Volume 229, number 1, 119–122 FEB 05590 February 1988

Similarity of protein conformation at low pH and high temperature observed for B-chains of two plant toxins: ricin and mistletoe lectin 1

T.L. Bushueva and A.G. Tonevitsky

Institute of Experimental cardiology, Cardiology Research Center of the USSR Academy of Medical Sciences, 3rd Cherepkovskaya Street 15a, Moscow 121552, USSR

Received 21 December 1987

A comparative study of subunits of two plant toxins, ricin (RC) and mistletoe lectin 1 (ML 1), has been undertaken. The study suggests that isolated B-chains of these toxins undergo structural transitions at low pH (from 5 to 4) and high temperature (45°C), and as a result of the guanidine hydrochloride denaturing effect (to 3 M). Our results indicate that the protein conformation observed at low pH and high temperature are similar, though not identical. These conformations differ from the native one (pH 7, 25°C), the protein in these conformations has a low fluorescence tryptophan intensity, and tryptophans are more exposed to aqueous solutions. However, these conformations differ also from the state unfolded by guanidine hydrochloride. An assumption is made that the partially denatured protein structure, exhibited at low pH and high temperature, is a functionally essential intermediate state of the toxin B-chain.

Ricin B-chain; Lectin B-chain; Protein fluorescence; (Mistletoe)

1. INTRODUCTION

The plant toxins ricin (RC) and mistletoe lectin 1 (ML 1) exhibit a pronounced similarity in their structural and functional characteristics. They consist of two polypeptide chains with almost similar molecular masses (about 30 kDa), bound by a disulfide bond. A-chains catalytically inactivate the 60 S subunit of ribosomes, whereas B-chains bind with galactose end residues on the cell surface [1–3], and promote A-subunit translocation into the cytoplasm [4]. Tryptophan residues are observed in both RC and ML 1 [5,6] and serve as useful reporters in protein structure studies [7]. The structural characteristics of both RC and ML 1 have recently been studied in our laboratory using intrinsic protein fluorescence [8,9]. We have demonstrated suggestive changes observed in the protein structure of total intact toxin molecues, and in isolated chains at pH < 5. The reported changes are exhibited with a noticeable prominence for B-chains of both proteins. The major purpose of this paper was to compare the conformational changes at low pH to those at high temperature and in denaturant. Protein conformations at low pH and high temperature are apparently similar.

Correspondence address: T.L. Bushueva, Institute of Experimental Cardiology, Cardiology Research Center of the USSR Academy of Medical Sciences, 3rd Cherepkovskaya Street 15a, Moscow 121552, USSR

Abbreviations: RC, ricin; ML 1, mistletoe lectin; Gnd HCl, guanidine hydrochloride

2. EXPERIMENTAL

Ricin from seeds (*Ricinus communis*) was obtained as described in [8,9]. Mistletoe lectin 1 (*Viscum album*) and its isolated B-chains were prepared by H. Franz as in [6]. Acrylamide and guanidine hydrochloride (Gnd Cl) from Sigma were used.

Fluorescence was measured with an Aminco SPF-500 spectrofluorometer (USA), equipped with a thermostated cuvette-holder, in a standard quartz cuvette (1 × 1 cm) as described [8].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

The toxin solutions were prepared in 25 mM Na-phosphate or Na-acetate buffer with 100 mM NaCl added. Final concentration of protein in the solution was from 15 to 30 μg/ml. Samples prepared at low pH, or high temperature, or with Gnd HCl were incubated 30 min before taking measurements. Temperature was recorded with time, each measurement with a digital probe thermometer.

3. RESULTS AND DISCUSSION

Fig.1 shows the pH effect on B-ricin conformation at 25°C and 60°C. The alterations observed for B-chains of both RC and ML 1 at 25°C were presented in detail in our previous papers [8,9]. At (and after) high temperature (60°C), the alteration is smoothed and the opposite character of spectral maximum shift is exhibited. A spectrum shift towards a shorter wavelength is observed at 60°C, while a shift towards a longer wavelength is observed at 25°C.

Thermal transitions for B-chains of both RC and ML 1 are shown in fig.1C,D. The midpoints of the transition, estimated by fluorescence intensity at 330 nm, are 45°C and 42°C for RC and ML 1, respectively. Thermal denaturation, as well as changes at low pH appear to result in irreversible changes.

Changes in fluorescence, which occur due to the guanidine hydrochloride effect [8,9] are qualitatively similar to those observed at low pH and high temperature, a fluorescence spectrum shift towards a longer wavelength (up to the position characteristic of free tryptophan maximum in water, λ = 353 nm), a drop of fluorescence intensity and an increase in the tryptophan residues accessibility to quenchers are exhibited (fig.2).

