Novel retinoid-binding proteins from filarial parasites

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The present study deals with the discovery and partial characterization of specific binding proteins for retinol and retinoic acid from filarial parasites (worms of the superfamily Filarioidea), including those from two species of *Onchocerca*. These binding proteins, which are distinct in their physicochemical properties and in the mode of ligand interactions from the host-tissue retinoid-binding proteins, may be involved in the mediation of the putative biological roles of retinoids in the control of parasitic growth, differentiation and reproduction. Parasite retinol-binding protein and retinoic acid-binding protein exhibited specificity for binding retinol and retinoic acid respectively. Both the binding proteins showed an $s_{20,w}$ value of 2.0 S. On gel filtration, both proteins were retarded to a position corresponding to the same molecular size (19.0 kDa). On preparative columns, the parasite binding proteins exhibited isoelectric points at pH 5.7 and 5.75. Unlike the retinoid-binding proteins of mammalian and avian origin, the parasite retinoid-binding proteins showed a lack of mercurial sensitivity in ligand binding. The comparative amounts of retinoic acid-binding protein in five parasites, *Onchocerca volvulus*, *Onchocerca gibsoni*, *Dipetalonema viteae*, *Brugia pahangi* and *Dirofilaria immitis*, were between 2.7 and 3.1 pmol of retinoic acid bound/mg of extractable protein. However, the levels of parasite retinol-binding protein were between 4.8 and 5.8 pmol/mg, which is considerably higher than the corresponding levels of cellular retinol-binding protein of mammalian and avian origin. Both retinol- and retinoic acid-binding-protein levels in *O. volvulus*-infected human nodules and *O. gibsoni*-infected bovine nodules were similar to their levels in mammalian tissues. Also, these nodular binding proteins, like the host-binding proteins, exhibited mercurial sensitivity to ligand interactions.

INTRODUCTION

Retinol (vitamin A) and its oxidized form, retinoic acid, are involved in the control of general growth and epithelial differentiation (Arens & VanDorp, 1946; Dowling & Wald, 1960; Wolbach & Howe, 1925). Retinoids have also been used successfully in the control of tumorigenesis (Saffiotti *et al.*, 1967; Bollag, 1972; Sporn *et al.*, 1984). The molecular mechanism of action of retinoids, other than in vision, is largely unknown, although it has been suggested that the well-known cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) may function as mediators of their action (Bashor *et al.*, 1973; Sani & Hill, 1974; Goodman, 1982; Sani & Banerjee, 1983; Chytil & Ong, 1984). These binding proteins for retinol and retinoic acid may mediate the nuclear translocation of the retinoid, and provide specificity for nuclear acceptor sites that may result in gene activation for the control of differentiation and growth (Sani, 1977; Wiggert *et al.*, 1977; Sani & Donovan, 1979; Jetten & Jetten, 1979; Liau *et al.*, 1981).

The importance and function of retinoids in parasites is a relatively unexplored area, although several investigators have described the occurrence and uptake of retinol, as well as its formation from β -carotene, in parasites (Juhász & Babos, 1969; Leutskaya, 1971; Comley & Jaffe, 1983; Comley *et al.*, 1983). Vitamin A in its phosphorylated forms, retinyl phosphate and retinyl phosphate mannose, have been implied as intermediates in filarial glycoprotein synthesis (Comley &

Jaffe, 1983). Storey (1982) and Turchetti-Maia *et al.* (1983) have illustrated that the growth of some developmental stages of helminth parasites is dependent on host levels of retinol. A link between vitamin A deficiency and helminthiasis has also been established (Mahalanabis *et al.*, 1976; Storey, 1982), indicating that the host vitamin A deficiency is concomitant with the development of onchocercal pathogenesis (Rodger, 1962). This may be a result of preferential absorption of vitamin A by the parasites. A significant observation was that of Sturchler *et al.* (1981, 1983), who show that the retinol concentration of adult *Onchocerca volvulus* was 8 times higher than that of the surrounding host tissues, suggesting that perhaps these parasites absorb and concentrate vitamin A. Since these parasites apparently lack visual function, the retinoids present in them may be utilized solely to support their growth, differentiation and reproduction.

