CIRCULAR DICHROIC STUDY OF RAT ALPHAFETOPROTEIN

J. P. AUBERT, J. P. KERCKAERT, B. BAYARD and M. H. LOUCHEUX-LEFEBVRE

Institut de Recherches sur les Cancer de Lille et Unité, No. 124 de l'INSERM, B. P. No. 3567, 59020 Lille Cédex, France

Received 6 April 1977

1. Introduction

Alphafetoprotein (AFP), a specific fetal serum protein found in association with primary hepatoma and teratoma (for review, see refs [1,2]), has been purified from various sources by several convenient methods [3–6]. Polyacrylamide gel electrophoresis of rat AFP has been used as a simple procedure in demonstrating its heterogeneity [3, 6–11] and in the purification of milligram quantities of the two variants [9] (AFP_A, electrophoretic 'slow' form and AFP_B 'fast' form).

Preliminary biochemical analysis failed to explain the difference in electrophoretic mobilities of these variants [9]. We report here a circular dichroic study undertaken to compare the secondary structure and the stability of these two AFP variants.

2. Materials and methods

2.1. *Source of AFP*

The two electrophoretic variants were obtained in pure form as previously described by polyacrylamide slab gel electrophoresis either directly from amniotic fluid [9] or from a whole immuno-adsorbed AFP preparation [4].

Rat albumin was isolated in pure form by polyacrylamide slab-gel electrophoresis.

2.2. *CD Measurements*

The dichroic spectra were recorded with a dichrograph Jobin-Yvon R. J. Mark III in 0.01 cm and 0.5 cm pathlength cell at an absorbance less than 1.5 optical density units.

The protein concentration was estimated by the Lowry's method [12] after dissolving it in 0.214 M NaF ($9^{\circ}/_{oo}$) pH 7.3.

The mean residual molar ellipticity $[\theta]$ was expressed in deg. cm^2 . $dmol^{-1}$. The ellipticity curves were constructed from at least five spectra. The average residual weights for AFP_A and AFP_B were 112.8 and 112.1 respectively.

2.3. *Ultraviolet measurements*

The ultraviolet spectra of the proteins were recorded with a spectrophotometer Varian Cary 118C in 1 cm pathlength cell. The protein concentration was about 1 mg/ml.

3. Results

3.1. *Dichroic study*

The dichroic spectra of both AFP dissolved in 0.214 M NaF were recorded. Between 180 nm and 250 nm the spectra showed two negative bands at 222 nm and 208 nm with an ellipticity of about $16 . 10^3$ deg. cm^2. $dmol^{-1}$, and one positive band at 193 nm with an ellipticity of $30 . 10^3$ deg. cm^2. $dmol^{-1}$ (fig.1). Between 250 nm and 350 nm the spectra showed a weak and large band centered at about 270 nm which corresponds to the dichroic contribution of the aromatic residues and disulfide bridges of the proteins. As it is shown in fig.1 the spectra obtained either from the two AFP prepared by the electrophoretic procedure or from the two AFP obtained by affinity chromatography were exactly the same with respect to their intrinsic and extrinsic bands. The nature of the dichroic spectra were typical of α-helix containing proteins. A value of about 60% α-helix was obtained by the method of Chen et al. [13]

