

Interaction of rat α -fetoprotein and albumin with polyunsaturated and other fatty acids: determination of apparent association constants

Alberto Anel, Miguel Calvo⁺, Javier Naval, María Iturralde, María A. Alava and Andrés Piñeiro

Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, 50009 Zaragoza and ⁺Tecnología y Bioquímica de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet, 177, 50013, Zaragoza, Spain

Received 17 February 1989

The interaction of fatty acids with rat α -fetoprotein and albumin was measured using a partition equilibrium method. α -Fetoprotein (AFP) displays one high-affinity binding site for fatty acids and albumin near two binding sites. The AFP association constants for most fatty acids were similar to those of albumin (in the 10^7 M⁻¹ range) whereas for docosahexaenoic acid it was 9.7×10^8 M⁻¹, about 50-fold higher than that corresponding to albumin. This difference justifies docosahexaenoic acid in fetal or neonatal serum being mainly bound to AFP and can indicate a highly specific role of AFP in the transport of this fatty acid.

α -Fetoprotein; Albumin; Docosahexaenoic acid

1. INTRODUCTION

α -Fetoprotein (AFP) is a major glycoprotein of embryonic and fetal plasma synthesized mainly in yolk sac and fetal liver [1]. This protein is internalized by many tissues during fetal [2] and tumoral growth [3]. The biological role for AFP may be related to its ability to bind and transport unesterified fatty acids [4]. The binding of fatty acids, first described in human AFP [5], has been observed in all other species studied, including rat [4], mouse [6], pig [7] and calf [8]. Comparative analyses show a greater proportion of saturated and monounsaturated fatty acids bound to albumin than in homologous AFP, whereas polyunsaturated fatty acids (mainly arachidonic and docosahexaenoic) are enriched in AFP preparations [4,5,7]. However, the interaction of polyunsaturated fatty acids with α -AFP seems to be more

sensitive to environmental changes than the interaction with saturated and monounsaturated fatty acids [9]. Long-chain polyunsaturated fatty acids, and particularly docosahexaenoic acid (C22:6n-3), could be essential for membrane structuration of many organs [4,10]. Studies on the interaction of AFP with different fatty acids by direct [6,8] or indirect [11-13] methods do not justify, however, the apparent specificity of AFP for polyunsaturated fatty acids observed in vivo.

The aim of the present work is to analyze the interaction of rat AFP and albumin with different fatty acids, including C22:6n-3, in order to conclude about the specificity of AFP as a polyunsaturated fatty acid transporter.

2. MATERIALS AND METHODS

2.1. Isolation and purification of proteins

Albumin and AFP were isolated, from the same lot of serum from neonatal rats (8-10 days of age), by a procedure previously described [14]. The fatty acids naturally bound to these proteins were eliminated by incubation with heptane.

Correspondence address: A. Piñeiro, Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, 50009 Zaragoza, Spain

2.2. Fatty acids

Solutions of ($1\text{-}^{14}\text{C}$)fatty acids (from Amersham, Buckinghamshire, England and New England Nuclear, Dreieich, FRG) in *n*-heptane were washed three times with saline solution to eliminate any water-soluble contaminants. The radiochemical purity of the fatty acids was higher than 98% after argentation thin-layer chromatography analysis [15]. The non-radiolabelled fatty acids (Sigma, London, England) were tested by gas-liquid chromatography [14].

2.3. Determination of the apparent affinity constants

Binding constants and number of high-affinity binding sites for the interaction between proteins and fatty acids were determined by a two phase partition method [16,17] with some modifications. The incubation (7 h) was performed at 37°C in Eppendorf cones. Each cone contained 300 μl of protein solution in 0.15 M NaCl, 0.01 M phosphate, pH 7.4, buffer and 700 μl of heptane containing the labelled fatty acids. The concentration of protein was around 3.5 μM for AFP and 3.7 μM for albumin. The initial concentration of fatty acids in heptane phase ranged between 5 and 150 μM . After centrifugation, the radioactivity in 100 μl samples of organic phase and aqueous phase (this obtained by puncture of Eppendorf cones with a hypodermic needle) was determined by liquid scintillation. The binding parameters were calculated by the graphic method of Scatchard, using as unbound fatty acid in the aqueous phase for palmitic, oleic and linoleic acids the values obtained by the application of equation and distribution parameters indicated in [16]. The corresponding values for arachidonic and docosahexaenoic acids were obtained from distribution isotherms of these fatty acids between *n*-heptane and the aqueous phase, in the absence of protein.