In our efforts to understand the mechanisms by which retinoids may function in the control of growth and differentiation of parasites, we studied the interactions *in vitro* between retinoids and proteins isolated from filarial parasites. We now report the presence and partial characterization of specific binding proteins for retinol and retinoic acid from these parasites. The physicochemical characteristics of these binding proteins are distinct from those of the cellular retinoid-binding proteins isolated and characterized from mammalian and avian tissues. A part of this study was presented at the Wellcome Foundation WHO Filariasis Seminar, held in London, U.K., in November 1984 (Sani & Comley, 1985).

* Abbreviations used: CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein; PRBP, parasite retinol-binding protein; PRABP, parasite retinoic acid-binding protein; phosphate-buffered saline, 10 mM-sodium phosphate, pH 7.2, containing 100 mM-NaCl.

MATERIALS AND METHODS

Materials

Retinoic acid, [11, 12-³H]₂retinoic acid (1.28 Ci/mmol) and retinol were supplied by the National Cancer Institute, Bethesda, MD, U.S.A. [15-³H] Retinol (1.66 Ci/mmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. Dithiothreitol and *p*-chloromercuribenzenesulphonic acid were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The protein standard kit for *M_r* determination was purchased from Boehringer-Mannheim Biochemicals, Indianapolis, IN, U.S.A. The LKB 8100 isoelectric-focusing column (110 ml) and Ampholines were purchased from LKB Instruments, Chapel Hill, NC, U.S.A.

Parasites, tissues and their extracts

Adult *Brugia pahangi* (mixed sexes) were obtained from the peritoneal cavities of laboratory-infected jirds (hamster-like animals) (10–16 weeks after infection), maintained at the Wellcome Research Laboratories, Beckenham, Kent, U.K. Adult *Dipetalonema viteae* (mixed sexes) were dissected from the subcutaneous tissues of laboratory-infected golden hamsters (8–16 weeks after infection), also maintained at the Wellcome Research Laboratories. *Dirofilaria immitis* (females) obtained from laboratory-infected dogs were purchased from TRS Laboratories, P.O. Box 5112, Athens, GA, U.S.A.

Bovine nodules containing *Onchocerca gibsoni* were provided by Dr. D. B. Copeman, Department of Tropical Veterinary Medicine, James Cook University of North Queensland, Qld. 4811, Australia. Nodules were air-freighted frozen on solid CO₂ to the U.K. Human nodules containing *Onchocera volvulus* were collected by Dr. J. P. Court (Wellcome Foundation Ltd.) while he was visiting Tamale, Ghana. Nodulectomies were performed, with informed consent, by Dr. K. Awadzi, on volunteers recruited from the surrounding countryside, at the Onchocerciasis Chemotherapeutic Research Centre, Tamale Hospital, Tamale, Ghana. After excision, nodules were immediately frozen by immersion in liquid N₂ and subsequently air-freighted to the U.K. in liquid N₂. Both human and bovine nodules were thawed in sterile ice-cold phosphate-buffered saline, and broken parts of the parasites (predominantly female worm tissue) dissected from the nodules. The material was collected in phosphate-buffered saline, and as much host contaminating tissue as possible removed with fine forceps before freeze-drying. Human and bovine nodule wall tissues were also collected for comparative purposes. This material was sliced from the walls of the *Onchocerca*-containing nodules and the material examined microscopically to ensure that it was absolutely free from contamination by *Onchocerca* tissue. The pieces of nodule wall were then washed in several changes of phosphate-buffered saline and minced into small lumps (2–4 mm²) and freeze-dried. The intact filarial parasites were processed similarly by washing free of host tissues in at least three changes of sterile phosphate-buffered saline before freeze-drying. Both parasite and host tissues were air-freighted to the U.S.A. frozen on solid CO₂.

Testes from 3–4-month-old Sprague–Dawley rats, and skins from 12–13-day-old embryonic chick were isolated. BALB/c mice were used for the implantation of

transplantable mouse colon tumour 26 (Sani & Corbett, 1977).