North-Holland Publishing Company – Amsterdam

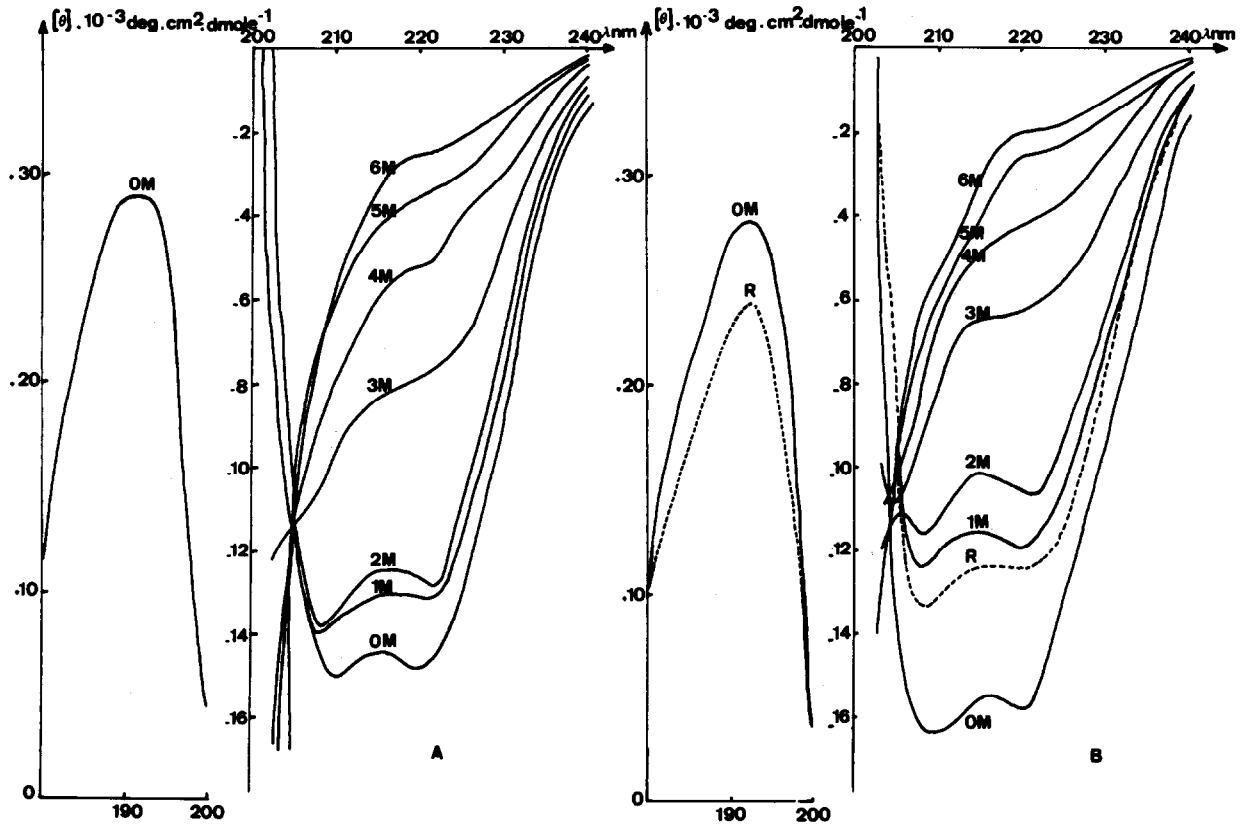

Fig.1. Dichroic spectra of AFP_A (A) and AFP_B (B) in different concentrations in guanidinium hydrochloride and after dialysis (R).

adjusted to our dichrograph. It is unusual for globular proteins to display such a high value. To observe the helix coil transition, the effect of the following factors were studied: ionic strength, pH, heat, addition of dioxan, addition of guanidinium hydrochloride (GuCl).

3.2. *Ionic strength*

The primary structure of AFP is characterized by a large ratio of Glu residues [9]. If their distribution was uneven, a modification of the secondary structure of the protein, that is a variation of the dichroic spectrum could be expected when the ionic strength is varied. Moreover, since the different electrophoretic mobilities of the two AFP variants might be due to a difference in the net charge of the α-amino acids residues, the dichroic spectra were recorded in different NaF solutions from 5 mM to 1 M. The spectrum remained exactly the same along the entire molarity gradient, and for the two variants.

3.3. *pH-Values*

The proteins were dissolved in 0.214 M NaF at pH 7.3. A variation in pH from 11–4.6 did not modify the spectra. Although a pH below 4.6 causes the AFP to precipitate, a subsequent rise in pH will not resolubilize it. The proteins go back into solution in a citrate buffer pH 2.5 but the spectra remain the same.

