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Binding of retinoids to uteroglobin

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Abstract

Uteroglobin, a progesterone-binding secretory protein, was shown to bind retinoic acid and retinol in a non-saturable manner, at least up to concentrations of retinoids of 20 μM. Binding is increased about 10-fold by previous reduction of uteroglobin with 10 mM dithothreitol and it is not affected by previous saturation of the progesterone binding site, suggesting different binding sites for the steroid and the retinoids. The results are discussed in relation to a possible physiological role for this protein.

Key words: Retinoid; Progesterone; Steroid-binding protein; Reproductive tract

1. Introduction

Uteroglobin (UG) is a small secretory protein composed of two identical subunits held together by disulfide bridges which was first isolated from the rabbit endometrium where its gene is transcriptionally activated by progesterone in early pregnancy (see [1,2] for reviews). UG is also synthesized in other tissues under the control of different steroid hormones: glucocorticoids in lung [3,4], estrogens in the oviduct [5] and androgens in the epididymis [6]. The protein appears to be widely distributed among mammals. In addition to species of *Lagomorpha* [7], UG is found in humans [8,9], rat [10,11], mouse and dog [12]. In the latter species the protein has been called CC10 (for Clara cell 10 kDa protein).

While the mechanisms of the expression of the UG/CC10 gene have been extensively studied in recent years [11,13–17] the physiological function of UG remains elusive. Rat CC10 binds polychlorinated biphenyl derivatives [10]. Rabbit UG seems to have immunosuppressive properties by inhibiting phospholipase A_2 [2] and also possesses a well-studied progesterone binding ability [18–20]. None of these activities have been conclusively related to a physiological role. Searching for other possible properties of UG, we have found that this protein is capable of binding both vitamin A and retinoic acid.

2. Materials and methods

[11,12-^{3}H]retinoic acid ([^{3}H]RA; 50.6 Ci/mmol) was obtained from New England Nuclear-Dupont. [11,12-^{3}H]vitamin A ([^{3}H]retinol; 60 Ci/mmol) was from Amersham International. All *trans*-retinoic acid (RA), vitamin A (retinol) and progesterone were obtained from Sigma. Purity of both labelled and unlabelled retinoids was assessed by HPLC on a Hypersil ODS column [21].

Uterine fluid from pseudopregnant rabbits was obtained by perfusion of the uterine lumen with phosphate-buffered saline, pH 7.5 (PBS) [22]. 1.5 ml of uterine perfusate was incubated for 1.5 h at 0°C with 1.5 μCi of either [^{3}H]RA or [^{3}H]retinol and then analyzed on a Sephadex G-100 column (1.5 × 47 cm) equilibrated with PBS. Fractions (1.3 ml) were assayed for protein content (A_{280}) and radioactivity. The UG peak was concentrated by lyophilization, incubated again with 0.5 μCi of the corresponding [^{3}H]retinoid and rapidly passed through a Sephadex G-25 column (5 ml of bed volume) equilibrated with 25 mM ammonium acetate, pH 4.8. Fractions containing protein were pooled and applied to a CM-cellulose column (0.9 × 3 cm) which was developed with an ammonium acetate gradient [22]. Protein and radioactivity were determined in the fractions as described above. All the steps were carried out at 0–4°C under subdued light and all solutions were de-aerated under vacuum and stored under N_2.

To study the parameters of the binding of retinoids to UG, protein-bound and free ligands were separated either by gel filtration or by dextran-coated charcoal (DCC) [18,23]. In gel filtration experiments, small portions of uterine fluid (0.2 ml, containing 80 μg of UG) were incubated with 0.5 μCi of radioactive retinoids and the radioactivity bound to UG was analyzed on a small Sephadex G-100 column (0.9 × 25 cm). In other experiments, homogeneous UG (30 μg in 0.1 ml of PBS) was incubated with 0.3 μCi of radioactive ligands. Free ligands were absorbed by adding 30 μl of DCC and leaving the samples at 0°C for 5 min. After centrifugation, bound radioactivity was measured in the supernatants. When pertinent, unlabelled retinoids were added up to final concentrations of 20 μM. UG and uterine fluid were reduced with 10 mM dithiothreitol (DTT) for 15 min at 37°C [18].

Radioimmunoassay for UG was carried out as described previously [24]. SDS-polyacrylamide gel electrophoresis was carried out in slab gels [25], using an acrylamide gradient from 10 to 20%. Cellular RA and retinol-binding proteins (CRABP and CRBP, respectively) were purified from rat testes and liver up to the step prior to Sephadex G-75 chromatography [26].