Freeze-dried samples of parasites and tissues were homogenized in phosphate-buffered saline by using a Teflon homogenizer, centrifuged at 100 000 *g* for 90 min, and the supernatants diluted to a protein concentration of 12 mg/ml. The human and bovine nodules were disintegrated in a PT Polytron homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.) before processing them in the Teflon homogenizer.

Sucrose-density-gradient sedimentation

Retinoid-binding proteins were detected by using this procedure (Sani & Banerjee, 1978). Portions of the [³H]-retinoid–protein complexes were centrifuged at 180 000 *g* for 18 h through 5–20% (w/v)-sucrose gradients, with bovine serum albumin and ovalbumin as external standards. The protein nature of retinoid-binding components in parasites was assessed by treatment of the extracts (2 mg of protein) with Pronase (100 µg) (Sani & Hill, 1976; Sani *et al.*, 1980b) before sucrose-gradient sedimentation. For studies on thiol functions in retinoid–protein interactions, portions of extracts (2.0 mg of protein) were incubated for 3 h with 300 pmol of [³H]retinoid in the presence or absence of 1–2 mM-*p*-chloromercuribenzenesulphonic acid and dialysed against phosphate-buffered saline for 22 h. The preparations were analysed by the sucrose-density-gradient-sedimentation technique described previously (Sani & Banerjee, 1978).

Molecular-size profiles of retinoid-binding proteins

Soluble extracts (50 mg of protein) from *D. immitis* were incubated with either [³H]retinol or [³H]retinoic acid for 1 h at room temperature and for 3 h at 4 °C, and filtered through a column (2.5 cm × 100 cm) of Sephadex G-75 equilibrated with phosphate-buffered saline. Both protein and radioactivity distributions of the elution profile were determined. Samples (5 mg each) of bovine serum albumin (*M_r* 68 000), ovalbumin (44 000), β-lactoglobulin (36 000), myoglobin (17 800) and cytochrome *c* (12 400), dissolved in 5 ml of the elution buffer, were run through the same column as standards for *M_r* determination.

Isoelectric focusing of retinoid-binding proteins

Fractions constituting radioactive peaks of [³H]retinoid–protein complexes resolved from the molecular-size profiles by using Sephadex G-75 columns from the above step were pooled, freeze-dried, and dialysed against 5 mM-Tris/HCl, pH 7.2. These preparations were applied to LKB isofocusing column (110 ml) in which Ampholines of pH 3–10 were employed. Electrofocusing of the protein sample was accomplished in 65 h at 5 °C, and fractions of volume 2.0 ml each were collected.

RESULTS

Sucrose-density-gradient patterns given by *O. volvulus* extracts (2 mg of protein) plus [³H]retinol with or without 200-fold molar excesses of unlabelled retinol or retinoic acid are shown in Fig. 1. The radioactivity profile of the parasitic extract plus [³H]retinol indicates a retinol-binding protein peak that corresponds to a calculated *s*_{20,w} value of 2.0 S. A 200-fold molar excess of unlabelled retinol almost eliminated the radioactive peak at 2 S,

Fig. 1. Sucrose-density-gradient-centrifugation patterns of *O. volvulus* extract (2 mg of protein) plus 300 pmol of [^3H]retinol, with or without a 200-fold molar excess of unlabelled retinoid

Δ, *O. volvulus* extract + [^3H]retinol; ○, extract + [^3H]retinol + retinol; ●, extract + [^3H]retinol + retinoic acid.