Fig.2 demonstrates fluorescence quenching by iodide and acrylamide, observed for ricin B-chain. Analogous data were obtained for the B-chain of ML 1 as well [9]. These data indicate that tryptophan residues of the proteins are relatively inaccessible in their native state and become accessible to quenchers in the presence of 3 M Gnd Cl.

At low pH or high temperature, the accessibility of tryptophan residues is intermediate or even approaches the native state.

Low pH and high temperature appear to be similar in their effect on protein conformation. Therefore, acting together, they are likely to serve as a driving force for protein transition from one state to another. Fig.3 demonstrates the thermal

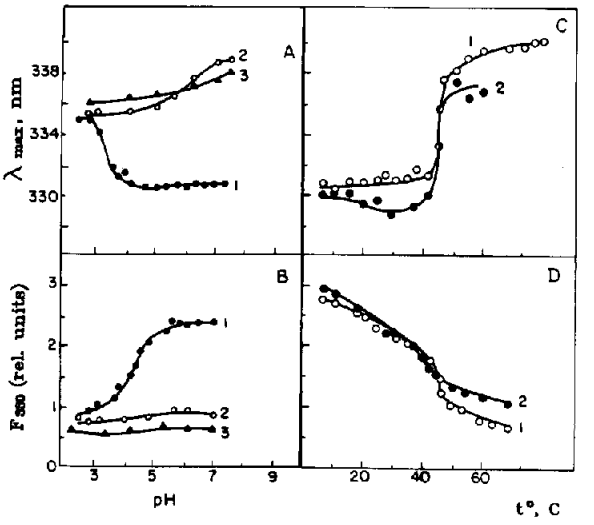

Fig.1. Effect of pH and temperature upon fluorescent parameters for B-chains of RC and ML 1. Panels: A,C, position of fluorescence spectral maximum; B,D, fluorescence intensity, $\lambda_{excitation}$ = 295 nm; $\lambda_{emission}$ = 330 nm; A,B, B-chain of RC (curves: 1, 25°C; 2, 25°C after heating to 60°C; 3, 60°C); C,D, curve 1, B-chain of RC; C,D, curve 2, B-chain of ML 1. Samples contained 15–30 μg/ml toxin in 25 mM buffer/100 mM NaCl. Buffers used were phosphate (pH > 6) and acetate (pH < 5.5).

changes observed at low temperature due to pH decrease. For RC B-chain the midpoint of the transition is shifted from 45°C (pH 7) to 27°C (pH 4). A further pronounced decrease in pH leads to the disappearance of thermal alteration (fig.3B, curve 4).

The data obtained show that low pH and high temperature conformations of the proteins are similar. The decreased fluorescence intensity as well as the fluorescence spectrum shift towards a longer wavelength, and tryptophan's prominent accessibility to a solvent serve as distinctive characteristic features of the intermediate state, as compared to the native one. However, the reported changes characterize only an insignificant unfolding of the protein structure. A certain difference exists in the states of protein structure at low pH and high temperature.

Similar intermediate states have recently been studied in detail and characterized for diphtheria toxin [10]. The intermediate states were found and investigated in quite different proteins (α-lactalbumin, carbonic anhydrase B) in the studies of Ptitsyn and colleagues [11,12]. The results ob-

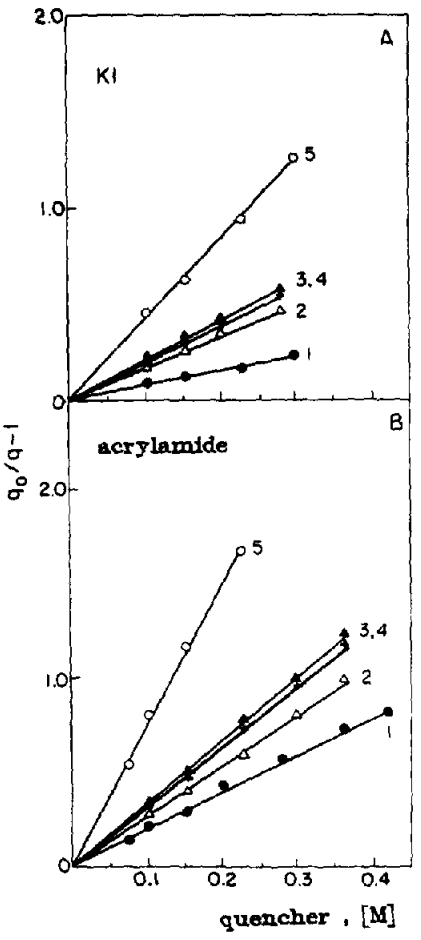

Fig.2. B-chain fluorescence quenching by iodide (A) and acrylamide (B). Stern-Volmer coordinates are used [14]. q_0 and q, the toxin fluorescence quantum yield in the presence or absence of the quencher. Curves: 1, pH 7, 25°C; 2, pH 3, 25°C; 3, pH 7, 60°C; 4, pH 7, 25°C after heating to 60°C; 5, in 3 M Gnd HCl.