3. Results

When uterine fluid, incubated in the presence of [^{3}H]RA, was analyzed on a Sephadex G-100 column, three peaks of radioactivity associated to proteins were observed (Fig. 1A). Peak I eluted at the void volume (V_o) of the column and contained unknown proteins of high M_r (inset, lane I). Peak II eluted with an apparent M_r (inset, lane I). Peak II eluted with an apparent M_r of approx. 65,000 and overlapped with a protein peak containing a major component of 65 kDa (inset, lane II) corresponding to serum albumin. Uterine fluid contains important amounts of serum albumin [27] and this protein binds RA and retinol in a non-saturable manner [28,29]

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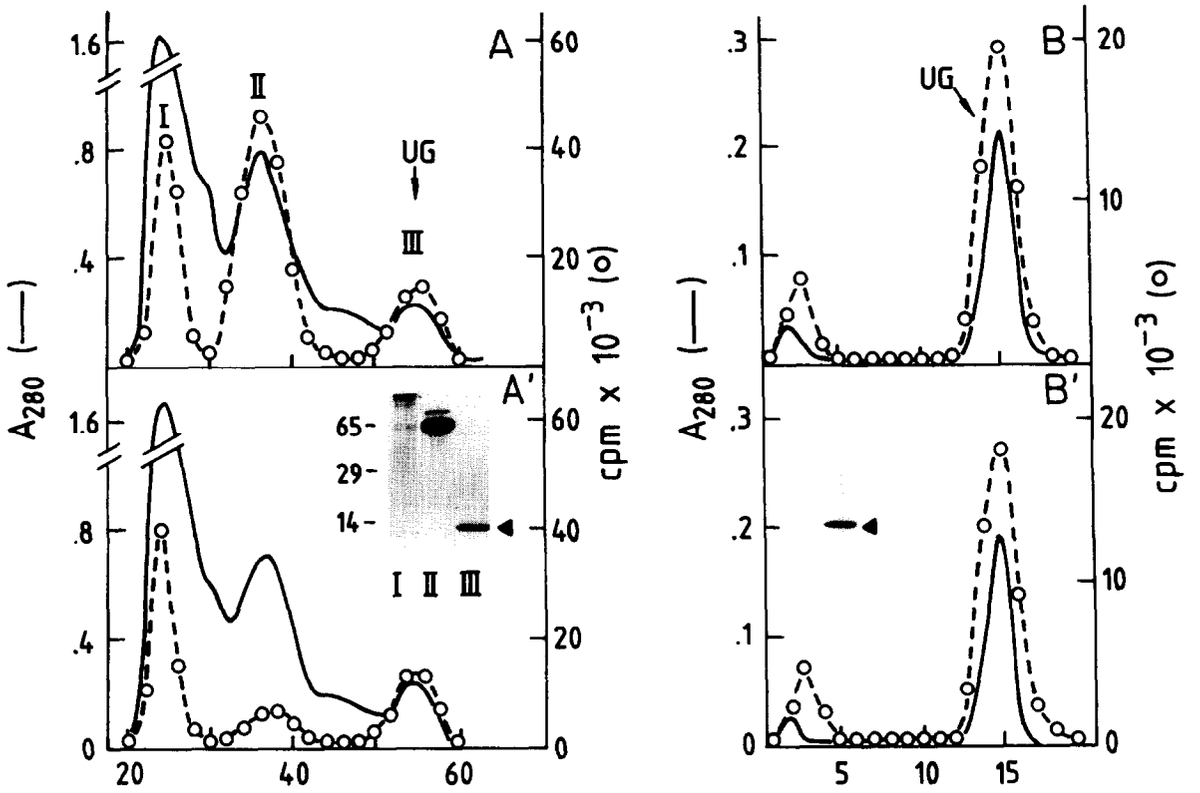

Fig. 1. Purification of UG from rabbit uterine fluid. A and A', Sephadex G-100 chromatography of uterine fluid incubated with [^{3}H]RA or [^{3}H]retinol, respectively. The inset shows a SDS-PAGE of material from peaks I, II and III (Coomassie blue staining). The arrowhead points to the UG band. Numbers to the left indicate the molecular mass (kDa) of the markers. B and B', ion-exchange chromatography of the UG (peak III) obtained in A and A', respectively. The inset shown the SDS-PAGE of the purified UG.

which could account for the radioactivity in Peak II. Peak III overlapped with a protein peak that was identified as UG on the basis of elution position [22], electrophoretic behaviour (inset, lane III) and immunoreactivity of the fractions in a specific radioimmunoassay (not shown). A duplicate sample of uterine fluid incubated with [^{3}H]retinol produced a similar pattern (Fig. 1A') although the radioactivity of peak II was considerably diminished, consistent with the low ability of serum albumin to bind retinol (our unpublished observations).