Fig. 2. Sucrose-density-gradient-sedimentation profiles of *O. volvulus* extract (2 mg of protein) plus 300 pmol of [^3H]retinoic acid with or without a 200-fold molar excess of unlabelled retinoid

Δ, Extract + [^3H]retinoic acid; ○, extract + [^3H]retinoic acid + retinoic acid; ●, extract + [^3H]retinoic acid + retinol; ▲, the same as Δ, plus 100 μg of Pronase.

whereas a similar fold excess of retinoic acid did not alter the binding of [^3H]retinol. Fig. 2 illustrates the radioactivity distribution patterns of [^3H]retinoic acid after incubation with *O. volvulus* extracts. Like Fig. 1, this Figure also reveals a 2 S radioactive peak. Competition with unlabelled retinoic acid, but not with retinol, eliminated the radioactive peak. This implies that binding of [^3H]retinol and [^3H]retinoic acid to their respective

Fig. 3. Molecular-size profiles of protein-[^3H]retinol complexes

Extracts from *D. immitis* (50 mg of protein) after incubation with [^3H]retinol (3 nmol) were resolved on a column (2.5 cm $\times$ 100 cm) of Sephadex G-75 equilibrated with phosphate-buffered saline, and fractions (5.0 ml each) were collected. For M_r determination, protein standards [bovine serum albumin (M_r 68000), ovalbumin (44000), β -lactoglobulin (36000), myoglobin (17800) and cytochrome *c* (12400)] were passed through the same column under identical conditions, and their respective elution positions are marked with arrows against their M_r values. Symbols: ●, radioactivity; ○, A_{280} . The position of elution of PRBP is indicated.

binding sites is specific. The protein nature of the parasitic [^3H]retinol- and [^3H]retinoic acid-binding components is revealed by Pronase digestion. The Pronase-treated preparations of both [^3H]retinol-binding protein complex (not shown) and [^3H]retinoic acid-binding protein complex (Fig. 2) after sucrose-density-gradient sedimentation did not show any 2 S radioactive peak. The parasitic retinol-binding protein and retinoic acid-binding protein, which have $s_{20,w}$ values of 2.0, are hereafter termed 'PRBP' and 'PRABP' respectively, as distinct from those (CRBP and CRABP) of mammalian and avian origin.

M_r values for both PRBP and PRABP were determined by column chromatography on Sephadex G-75. Either [^3H]retinol or [^3H]retinoic acid (3 nmol) was incubated with 50 mg of protein of *D. immitis* extracts for 4 h at 4 °C, dialysed against phosphate-buffered saline for 5 h, and resolved on a column (2.5 cm $\times$ 100 cm) of Sephadex G-75 equilibrated and developed with phosphate-buffered saline at a flow rate of 25 ml/h. Fig. 3 presents the chromatographic profiles of radioactivity distribution and A_{280} . M_r values corresponding to the radioactive peaks were assessed from the relative elution volumes of the molecular-size markers, which were resolved in a separate run. The second radioactive peak, which represents the 2 S PRBP-[^3H]retinol complex, corresponds to M_r 19000. The protein concentration in the area of this peak is minimal, indicating a high specificity of the ligand for the binding protein. The first radioactive peak corresponded to M_r > 68000, and is located in fractions with the highest protein concentration. In a separate run under similar conditions, the 2 S [^3H]retinoic acid-PRABP complex was also retarded to a position corresponding to M_r 19000 (results not shown).

Fig. 4. Sedimentation patterns of sucrose gradients of *O. volvulus* extract (2 mg of protein) and chick-embryo skin extract (2 mg of protein) plus [³H]retinoic acid (300 pmol) in the presence or in the absence of *p*-chloromercuribenzenesulphonic acid

●, Chick-embryo skin extract + [³H]retinoic acid; ●, chick-embryo skin extract + [³H]retinoic acid + 1 mM-*p*-chloromercuribenzenesulphonic acid; ○, *O. volvulus* extract + [³H]retinoic acid; ▲, *O. volvulus* extract + [³H]retinoic acid + 2 mM-*p*-chloromercuribenzenesulphonic acid.

Fig. 5. Sucrose-density-gradient profiles showing the effects of *p*-chloromercuribenzenesulphonic acid on the binding of [³H]retinoic acid (300 pmol) to the proteins of *O. volvulus* extract (2 mg of protein) and *O. volvulus* nodular extract (2 mg of protein)

△, *O. volvulus* extract + [³H]retinoic acid; ▲, *O. volvulus* extract + [³H]retinoic acid + 2 mM-mercury reagent; ○, *O. volvulus*-infected nodular extract + [³H]retinoic acid; ●, *O. volvulus*-infected nodular extract + [³H]retinoic acid + 1 mM-mercury reagent.