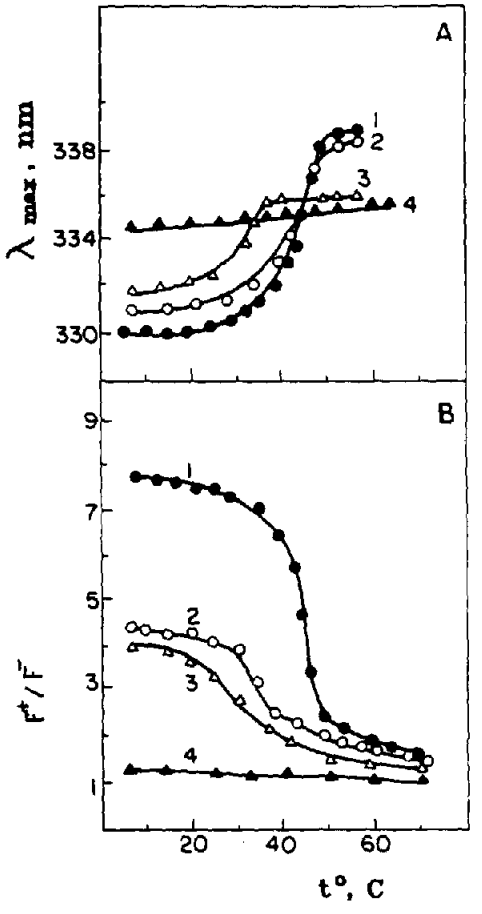

Fig.3. Effect of pH upon the temperature dependence of toxin fluorescence. (A) Temperature dependence of λ_{max} at various pH values; (B) fluorescence intensity (330 nm) vs temperature at various pH values. Fluorescence upon increasing temperature (F^+) was divided by fluorescence obtained during decreasing temperature (F^-) to cancel out ordinary temperature dependence of fluorescence. Curves: 1, pH 7.3; 2, pH 4.7; 3, pH 4.0; 4, pH 3.0.

tained allowed them to suggest a model for an intermediate state. The model was presented in the form of a so-called compact 'molten globule' with a secondary structure, though with fluctuating tertiary structure. By its physical characteristics, the molten globule contains the properties of both native and unfolded states. The decrease of specific interactions, as well as unfreezing of structure fluctuations are used as the explanation for the existence of the intermediate state, which is in accordance with the theory of conformational transitions [13].

The intermediate states, observed for the B-chains of toxin molecules, are presumably in close connection with functional properties of these proteins.

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.
[2] Olsnes, S., Stirpe, F., Sandvig, K. and Pihl, S. (1982) J. Biol. Chem. 257, 13263–13270.

[3] Sandvig, K. and Olsnes, S. (1982) J. Biol. Chem. 257, 7504–7513.
[4] Olsnes, S. and Sandvig, K. (1985) in: Endocytosis (Pastan, J. and Wilingham, M.C. eds) ch.7, pp.195–234.
[5] Funatsu, G., Kimura, M. and Funatsu, M. (1979) Agric. Biol. Chem. 43, 2221–2224.
[6] Franz, H. (1986) Onkology 43, 23–34.
[7] Burstein, E.A. (1977) Intrinsic Protein Luminescence. Nature and Applications (Russ.), Ser. Biofizika, vol.7, VINITI, Moscow.
[8] Bushueva, T.L. and Tonevitsky, A.G. (1987) FEBS Lett. 215, 155–159.
[9] Bushueva, T.L., Tonevitsky, A.G., Kindt, A. and Franz, H. (1988) Mol. Biol. (USSR), in press.
[10] Zhao, J.-M. and London, E. (1986) Proc. Natl. Acad. Sci. USA 83, 2002–2006.
[11] Dolgikh, D.A., Gilmanshin, R.J., Brazhnikov, E.V., Bychkova, V.E., Semisotnov, G.V., Venyaminov, S.Yu. and Ptitsyn, O.B. (1981) FEBS Lett. 136, 311–315.
[12] Ptitsyn, O.B., Dolgikh, D.A., Gilmanshin, R.T., Shakhnovich, E.I. and Finkelstein, A.V. (1983) Mol. Biol. (USSR) 17, 569–576.
[13] Shakhnovich, E.A. and Finkelstein, A.V. (1982) Dokl. AN USSR 5, 276–270.
[14] Stern, O. and Volmer, M. (1919) Phys. Z. 20, 183–188.