Peak III was further purified by ion-exchange chromatography to ensure that the observed binding was not due to a minor contaminant co-eluting with UG. Fig. 1B and B' show that either RA or retinol were bound to homogeneous UG.

Some parameters of the binding of both retinoids were then studied and the results are summarized in Table 1. Binding of either [^{3}H]RA or [^{3}H]retinol to UG was not displaced by a 500-fold in excess of the corresponding unlabelled retinoid, indicating a non-saturable binding at concentrations of 20 μM. This finding was confirmed by Scatchard plot analysis of the binding of the performed at different concentrations of the ligands (not shown). Higher concentrations of retinoids were not tested due to the low solubility of these compounds. Control experiments, performed in similar way but using CRABP and CRBP (see section 2), demonstrated that the binding of radioactive RA and retinol to these proteins could be completely displaced by an excess of unlabelled ligands (not shown). Therefore, our experimental conditions allowed for discrimination between saturable and non-saturable bindings. Binding of RA and retinol to UG was increased approx. 10-fold by previous treatment of the protein with DTT but was again unsaturable (Table 1). This enhancement of binding was specific for UG since the radioactivity peaks I and II were not affected by DTT as determined by gel filtration experiments (not shown). Since DTT also enhances the satura-

Table 1
Binding of labelled retinoids to UG

Treatment	RA	Retinol
Native UG	14.3 ± 2.6	9.7 ± 2.3
Native UG + unlabelled retinoid	13.4 ± 2.3	10.2 ± 2.4
Native UG + progesterone	12.2 ± 4.1	10.7 ± 3.7
Reduced UG	113.3 ± 13.3	94.3 ± 13
Reduced UG + unlabelled retinoid	130 ± 8.7	106.6 ± 11
Reduced UG + progesterone	135 ± 8.2	103.3 ± 13

Values, expressed as cpm/μg of UG, are means ± S.D. for three different experiments performed by the DCC method.

ble binding of progesterone to UG [18,20], we investigated whether the steroid and the retinoids might share the same binding site. Preincubation of either native or reduced UG with excess of progesterone (to saturate the progesterone-binding site) was without effect on the binding of either RA or retinol to UG (Table 1), suggesting different binding sites for progesterone and retinoids.

4. Discussion

In this report we show that the progesterone-binding protein UG also binds RA and retinol. This binding was greatly enhanced by prior reduction of the protein with DTT, a treatment that also increases the binding of progesterone to UG [18,20]. The effect of DTT on progesterone binding to UG appears to involve the disulfide bonds joining the two subunits of the protein [30]. In any case, reduction or blocking of these disulfide groups does not result in the dissociation of the two monomers of UG [22] although it produces conformational changes in the protein [31]. Although the UG binding site(s) for retinoids remain to be characterized, they appear to be different to that for progesterone since previous saturation of the progesterone binding site with an excess of steroid has no effect on the binding of retinoids.

Several retinoid-binding proteins have been described so far in the secretions of the mammalian genital tract including four retinol-binding proteins isolated from the secretions of pig uterus [23] and two 18 kDa secretory polypeptides of the rat epididymis [32,33], all of them being regulated by sexual steroid hormones. UG, synthesized in several organs of the male and the female genital tract under the control of sexual steroid hormones, appears to be another member of that class of retinoid-binding secretory proteins. The physiological function of these mentioned proteins remains to be elucidate but it is well established that retinoids play a crucial role in the normal function of the reproductive system [32,34] and in the development of the embryo [35]. The 18 kDa epididymal protein has been tentatively implicated in the transport of RA to the spermatozoa [36] and, in relation to this, it is noteworthy that this protein associates to defined regions of the spermatozoa [36] as UG does [37].

On the other hand, given the number of ligands able to interact with UG (progesterone, polychlorinated biphenyls, RA and retinol), it is possible that UG could act as a multi-carrier protein for several compounds in different organs.