We have shown previously that binding of both retinol and retinoic acid to CRBP and CRABP respectively is completely inhibited by *p*-chloromercuribenzenesulphonic acid (Sani & Banerjee, 1978; Sani *et al.*, 1980a). Thus it is likely that thiols are involved in the ligand-

Fig. 6. Isoelectric-focusing profiles of fractions containing PRBP-[³H]retinol complex obtained after chromatography on a column (2.5 cm × 100 cm) of Sephadex G-75

The proteins (8 mg) were electrofocused at 5°C for 65 h. The pH profile (○), A₂₈₀ (△) and radioactivity (●) distribution of the fractions (2 ml) are plotted. The sharp radioactive peak at pH 5.7 is due to the parasite retinol-binding protein (PRBP).

protein interactions. We presently examined whether such mercurial-sensitive binding sites are also present in the parasitic binding proteins. Fig. 4 illustrates the effect of 1–2 mM-*p*-chloromercuribenzenesulphonic acid on the binding of [³H]retinoic acid on CRABP and PRABP. The binding of retinoic acid to chick-embryo skin CRABP was completely abolished in the presence of 1 mM-mercurial. However, the PRABP–ligand interactions in *O. volvulus* preparations were totally insensitive up to 2 mM-mercurial. In fact, 2 mM-*p*-chloromercuribenzenesulphonic acid slightly stimulated binding of [³H]retinoic acid to PRABP. We also tested four other parasites, *O. gibsoni*, *D. viteae*, *B. pahangi* and *D. immitis*, for mercurial sensitivity in retinoid binding: none of them expressed any sensitivity (results not shown). A systematic examination of the sensitivity of [³H]retinol–PRBP interactions to *p*-chloromercuribenzenesulphonic acid revealed that, in contrast with mammalian CRBP, the parasitic binding protein expressed a complete lack of mercurial sensitivity (results not shown).

We also decided to examine the human and bovine nodular preparations for the presence of retinoid-binding proteins as well as for the sensitivity of ligand–protein interactions to mercury compounds. Both *O. volvulus* nodules (human origin) and *O. gibsoni* nodules (bovine origin) showed the presence of specific 2S [³H]retinol-binding protein and [³H]retinoic acid-binding protein. Fig. 5 shows the sucrose-density-gradient profile of [³H]retinoic acid binding by *O. volvulus*-infected nodular extract in comparison with that of binding by *O. volvulus* extracts. The 5 S peaks of the nodular preparations may be due to [³H]retinoic acid bound to traces of contaminating serum albumin (Sani *et al.*, 1978), which is also observed in the sucrose-density-gradient profiles of chick-embryo skin extracts (Fig. 4). In sharp contrast with *O. volvulus* extracts, the binding protein from nodular-wall materials demonstrated totally mercurial-sensitive protein–ligand interactions that are typical of host-tissue binding proteins. The bovine nodular prepara-

Table 1. Cellular binding proteins for retinol and retinoic acid in (a) parasites and (b) tissues

Retinoid-binding proteins were measured by sucrose-density-gradient analysis. Specific binding by [³H]retinol or [³H]retinoic acid under their respective binding protein peaks was determined.

		[³ H]Retinoid bound (pmol/mg of extracted protein)	
(a) Parasite	Retinoid... Binding protein...	Retinol PRBP	Retinoic acid PRABP
<i>O. volvulus</i>		5.5	3.0
<i>O. gibsoni</i>		5.8	3.1
<i>D. viteae</i>		5.4	2.9
<i>B. pahangi</i>		4.8	2.7
<i>D. immitis</i>		5.0	3.1
(b) Tissue	Binding protein...	CRBP	CRABP
Rat testes		3.0	3.1
Chick-embryo skin		0.6	3.3
Mouse colon tumour 26		1.8	4.4
<i>O. volvulus</i> -infected nodule-wall material		1.7	2.3
<i>O. gibsoni</i> -infected nodule-wall material		1.9	2.6

tions from *O. gibsoni* also behaved like the human nodules with respect to mercurial sensitivity. Thus, as expected, both the retinol- and the retinoic acid-binding proteins of nodular preparations acted as CRBP and CRABP.

