chain is equal to 20% of the total emission, and all of its fluorescence parameters are significantly changed (see table 1).

Addition of 50 mM lactose to the solutions of ricin and its subunits does not affect their spectral parameters practically. Change in ionic strength of

Table 1

Fluorescence parameters of ricin, its subunits and diphtheria toxin at pH 7.5

Protein	λ_{ex} (nm)	λ_{max} (± 1 nm)	$\Delta\lambda$ (± 1 nm)	Quantum yield, q (± 10%)
Ricin	295	329	50	0.28
	280	329	52	0.21
B-chain	295	330	50	0.20
	280	330	51	0.20
A-chain	295	328	46	0.29
	280	323	52	0.11
Diphtheria toxin	295	327	50	0.065

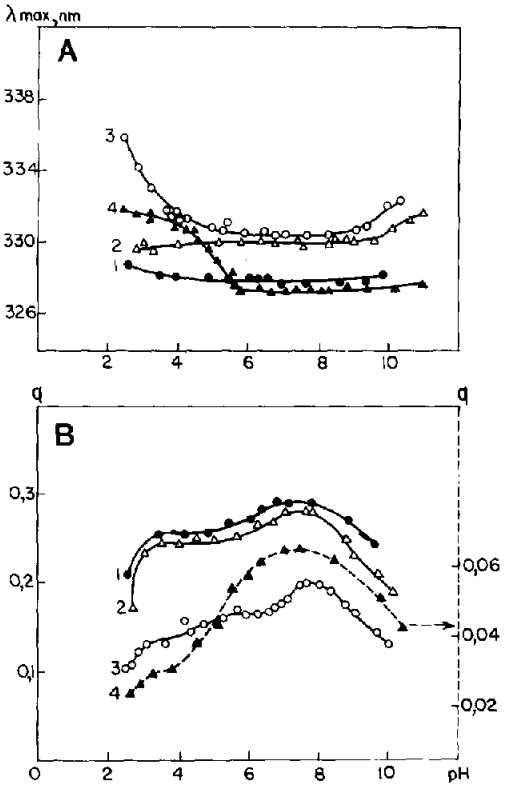

Fig.1. Effect of pH on toxin fluorescence. (A) Position of spectral maximum; (B) fluorescence quantum yield: 1, ricin A-chain; 2, ricin; 3, ricin B-chain; 4, diphtheria toxin. Measurement conditions are described in section 2. Protein concentrations ~20 μM.

the solution from 0.05 to 0.4 at pH 7.5 also has no influence on the fluorescence characteristics.

The short-wavelength position of the spectral maxima (328–330 nm) and high values of the fluorescence quantum yield indicate that tryptophan residues in ricin and its subunits are localized in the region of protein molecules, which are inaccessible to water molecules and have rather low mobility.

3.2. *Effect of pH*

Fig.1 shows the dependence of the spectral parameters of ricin, its A- and B-chains and diphtheria toxin on pH. The pH-induced changes in fluorescence quantum yield for ricin and its A-chain are identical (fig.1B, curves 1,2). These changes occur, in fact, without change in the position and shape of the spectra over a wide range of pH from 3 to 10 (fig.1A, curves 1,2), which evidently indicates the absence of significant structural rearrangement in the environment of tryptophan residues in this pH range. A drop of fluorescence quantum yield by about 15% in the pH region from 8 to 5 for all studied proteins is, presumably, caused by protonation of the imidazole rings of histidine residues (pK 6.5) [18]. For ricin B-chain, this is most likely caused by protonation of the imidazole of His-251 located near Trp-249. Further decrease in fluorescence yield at pH < 3 seems to be associated with quenching by free protons in the solution [19].

In the alkaline range of pH from 8 to 10 the quantum yield decreases for all studied proteins (fig.1B). For ricin and its B-chain this is accompanied by an insignificant red shift of their spectra (2 nm) (fig.1A, curves 2,3) which is probably caused by deprotonation of the amino groups of lysine residues, guanidine groups of arginine residues and the phenol group of tyrosine residues which quench the fluorescence of tryptophan residues in the deprotonated form [20,21].

The pH dependence of the fluorescence parameters for ricin B-chain exhibits peculiarities in the pH region below 5. A decrease in quantum yield of the B-chain fluorescence in this region (fig.1B, curve 3) is accompanied by a shift of the spectrum towards longer wavelengths (6 nm) (fig.1A, curve 3), which suggests unfolding of the protein structure near tryptophan residues. Similar changes also occur in diphtheria toxin at pH < 6 (fig.1A,B, curves 4), which is in good agreement with the data in [12] and data for cholera toxin [22]. As shown in table 2, the pH dependence of the fluorescence of ricin and its fragments is less pronounced than that for other toxins. However, the present data demonstrate that the ricin B-chain undergoes a structural transition in acid media (pK 4). The binding of ricin B-chain to lactose does not affect the pH dependence of fluorescence.

