Structural properties of α-fetoprotein from human cord serum: the protein molecule at low pH possesses all the properties of the molten globule

Vladimir N. Uversky[a,*], Marina D. Kirkitadze[a], Natalya V. Narizhneva[a], Sergey A. Potekhin[a], Andrey Yu. Tomashevski[b]

[a]*Institute of Protein Research, Russian Academy of Sciences, Pushchino, Moscow Region, Russian Federation*
[b]*Institute of Biology and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russian Federation*

Received 1 March 1995; revised version received 20 March 1995

Abstract Structural studies of α-fetoprotein (AFP) from human cord serum have shown that a decrease in pH to 3.1 leads to a considerable conformational rearrangement of the protein molecule. The acid form of AFP belongs to the class of denatured conformations and fulfills all the requirements of the molten globule state. The possible functional role of such a transformation is discussed.

Key words: α-Fetoprotein; Protein stability; Scanning microcalorimetry; Circular dichroism

1. Introduction

α-Fetoprotein (AFP) is a glycoprotein which consists of ~600 amino acid residues. This protein is present at a high concentration in embryonal and fetal sera, while only traces of AFP are detected in adult sera. On the other hand, it has been shown that a considerable increase in AFP content in adults reflects the development of pathological conditions [1], such as hepatocellular carcinoma or germ cell teratocarcinoma. It has been established that AFP possesses a lot of biological activity – it participates in the transport of various hydrophobic ligands such as unsaturated fatty acids [2] and estrogens [3,4], it takes part in cell multiplication [5], it can affect cell metabolism [6] and interact with T-lymphocytes [7,8] and macrophages [9,10], it binds bilirubin as well as Ni^{2+} and Cu^{2+} ions, and, finally, it possesses immunosuppressive properties [9,11]. The biochemical and structural properties of AFP and its numerous biological functions have been reviewed [1,12–14].

The molten globule (see [15–19] for reviews) is the third thermodynamic state of the protein molecule [20–22] which exists as an equilibrium intermediate of many proteins under mild denaturing conditions. It accumulates as a universal kinetic intermediate during protein folding. The protein molecule in the molten globule state is almost as compact as in the native state, has a pronounced secondary structure, and differs from the native molecule mainly by the absence of tight packing of side chains in the protein core and by a substantial increase in fluctuations [15–19]. It has been suggested [23] and confirmed experimentally [24–27] that the molten globule state of a protein molecule can be of large physiological importance (see [28] for review).

*Corresponding author. Fax: (7) (095) 924 0493.
E-mail: protres@ipr.serpukhov.su

In the present paper we show that the acid form of human AFP is denatured (i.e. it lacks a rigid tertiary structure), but is practically as compact as the native protein molecule and has a native-like content of secondary structure. Its affinity to the hydrophobic fluorescent probe (ANS) is much higher than that observed for the native or completely unfolded states of AFP. All this means that at low pH the AFP molecule is transformed into the molten globule state. We suggest that this observation can be of functional significance.

2. Materials and methods

2.1. Materials

AFP was isolated from human cord serum with the use of metal-chelate and reverse-phase chromatography. The procedure of protein isolation and purification will be described elsewhere.

The method of immunorocketry electrophoresis was used for the identification of chromatographic fractions containing AFP. The protein purity was checked by HPLC reverse-phase chromatography, SDS and native polyacrylamide gel electrophoresis (PAGE) with immunoblotting, and by the ELISA test with antibodies against human serum albumin, α-antitrypsin and transferrin. The protein purity was no less than 98% according to all these tests.

Electrophoresis reagents were from Bio-Rad. Buffer compounds were analytical or extra pure grade and were used without additional purification. Desirable pH values were adjusted by the addition of 0.5–1.0 N HCl or NaCl. All solutions were prepared on bi-distilled water and contained 0.15 M NaCl.

2.2. Experimental procedures

Calorimetric measurements were carried out by a precision scanning microcalometer DASM-5M (Bureau of Biological Instrumentation, Pushchino, Russia), with a cell volume of 1 ml. The rate of heating was 1 K/min and the excess pressure was kept equal to 3.6 atm. The protein concentration was 0.5–1.0 mg/ml. In calculations the AFP partition volume of 0.72 cm^3/g (see [33]) was used. The excess heat capacity of the protein in solution was determined as described in [34].

All solutions contained 0.15 M NaCl and were equilibrated overnight against a corresponding buffer by dialysis.

Circular dichroism spectra were obtained on a Jasko-600 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) equipped with a temperature-controlled holder. The cell pathlength was 0.15 mm. The protein concentration was 0.8 mg/ml. All solutions contained 0.15 M NaCl. Measurements were done at 23°C.