3.4. *Heat*

The dichroic spectra were recorded after heating the protein from 20–90°C. Figure 2 gives the variation of $[\theta]_{222}$, the ellipticity at 222 nm, against the temperature. The curve is exactly the same for the two variants. As shown in fig.2 drastic changes occured when protein was heated since AFP lost about 40% of

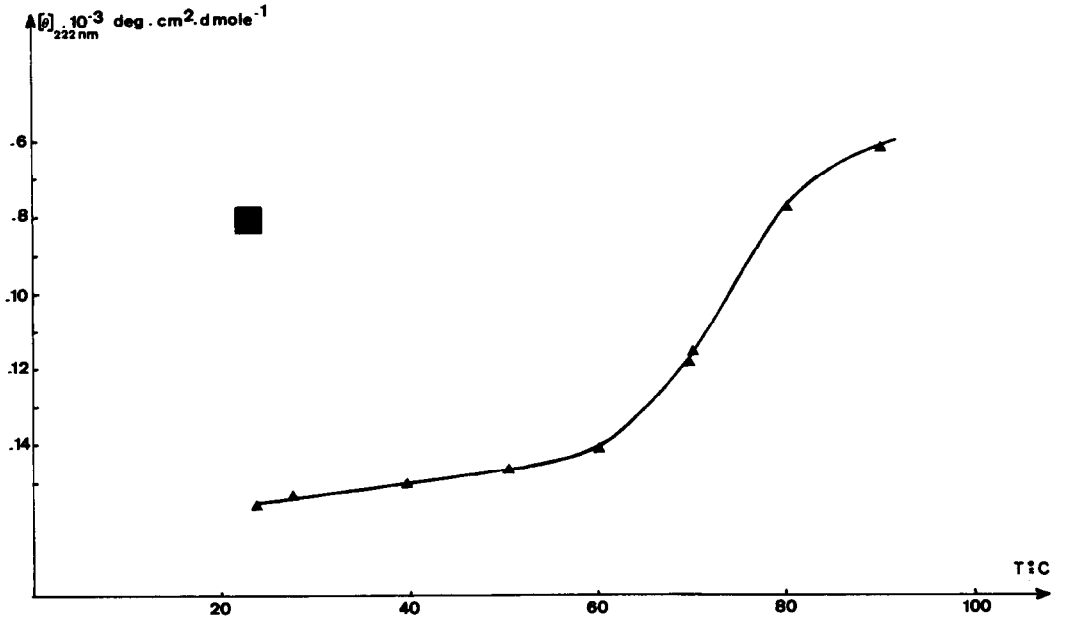

Fig.2. Variation of $[\theta]_{222}$ of AFP versus the temperature. (■) Value of $[\theta]_{222}$ obtained for the heat denaturated solution after cooling.