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References

[1] Westphal, U. (1986) in: Steroid-protein interactions II (Gross, F. Ed.) pp. 357–386. Springer-Verlag, Berlin, Heidelberg.
[2] Miele, L., Cordella-Miele, E. and Mukherjee, A.B. (1987) Endocrine Rev. 8, 474–490.
[3] Torkkeli, T., Krusius, T. and Jänne, O. (1978) Biochim. Biophys. Acta 544, 578–592.
[4] Fernández-Renau, D., Lombardero, M. and Nieto, A. (1984) Eur. J. Biochem. 44, 523–527.
[5] Kay, E. and Feigelson, M. (1972) Biochim. Biophys. Acta 271, 436–441.
[6] López de Haro, M.S., Alvarez, L. and Nieto, A. (1988) Biochem. J. 250, 647–651.
[7] López de Haro, M.S. and Nieto, A. (1986) Biochem. J. 235, 895–898.
[8] Singh, G., Katyal, S.L., Brown, W.E., Kennedy, A.L., Singh, U. and Wong-Chong, M.L. (1990) Biochim. Biophys. Acta 1039, 348–355.
[9] Mantile, G., Miele, L., Cordella-Miele, E., Singh, G., Katyal, S.L. and Mukherjee, A.B. (1993) J. Biol. Chem. 268, 20343–20351.
[10] Nordlund-Moller, L., Anderson, O., Ahigren, R., Schiling, J., Gilner, M., Gustaffson, J.-A. and Lund, J. (1990) J. Biol. Chem. 21, 12690–12693.
[11] Hagen, G., Wolf, M., Katyal, S.L., Singh, G., Beato, M. and Suske, G. (1990) Nucleic Acid Res. 18, 2939–2946.
[12] Ray, M.K., Magdaleno, S., O'Malley, B.W. and DeMayo, F.J. (1993) Biochem. Biophys. Res. Commun. 197, 163–171.
[13] Sandmöller, A., Voss, A.K., Hahn, J., Redemann-Fibi, B., Suske, G. and Beato, M. (1991) Mech. Dev. 34, 57–68.
[14] DeMayo, F.J., Damak, S., Hansen, T.N. and Bullock, D.W. (1991) Mol. Endocrinol. 5, 311–318.
[15] Gómez Lahoz, E., López de Haro, M.S., Esponda, P. and Nieto, A. (1992) Gene 117, 255–258.
[16] Sawaya, P.L., Stripp, B.R., Whitsett, J.A. and Luse, D.S. (1993) Mol. Cell. Biol. 13, 3860–3871.
[17] Bingle, C.D. and Gitlin, J.D. (1993) Biochem. J. 295, 227–232.
[18] Beato, M. (1976) J. Steroid Biochem. 7, 327–334.
[19] Morize, I., Surcouf, E., Vaney, M.C., Epelboin, Y., Buehner, M., Fridlansky, F., Milgrom, E. and Mornon, J.P. (1987) J. Mol. Biol. 194, 725–739.
[20] Fridlansky, F. and Milgrom, E. (1976) Endocrinology 99, 1244–1251.
[21] McCormick, A.M., Napoli, J.L. and De Luca, H.F. (1980) Methods Enzymol. 67, 220–233.
[22] Nieto, A., Ponstingl, H. and Beato, M. (1977) Arch. Biochem. Biophys. 180, 82–92.
[23] Clawitter, J., Trout, W.E., Burke, M.G., Araghi, S. and Roberts, R.M. (1990) J. Biol. Chem. 265, 3248–3255.
[24] Nieto, A. and Beato, M. (1980) Mol. Cell. Endocrinol. 17, 25–39.
[25] Laemmli, U.K. (1970) Nature 227, 680–685.
[26] Ong, D. and Chytil, F. (1990) Methods Enzymol. 67, 288–296.
[27] Beier, H.M. (1974) J. Reprod. Fertil. 37, 221–237.
[28] Bashor, M.M. and Chytil, F. (1975) Biochim. Biophys. Acta 411, 87–96.
[29] Ong, D.E. and Chytil, F. (1975) J. Biol. Chem. 250, 6113–6117.
[30] Tancredi, T., Temussi, P.A. and Beato, M. (1982) Eur. J. Biochem. 122, 101–104.
[31] Puigdomenech, P. and Beato, M. (1977) FEBS Lett. 83, 217–221.
[32] Ong, D.E. and Chytil, F. (1988) Arch. Biochem. Biophys. 267, 474–478.
[33] Newcomer, M.E. and Ong, D.E. (1990) J. Biol. Chem. 265, 12876–12879.
[34] Moore, T. (1967) in: The Vitamins (Sebrell, W.H. and Harris, R.S., Eds.) vol. I, pp. 245–266, Academic Press, New York.
[35] Wedden, S., Thaller, C. and Eichele, G. (1990) Methods Enzymol. 190, 201–209.
[36] Brooks, D.E. (1983) Mol. Cell. Endocrinol. 29, 255–270.
[37] García, C., Regalado, F. and Nieto, A. (1988) Histochem. J. 20, 708–714.