3. RESULTS AND DISCUSSION

The equilibrium partition method has been widely used in the study of fatty acid interactions with albumin [16–18] and other proteins [19]. The modification described here allows the use of small quantities of both protein and radiolabelled fatty acids and the achievement of equilibrium between fatty acid bound to the protein and fatty acid free in aqueous and organic phases in a short time. The Scatchard plots obtained for the interaction of rat AFP and albumin with oleic and docosahexaenoic acids are shown in fig.1. AFP displays one high-affinity binding site for this and other fatty acids tested (table 1). Albumin displays a fractional number of binding sites (1.5 to 1.9), depending on the fatty acid. The level of fatty acids bound to this protein isolated in mild conditions [14] suggests, however, that the number of high-affinity binding sites for fatty acids in rat albumin may be actually two.

The apparent affinity constants (k_a) of albumin

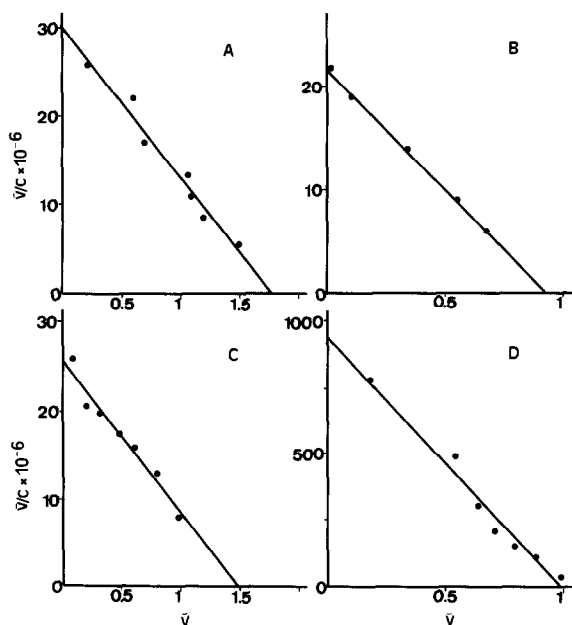


Fig.1. Scatchard plots obtained for the interaction of oleic (A and B) and docosahexaenoic (C and D) acids with rat albumin (A and C) and AFP (B and D). \bar{v} , concentration of fatty acid bound to the protein/protein concentration; c , concentration of fatty acid unbound to the protein in the aqueous phase.

and AFP were similar for palmitic, oleic and linoleic acids and in the same magnitude order (10^7 M^{-1}) as others previously published for different albumins [6,8,16–18] and AFPs [6,8,11,13]. The k_a values for more polyunsaturated fatty acids were, by contrast, higher with AFP than albumin, mainly for docosahexaenoic acid, which was around 50-fold higher (table 1). The previous k_a values for the interaction of C22:6n–3 with AFP,

Table 1

Apparent affinity constants (k_a) between rat albumin and AFP and several fatty acids

Fatty acid	Albumin ($k_a \times 10^{-7} \text{ M}^{-1}$)	AFP ($k_a \times 10^{-7} \text{ M}^{-1}$)
Palmitic	1.54 (1.86)	1.95 (0.88)
Oleic	1.76 (1.79)	2.41 (0.92)
Linoleic	1.43 (1.61)	2.67 (1.06)
Arachidonic	1.15 (1.68)	4.32 (1.06)
Docosahexaenoic	1.80 (1.48)	97.00 (0.99)

The value in brackets is the calculated number of high-affinity binding sites for fatty acids by molecule of protein. Data are the mean of two independent Scatchard plots for each interaction.

SD was always less than 10% of the mean

obtained by indirect measurements [11,12] are clearly lower than that reported here. Moreover, there are no comparative studies of the k_a value of albumin and AFP in their interaction with docosahexaenoic acid.