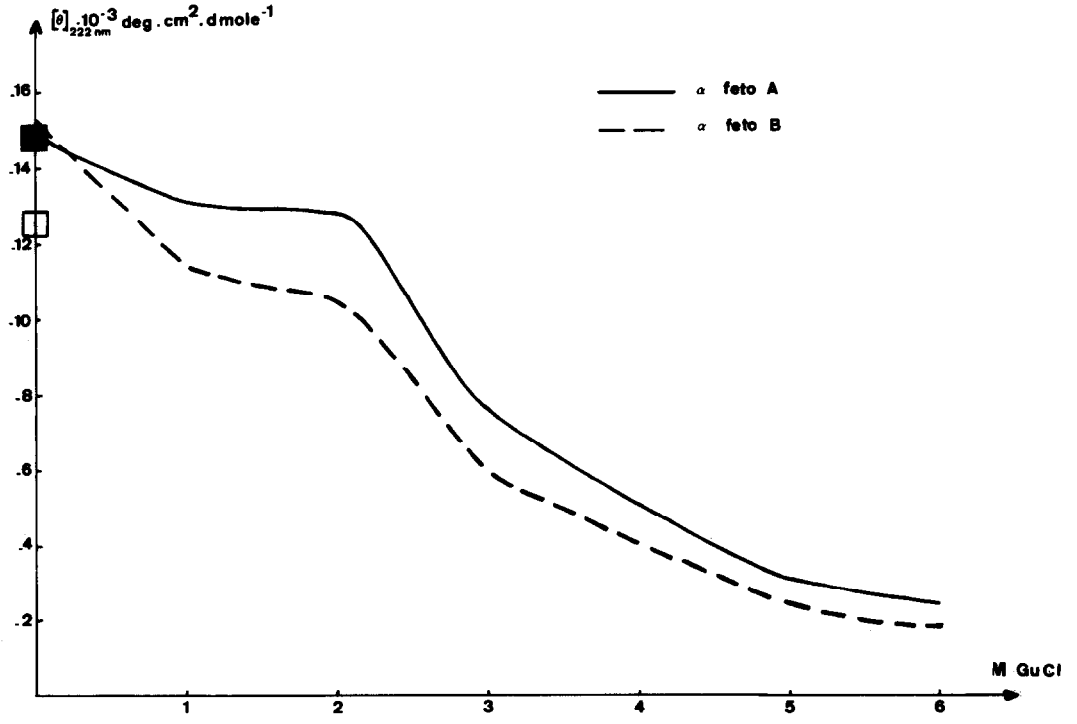

Fig.3. Transition of $[\theta]_{222}$ versus the molarity of guanidinium hydrochloride. (———) AFP_A; (– – – –) AFP_B.

ellipticity between 20°C and 90°C. The thermal denaturation is not very cooperative. Moreover, the reversibility of this phenomenon was controlled as observed fig.2: the protein does not find again its secondary structure after cooling of the heat denatured solution.

3.5. *Addition of dioxanne*

Spectra obtained in dioxanne–water solutions remained exactly the same when the quantity of dioxanne increased to 35% in volume.

3.6. *Addition of guanidinium hydrochloride*

Upon addition of 3 M GuCl, the spectrum became characteristic of a random coil structure (fig.1). The variation of the ellipticity at 222 nm, $[\theta]_{222}$, versus the molarity of GuCl showed that the transition took place between 2 M and 3 M (fig.3). Furthermore it is interesting to point out that the denaturation of AFP_B appeared at a lower molarity than AFP_A. As is shown in fig.1 only AFP_A is completely renatured after 48 h dialysis against 0.214 M NaF.

3.7. *Differential ultraviolet study*

In order to detect a possible interaction between the two variants, the differential ultraviolet absorption was measured with a four-cell system while adding AFP_B to AFP_A until equimolar ratios were obtained. Of the two beams, one passes through NaF and then a AFP_A, AFP_B mixture, while the other traverses first AFP_A followed by AFP_B. Since no difference in the spectra was observed we conclude that no interaction took place between AFP_A and AFP_B.

4. Discussion

The stability of these two variants towards denaturation is consistent with a high degree of helicity. If the two variants are denatured by 3 M GuCl, the renaturation of AFP_A is total while that of AFP_B is incomplete. An effort was made to see if Fe^{3+}, Mg^{2+} or Ca^{2+} were bound. No Na^+, Cl^- or Ca^{2+} was found in either case, while trace amounts of Fe^{3+} and Mg^{2+} were revealed in AFP_A. For AFP_B, Mg^{2+} was present in significant amounts and Fe^{3+} was also seen to a lesser extent. It is tempting to hypothesize that dialysis after denaturation of AFP_B removes the Mg^{2+} which is then no longer available for complete renaturation.

In addition it seems that there is no interaction between the two AFP variants as shown by differential ultraviolet absorption. This result agrees with the dichroic spectra which did not vary when the ionic concentration changed. Therefore, no electrostatic interactions take place between AFP_A and AFP_B. It has been suggested that AFP may be the fetal counterpart of serum albumin [2] and in addition, these two proteins have some sequence homologies [14]. For these reasons, we have recorded the spectrum of rat serum albumin (fig.4). Our results are consistent with the well established secondary structure of this protein. The unexpected high percentage of helicity of AFP is not an artefact which should be due to the method of preparation, since the dichroic spectrum observed for serum albumin (fig.4) obtained by the same procedure that AFP is in excellent agreement with the ellipticity of bovine serum albumin given