Cellular retinoid-binding proteins of mammalian and avian origin have been reported to be acidic proteins with isoelectric pH values ranging between 4.5 and 4.8 (Sani & Hill, 1976; Sani *et al.*, 1980a; Ross *et al.*, 1978). Isoelectric focusing with Ampholines of pH range 3.0–10 was performed with protein fractions containing PRBP–[³H]retinol complex corresponding to *M_r* 19000 from the Sephadex G-75 column-chromatographic step. A sharp radioactive peak at pH 5.7 (Fig. 6) was observed that corresponded to the binding protein–[³H]retinol complex. Isoelectric focusing of PRABP–[³H]retinoic acid complex under similar conditions revealed a similar radioactive peak at pH 5.75 (result not shown). Thus both the parasitic binding proteins showed similar pI, at about 5.7.

A comparative study on the quantitative amounts of PRBP and PRABP in different parasites and the CRBP and CRABP levels in mammalian and avian tissues are presented in Table 1. Levels of ligand bound to the extractable proteins (amounts/mg) were assumed to be directly related to the amounts of the binding protein. All the five parasites that were analysed contained between 2.7 and 3.1 pmol of retinoic acid bound/mg of protein. These levels were similar to the CRABP levels in rat testes, mouse colon tumour 26 and chick-embryo skin. *O. volvulus*- and *O. gibsoni*-infected nodular-wall materials showed 2.3 and 2.6 pmol of bound ligand, values that are slightly lower than those in the above-mentioned tissues. On the other hand, the levels of PRBP in parasites (4.8–5.8 pmol) on average were much higher than the corresponding CRBP levels (0.6–3.0 pmol) in other tissues. CRBP levels in the human and bovine nodular preparations (1.7 and 1.9 pmol) were similar to those in rat, mouse, and chick-embryo tissues, but these levels constituted only about a third of the binding-protein levels in filarial parasites. However, no significant differences were observed between the CRABP

levels of the nodular preparations and of the parasitic or host-tissue preparations (Table 1).

DISCUSSION

Emphasis of research in the area of retinoids has been enhanced considerably during the past decade, primarily on account of their chemopreventive actions against cancer (Sporn *et al.*, 1984); however, the importance and function of retinoids in parasites have been relatively unexamined. Since it is believed that parasites lack a visual role for vitamin A, the retinoids that are absorbed by parasites may be utilized for other important functions of vitamin A such as control of normal differentiation of epithelial tissues, maintenance of growth, and reproduction. Since retinoic acid, which cannot be biologically reduced to either retinol or retinal, can substitute for retinol in vitamin A-deprived animals (Arens & VanDorp, 1946; Dowling & Wald, 1960), it may be assumed that this form of the retinoid is functional in parasites as well. Discovery of the presence of large quantities of retinoids in several parasites (Juhász & Babos, 1969; Comley & Jaffe, 1983; Sturchler *et al.*, 1981, 1983) paved the way for studies pertaining to their mode of action. Various attempts designed to determine the molecular mode of action of retinoids in the control of epithelial differentiation and growth have centred on intracellular retinoid-binding proteins, CRBP and CRABP (Bashor *et al.*, 1973; Sani & Hill, 1974; Goodman, 1982; Sani & Banerjee, 1983; Chytil & Ong, 1984). These functions of retinoids are believed to be exerted via the cell nucleus through the mediation of the binding proteins (Sani, 1977; Wiggert *et al.*, 1977; Liao *et al.*, 1981). By analogy to host-tissue binding proteins, the parasitic binding proteins, PRBP and PRABP, described in the present paper may mediate the biological functions of both retinol and retinoic acid in differentiation, growth and reproduction of the parasites.