Fluorescence measurements were made with an Aminco (SPF-100CS) corrected spectrofluorimeter (American Instrument Corp., Silver Spring, MD). The cell pathlength was 10.0 mm. Protein and ANS concentrations were 0.005 mg/ml. ANS binding was registered by the characteristic increase in the ANS fluorescence intensity [32]. All solutions contained 0.15 M NaCl. Measurements were done at 23°C.

Protein concentrations were determined by the absorbance at 280 nm on a Cary spectrophotometer. The extinction coefficient was determined according to a well-known method based on the nitrogen determination [35] and was $\varepsilon_{1\,cm,280}^{1\,mg/ml} = 0.365$.

SSDI 0014-5793(95)00379-7

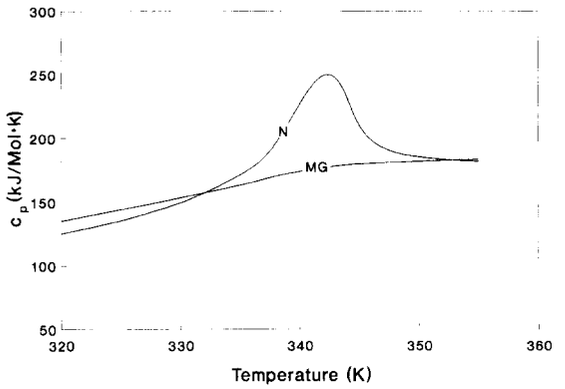

Fig. 1. Temperature dependence of partial molar heat capacity of human α-fetoprotein at pH 7.2 (N) and 3.1 (MG). The solutions contained 0.15 M NaCl.

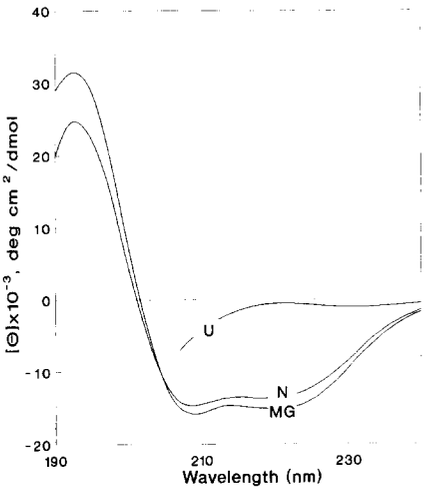

Fig. 2. Far UV circular dichroism spectra of AFP in the native state at pH 7.2 (N), acid form at pH 3.1 (MG) and completely unfolded state at pH 7.2, 9 M urea (U). The solutions contained 0.15 M NaCl. All measurements were done at 23°C.

3. Results

3.1. The acid form of AFP is denatured

Since the AFP polypeptide chain contains only one tryptophan among 600 amino acid residues, it was not a surprise that its circular dichroism (CD) spectra in the near ultra violet (UV) region are weakly pronounced and do no differ much in water and 9 M urea (data not shown). This was the reason for the use of other structural methods to find out whether AFP has a rigid tertiary structure at given conditions. One such approach is scanning microcalorimetry [29] (the presence of a heat absorption peak on the heat capacity vs. temperature curves under the temperature-induced denaturation of protein molecules is usually considered as direct evidence for the cooperative melting of the protein rigid tertiary structure [29]).

Fig. 1 presents the partial molar heat capacity vs. temperature curves for human α-fetoprotein at pH 7.2 and 3.1. One can see that at pH 7.2 the curve contains the characteristic heat absorption peak. From the analysis of this curve with the use of well-known equations [29] it is possible to determine calorimetric (ΔH^{cal}) and effective (ΔH^{eff}) enthalpy values, a comparison of which can provide information on the mechanism of the given temperature-induced transition [29]. In our case, the calorimetric enthalpy is practically equal to the effective one (ΔH^{cal} = 476 kJ/M, ΔH^{eff} = 514 kJ/M; and $\Delta H^{eff}/\Delta H^{cal}$ = 1.08). This means that temperature-induced denaturation of AFP at pH 7.2 can be considered as a two-state process. In other words, a cooperative unit coincides with a protein molecule, i.e. a protein molecule denatures as a whole without the coexistence of native and denatured parts in the same molecule.

Another situation is observed for AFP molecules at pH 3.1. As follows from Fig. 1, in this case the characteristic heat absorption peak is absent. This means that at low pH the AFP molecule has no rigid tertiary structure, i.e. it is already denatured.

The pecularities of thermodynamic studies of AFP denaturation will be described elsewhere.