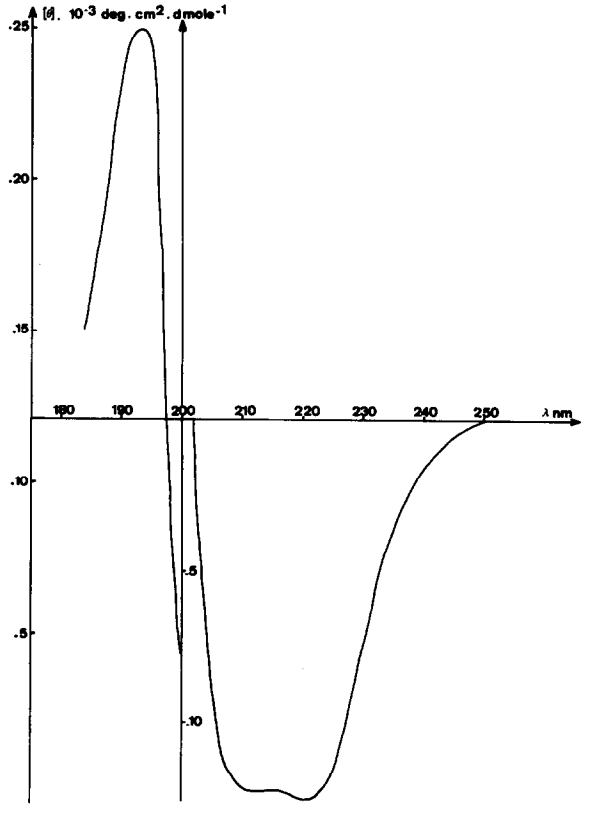

Fig.4. Dichroic spectra of rat serum albumin in 0.214 M NaF solution.

in the literature [15]. The difference between the secondary structure of AFP and serum albumin is significant, but no correlation can be established between these two proteins.

Acknowledgements

The authors thank Mrs M. P. Hildebrand, S. Quief and D. Roux for their excellent technical assistance. This work was supported by the Institut National de la Santé et de la Recherche Médicale (U. 124) and the Fondation pour la Recherche Médicale Française.

References

[1] Masseyeff, R. (1972) Pathol. Biol. 20, 703–725.
[2] Abelev, G. I. (1971) Adv. Cancer Res. 14, 295–358.
[3] Gusev, A. I. and Yazova, A. K. (1970) Biokhimiya 35, 172–181.
[4] Nishi, S. and Hirai, H. (1972) Biochim. Biophys. Acta 278, 293–298.
[5] Alpert, E., Drysdale, J. W. and Isselbacher, K. J. (1973) Ann. NY Acad. Sci. 209, 387–396.
[6] Cittanova, N., Grigorova, A. M., Benassayag, C., Nunez, E. and Jayle, M. F. (1974) FEBS Lett. 41, 21–24.
[7] Aussel, C., Uriel, J. and Mercier-Bodard, C. (1973) Biochimie 55, 1431–1437.
[8] Watabe, H. (1974) Int. J. Cancer 13, 377–388.
[9] Kerckaert, J. P., Bayard, B., Quief, S. and Biserte, G. (1975) FEBS Lett. 53, 234–236.
[10] Watanabe, A., Taketa, K. and Kosaka, K. (1975) Ann. NY Acad. Sci. 259, 95–108.
[11] Lai, P. C. W., Forrester, P. I., Hancock, R. L., Hay, D. M. and Lorscheider, F. L. (1976) J. Reprod. Fert. 48, 1–8.
[12] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Chen, Y. H., Yang, J. T. and Chau, K. H. (1974) Biochemistry 13, 3350–3359.
[14] Ruoslahti, E. and Seppälä, M. (1972) Nature 235, 161–162.
[15] Timasheff, S. N., Susi, H., Townend, R., Stevens, L., Gorbunoff, M. J. and Kumosinski, T. F. (1967) in: Conformation of Biopolymers (Ramachandran, G. N. ed) Vol. 1, pp. 173–196, Academic Press, London, New York.