Mammalian and avian CRBP, which is physicochemically distinct from the serum retinol-binding protein, exhibited specific binding sites for retinol; retinoic acid

or retinal did not show competition for these binding sites (Ong & Chytil, 1975). Likewise a retinoic acid-like structure with a free carboxy group is essential for maximal binding to CRBP (Sani & Hill, 1974, 1976). The specificity of PRBP and PRABP for retinol and retinoic acid respectively was retained in the same manner as was demonstrated by host tissue-binding proteins. However, we have noticed remarkable differences in the binding affinities of several synthetic retinoids to the parasitic binding proteins, compared with their affinities to the binding proteins of the host system (B. P. Sani, M. I. Dawson, J. C. W. Comley & A. Vaid, unpublished work). These differences may be attributed to the differential physicochemical properties exhibited by the parasite-binding proteins.

On filtration through columns of Sephadex G-100, both CRBP and CRABP were retarded to the same molecular-size positions (M_r 17800). However, on polyacrylamide-gel electrophoresis in presence of sodium dodecyl sulphate, CRBP and CRABP acted as monomers, with M_r values of 15500 and 14000 respectively (Ong & Chytil, 1978; Ross *et al.*, 1978; Sani & Banerjee, 1978; Sani *et al.*, 1980a). On the other hand, both PRBP and PRABP on gel filtration showed slightly higher M_r values, of about 19000. Since the M_r values of host-tissue binding proteins by Sephadex-column chromatography were not presently determined, comparison of these values with M_r values of parasitic binding proteins may involve some uncertainty. Although our initial observations indicate that both the proteins are monomers, determination of their subunit M_r values on SDS-containing gels must await a purified preparation of the parasitic binding proteins. Isoelectric-focusing profiles of PRBP and PRABP were quite different from those of CRBP and CRABP. They both revealed a similar pI (5.7–5.75) which is distinct from the reported pI 4.6–4.8 for the host binding proteins (Ross *et al.*, 1978; Sani & Hill, 1976; Sani *et al.*, 1980a). Although both CRBP and CRABP have been resolved from one another by means of DEAE-cellulose chromatography, a similar approach to resolve PRBP and PRABP has been unsuccessful, probably owing to the fact that these proteins are less acidic than the host binding proteins, and therefore less resolvable on anion-exchange column.

We established previously that retinol and retinoic acid binding to CRBP and CRABP are sensitive to mercurials and that the inhibition is reversible in the presence of dithiothreitol (Sani & Banerjee, 1978). We therefore postulated that thiol functions may be involved in the protein-ligand interactions, although the exact mode of interaction of these retinoids with their binding proteins still needs to be elucidated by more careful studies. Interestingly, our present data indicate that the parasite binding protein-ligand interactions are totally insensitive to the mercury compound. All the five parasites (*O. volvulus*, *O. gibsoni*, *D. viteae*, *B. pahangi* and *D. immitis*) that were tested expressed similar lack of sensitivity to mercury compounds. In contrast, as one would expect, CRBP and CRABP from *O. volvulus*-infected nodules from human tissues and *O. gibsoni*-infected nodules from bovine tissues exhibited mercurial-sensitive retinoid-protein interactions. These observations further establish that, in addition to the differential physicochemical properties of the retinoid-binding proteins, fundamental differences also exist between the host and parasites with regard to their binding protein-ligand interactions.

Since we believe that retinoids are important molecules that may be involved in the control of normal differentiation, growth and reproduction of the parasites, and that their binding proteins may be important mediators of these biological functions, the specific biochemical differences in molecular charge, size and the mode of ligand interactions that were observed between the host and parasite binding proteins could be employed advantageously in the selective control of parasitic growth. Through the use of the differential physicochemical properties of the binding proteins of the host and parasite, we may be able to develop synthetic retinoids that might bind parasite retinoid-binding proteins with high affinity. If such retinoids possess low biological potency and low affinity for host-binding proteins, then those compounds may form selective agents to block parasitic growth. Binding affinity studies designed to discover synthetic retinoids that exhibit differential binding affinity and biological potency are needed.

This work was supported by Grant No. 83001 from the World Health Organization (Onchocerciasis Chemotherapy Project of the Onchocerciasis Control Programme in the Volta River Basin Area).

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Received 9 May 1985/29 July 1985; accepted 8 August 1985