3.2. The acid form of AFP has pronounced secondary structure

Fig. 2 shows AFP CD spectra in the far UV region in the native state (pH 7.2), the acid state (pH 3.1) and in 9.0 M urea. One can see that the CD spectrum in 9 M urea is typical of the completely unfolded state, while the CD spectrum at pH 3.1 is comparable with that of the native state. This indicates that in the acid form AFP has a native-like content of secondary structure.

3.3. The acid form of AFP is compact

It is known that unfolding of the protein molecule is accompanied by a red shift of the tryptophan (Trp) fluorescence maximum [30]. Fig. 3 shows Trp fluorescence spectra of human AFP in the native state (pH 7.2), acid form (pH 3.1) and in 9.0 M urea. It shows that the maximum of spectrum for the acid form (curve 2) is only slightly shifted to the red region as compared with the native state (curve 1) and is far from that of the completely unfolded state (curve 3). This indicates that the Trp residue of AFP in the acid form is inaccessible to the solvent, which may suggest that at least the Trp-containing domain of the protein molecules in this state are compact.

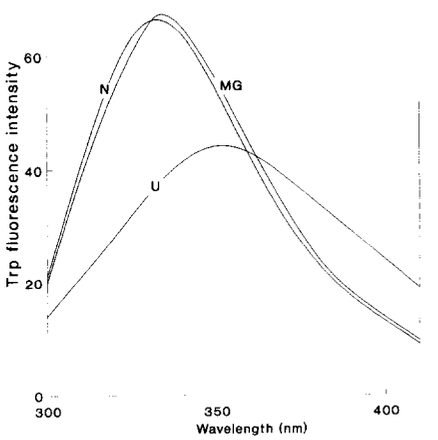

Fig. 3. Trp fluorescence spectra of AFP in different conformational states (see legend to Fig. 2). The solutions contained 0.15 M NaCl. All measurements were done at 23°C.

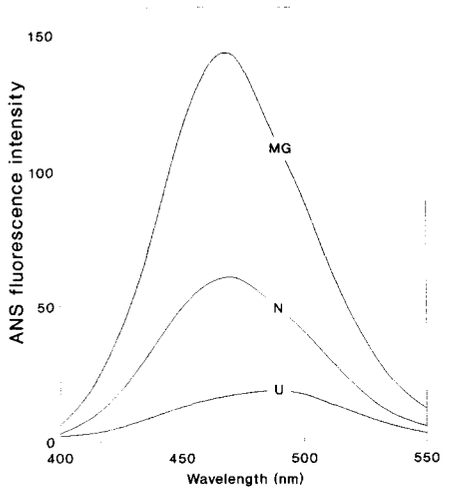

Fig. 4. Fluorescence spectra of ANS in the presence of AFP in different conformational states (see the legend to Fig. 2). The solutions contained 0.15 M NaCl. Protein and ANS concentrations were 0.005 mg/ml. All measurements were done at 23°C.

3.4. *The acid form of AFP binds strongly to ANS*

Fig. 4 presents the results of 8-anilinonaphthalene-1-sulphonate (ANS) fluorimetric studies of AFP in the native, completely unfolded and acid states. It is known that the binding of ANS is accompanied by a strong increase in its fluorescence intensity and by a blue shift of the spectrum maximum [31]. Fig. 4 shows that both native and acid forms of AFP bind ANS, but the affinity of the acid form to ANS is essentially higher. It has been shown that in some cases ANS binds to solvent-accessible clusters of non-polar groups in the native state [31,32], but its binding to the molten globule state is much stronger [32].

4. Discussion

The results presented in this paper show that the acid form of AFP from human cord serum possesses all the properties of the molten globule state. Indeed, at pH 3.1 the AFP molecule is denatured. On the other hand, it is practically as compact as the native one and preserves a pronounced secondary structure. At last, the acid form of AFP has a larger affinity to ANS as compared with the native and completely unfolded states, which is the general sensitive test for molten globule formation [32].

We suggest that the conformational transition of the AFP molecule from the native to the molten globule state can play an important role in the functioning of this protein. It is known that AFP, being a transport protein, binds its ligands (e.g. unsaturated fatty acids) with a high association constant [2]. This permits it to avoid their spontaneous release in serum. On the other hand, near (or within) the target cells these ligands must leave the AFP molecule. We assume that this process is connected with the transformation of a rigid native protein molecule into the molten globule, which results in a substantial increase in flexibility and facilitates the release of ligands [28]. It is necessary to emphasize that the native–molten globule transition can occur in a living cell where a protein molecule can be strongly influenced by electrostatic fields and the organic moiety of membranes [28].