3.3. *Denaturation by guanidine hydrochloride*

Figs 2 and 3 demonstrate that the increase in

Table 2

Effect of pH on quantum yield of fluorescence of various toxins (excitation wavelength 295 nm)

Protein	$(q_{pH7.5} - q_{pH5})/q_{pH7.5}$	$(q_{pH7.5} - q_{pH3.5})/q_{pH7.5}$	The amount of residues per molecule	
			Trp	His
Ricin	0.125	0.13	7	6
Ricin A-chain	0.12	0.12	1	4
Ricin B-chain	0.20	0.325	6	2
Diphtheria toxin	0.31	0.54	5	14
Cholera toxin[a]	0.30	0.64	7	30
Cholera toxin[a] B-chain	0.45	0.64	1	4

[a] Data from [22]

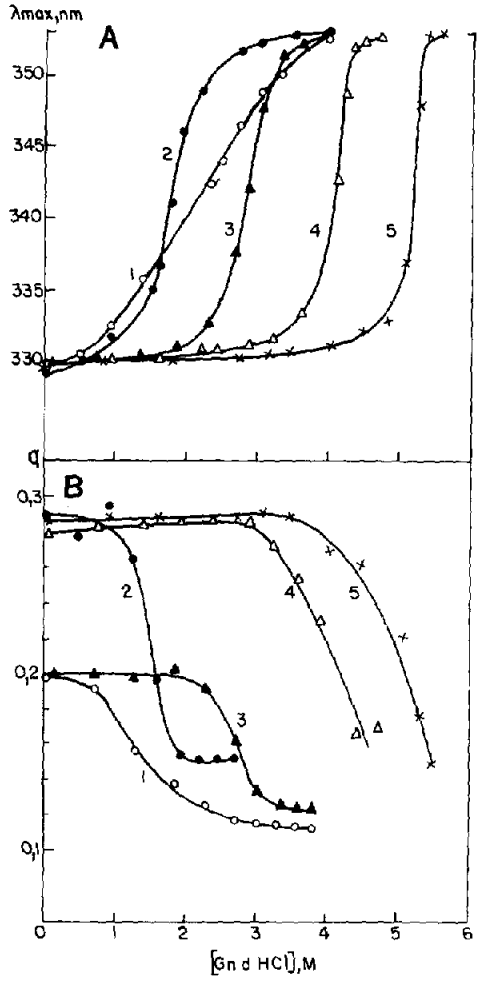

Fig.2. Effect of guanidine hydrochloride on fluorescence of ricin and its subunits at pH 7.5. (A) Position of spectral maximum; (B) fluorescence quantum yield; 1, ricin B-chain; 2, ricin A-chain; 3, ricin B-chain + 50 mM lactose; 4, ricin; 5, ricin + 50 mM lactose.

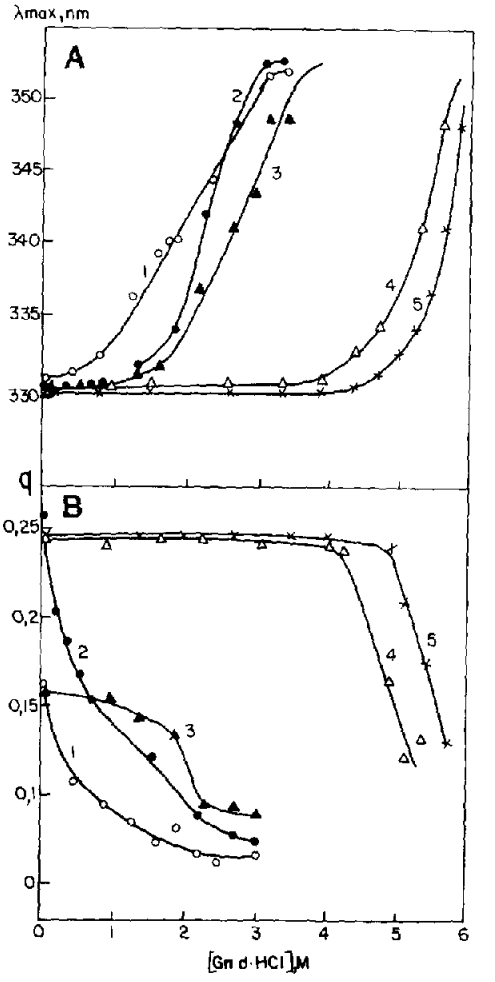

Fig.3. Effect of guanidine hydrochloride on fluorescence of ricin and its subunits at pH 5.0. (A) Change in position of spectral maximum; (B) change in quantum yield; 1, ricin B-chain; 2, ricin B-chain + 50 mM lactose; 3, ricin A-chain; 4, ricin; 5, ricin + 50 mM lactose.