It has been proposed that AFP is a specialized transporter of docosahexaenoic acid and, perhaps, depending on the species, of other polyunsaturated fatty acids [20]. However, the available binding data [11,12] did not allow one to conclude about this specialization. The results of the present work demonstrate that the preference of AFP for C22:6 n -3 and other polyunsaturated fatty acids observed in vivo [4,5,7] is due to a direct thermodynamic control. This fact may be related with the essential role of docosahexaenoic acid in the fetal development of organs as the central nervous system [10]. AFP could guarantee not only the maternal-fetal transfer of C22:6 n -3 but also the transport of this fatty acid and its uptake by specialized cells [21].

Acknowledgements: This work has been supported by Fondo de Investigaciones Sanitarias de la Seguridad Social and by Diputación General de Aragón.

REFERENCES

- [1] Gitlin, D. and Perricelli, A. (1970) *Nature* 228, 995-997.
- [2] Laborda, J., Naval, J., Calvo, M., Lampreave, F. and Uriel, J. (1989) *Biol. Neonate*, in press.
- [3] Naval, J., Laborda, J., Villacampa, M.J., Calvo, M., Geuskens, M. and Uriel, J. (1986) in: *Binding Proteins of Steroid Hormones* (Forest, M.G. and Pugeat, M. eds) Colloque INSERM, vol. 149, pp. 523-536, John Libbey Eurotext, London, Paris.
- [4] Piñeiro, A., Olivito, A.M. and Uriel, J. (1979) *CR Acad. Sci. Paris* 2890, 1053-1056.
- [5] Parmelee, D.C., Evenson, M.A. and Deutsch, H.F. (1978) *J. Biol. Chem.* 253, 2114-2119.
- [6] Savu, L., Benassayag, C., Vallette, G., Christeff, N. and Nunez, E. (1981) *J. Biol. Chem.* 256, 9414-9418.
- [7] Lampreave, F., Calvo, M., Naval, J. and Piñeiro, A. (1982) *Comp. Biochem. Physiol.* 738, 823-827.
- [8] Carlsson, R.N.K., Estes, T., Degroot, T., Holden, T. and Ruoslahti, E. (1980) *Biochem. J.* 190, 301-305.
- [9] Calvo, M., Naval, J., Lampreave, F. and Piñeiro, A. (1985) *J. Chromatogr.* 328, 392-395.
- [10] Neuringer, M., Anderson, G.J. and Connor, W.E. (1988) *Annu. Rev. Nutr.* 8, 517-541.
- [11] Aussel, C. and Masseyeff, R. (1983) *Biochem. Biophys. Res. Commun.* 115, 38-45.
- [12] Aussel, C. and Masseyeff, R. (1983) *Biochim. Biophys. Acta* 752, 324-328.
- [13] Benassayag, C., Valette, J., Delorme, J., Savu, L. and Nunez, E.A. (1980) *Oncodev. Biol. Med.* 1, 27-36.
- [14] Calvo, M., Naval, J., Lampreave, F., Uriel, J. and Piñeiro, A. (1988) *Biochim. Biophys. Acta* 959, 238-246.
- [15] Christie, W.W. (1982) in: *Lipid Analyses*, Pergamon Press, Oxford, pp. 63-92.
- [16] Ashbrook, J.D., Spector, A.A., Santos, E.C. and Fletcher, J.E. (1975) *J. Biol. Chem.* 250, 2333-2338.
- [17] Spector, A.A., John, K. and Fletcher, J.E. (1969) *J. Lipid Res.* 10, 56-67.
- [18] Goodman, D.S. (1958) *J. Am. Chem. Soc.* 80, 3892-3898.
- [19] Spector, A.A. and Fletcher, J.E. (1970) *Lipids* 5, 403-411.
- [20] Uriel, J., Trojan, J., Moro, R. and Piñeiro, A. (1983) *Ann. NY Acad. Sci.* 417, 321-329.
- [21] Laborda, J., Naval, J., Calvo, M., Lampreave, F. and Uriel, J. (1985) *Tumour Biol.* 6, 407.