Acknowledgements: This work was supported in part (V.N.U.) by a grant from Human Frontier Scientific Foundation Program (RG-331/93). We want to express our deepest gratitude to Professors O.B. Ptitsyn and G.I. Abelev for valuable discussions.

References

[1] Abelev, G.I. (1971) Adv. Cancer Res. 14, 295–358.
[2] Parmelee, D., Evenson, M.A. and Deutsch, H.F. (1978) J. Biol. Chem. 253, 2114–2119.
[3] Uriel, J., de Nechaud, B. and Dupiers, M. (1972) Biochem. Biophys. Res. Commun. 46, 1175–1180.
[4] Nishi, S., Matsue, H., Yoshida, H., Yamamoto, R. and Sakai, M. (1991) Proc. Natl. Acad. Sci. USA 88, 3102–3105.
[5] Hirai, H. (1985) in: Biochemical Markers for Cancer (Ming Chu, T., ed.) p. 25, Marcel Dekker, New York.
[6] Nunez, E.A., Cristef, N., Auclair, M.C., Benassay, C. and Carli, A. (1987) Tumor Biol. 8, 273–280.
[7] Parmelee, M.J. and Hsu, H.F. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 979.
[8] Torres, J.M., Laborda, J., Naval, J., Darracq, N., Calvo, M., Mishal, Z. and Uriel, J. (1989) Mol. Immunol. 26, 851–857.
[9] Lu, C.Y., Changelian, P.S. and Unanue, E.R. (1984) J. Immunol. 132, 1722–1727.
[10] Lu, C.Y., Redline, R.W., Shea, C.M., Dustin, L.B. and McKay, D.B. (1989) Transplant 48, 848–855.
[11] Calwell, J.L., Severson, Ch.D. and Thompson, J.S. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 979.
[12] Abelev, G.I. (1993) Sov. Sci. Rev. D. Physicochem. Biol. 11, 85–109.
[13] Ruoslahti, E. and Seppala, M. (1979) Adv. Cancer Res. 29, 275–346.
[14] Deutsch, H.F. (1991) Adv. Cancer Res. 56, 253–312.
[15] Ptitsyn, O.B. (1987) J. Prot. Chem. 6, 273–293.
[16] Kuwajima, K. (1989) Proteins: Struct. Funct. Genet. 6, 87–103.
[17] Christensen, H. and Pain, R.H. (1991) Eur. Biophys. J. 19, 221–229.
[18] Baldwin, R.L. (1991) Chemtracts 20–Biochem. Mol. Biol. 2, 379–389.
[19] Ptitsyn, O.B. (1992) in: Protein Foldin (Creighton, T.E., ed.) pp. 243–300, Freeman, New York.
[20] Uversky, V.N., Semisotnov, G.V., Pain, R.H. and Ptitsyn, O.B. (1992) FEBS Lett. 314, 89–92.
[21] Uversky, V.N., Semisotnov, G.V. and Ptitsyn, O.B. (1993) Biophysics 38, 31–39.
[22] Ptitsyn, O.B. and Uversky, V.N. (1994) FEBS Lett. 341, 15–18.
[23] Bychkova, V.E., Pain, R.H. and Ptitsyn, O.B. (1988) FEBS Lett. 238, 231–234.
[24] Martin, J., Langer, T., Boteva, R., Schamel, A., Horwich, A.L. and Hartl, F.-U. (1991) Nature 352, 36–42.
[25] Van der Goot, F.G., Gonzalez-Manes, J.M., Lakey, J.H. and Pattus, F. (1991) Nature 359, 408–410.
[26] De Jongh, H.H.J., Killian, J.A. and de Kruijff, B. (1992) Biochemistry 31, 1631–1643.
[27] Hua, Q.X., Ladbury, T.E. and Weiss, M.A. (1993) Biochemistry 32, 1433–1442.
[28] Bychkova, V.E. and Ptitsyn, O.B. (1993) Chemtracts Biochem. Mol. Biol. 4, 133–163.
[29] Privalov, P.L. (1979) Adv. Protein Chem. 33, 167–241.
[30] Stryer, L. (1966) Science 162, 526–540.
[31] Stryer, L. (1965) J. Mol. Biol. 13, 482–495.
[32] Semisotnov, G.V., Rodionova, N.A., Razgulyaev, O.I., Uversky, V.N., Gripas, A.F. and Gilmanshin, R.I. (1991) Biopolymers 31, 119–128.
[33] Zamyatnin, A.A. (1973) Dilatometry of Protein Solutions. Nauka, Moscow, Russia (in Russian).
[34] Privalov, P.L. and Potekhin, S.A. (1986) Methods Enzymol. 131, 4–51.
[35] Jaenicke, L. (1974) Anal. Biochem. 61, 623–627.