concentration of guanidine hydrochloride results in a typical denaturational shift of the protein spectra towards a position characteristic of free tryptophan (353 nm) (figs 2A,3A). From the data in figs 2 and 3, it is clearly seen that at pH 5 the effect of the binding of lactose on protein denaturation is less pronounced in comparison with that at pH 7.5.

Thus, the above data show that at two different pH values, 7.5 and 5.0, the stability of the structure of ricin and its subunits is significantly different and the decrease of pH up to 5.0 results in increased stability of the ricin and A-chain structure and decreased stability of the B-chain structure. These results demonstrate the interdependence of the A- and B-chains of ricin.

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REFERENCES

[1] Olsnes, S. and Phil, A. (1982) in: Molecular Action of Toxin and Viruses (Cohen, P. and Van Heyningen, S. eds) pp.51–105, Elsevier, Amsterdam, New York.

[2] Benson, S., Olsnes, S., Phil, A., Skorve, J. and Abraham, A.K. (1975) Eur. J. Biochem. 59, 573–580.

[3] Olsnes, S. and Phil, A. (1982) Pharmacol. Ther. 15, 355–367.

[4] McIntosh, D.P., Edwards, D.C., Cumber, A.J., Parnell, G.D., Dean, C.J., Ross, W.C.J. and Forrester, J.A. (1983) FEBS Lett. 164, 17–20.

[5] Sandvig, K. and Olsnes, S. (1982) J. Biol. Chem. 257, 7495–7513.

[6] Sandvig, K. and Olsnes, S. (1980) J. Cell Biol. 87, 828–832.

[7] Sandvig, K., Olsnes, S. and Phil, A. (1979) Biochem. Biophys. Res. Commun. 90, 648–655.

[8] Mekada, E., Uchida, T. and Okada, Y. (1981) J. Biol. Chem. 256, 1225–1228.

[9] Jansen, F.K., Blythman, H.E., Carriere, D., Casellas, P., Gros, O., Laurent, J.C., Paolucci, F., Pau, B., Poncelet, P., Richer, G., Vidal, H. and Voisin, G.A. (1982) Immunol. Rev. 62, 185–216.

[10] Blewitt, M.G., Zhao, J.-M., McKeever, B., Sarma, R. and London, E. (1984) Biochem. Biophys. Res. Commun. 120, 286–290.

[11] Blewitt, M.G., Chung, L.A. and London, E. (1985) Biochemistry 24, 5458–5464.

[12] Nicolson, G.L. and Blaustein, J. (1972) Biochim. Biophys. Acta 2666, 543–547.

[13] Olsnes, S. and Phil, A. (1973) Biochemistry 12, 3121–3126.

[14] Anderson, C.W., Baum, P.R. and Gesteland, R.F. (1973) J. Virol. 12, 241–254.

[15] Bale, W.F., Helkamp, R.W., Davis, T.P., Izzo, M.J., Goodland, R.L., Contreras, M.A. and Spar, I.L. (1966) Proc. Soc. Exp. Biol. Med. 122, 407–414.

[16] Parker, C.A. and Rees, W.F. (1960) Analyst 85, 587–600.

[17] Teale, F.W.J. and Weber, G. (1957) Biochem. J. 65, 472–482.

[18] Bushueva, T.L., Busel, E.P., Bushuev, V.N. and Burstein, E.A. (1974) Stud. Biophys. 44, 129–139.

[19] Vladimirov, Yu.A. and Li, C.-G. (1962) Biofizika 7, 270–280.

[20] Bushueva, T.L., Busel, E.P. and Burstein, E.A. (1975) Stud. Biophys. 52, 41–52.

[21] Steinberg, J.Z. (1971) Annu. Rev. Biochem. 40, 83–160.

[22] De Wolf, M., Van Dessel, G., Lagron, A., Hilderson, H.J. and Dierick, W. (1985) Biochim. Biophys. Acta 832, 165–174.