Vol. 118, No. 3, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1984 Pages 789-796

SYNTHESIS OF INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN (IRBP) BY MONKEY RETINA IN ORGAN CULTURE: EFFECT OF MONENSIN

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Received December 23, 1983

SUMMARY: Whole monkey retinas were incubated in short-term organ culture with either radiolabeled amino acids or glucosamine. Soluble retinal proteins and proteins in the culture medium were analyzed by SDS-polyacrylamide gel electrophoresis. Fluorography showed that the interphotoreceptor retinoid-binding protein (IRBP), a 146,000 M_r glycoprotein localized in the extracellular matrix, is synthesized by the neural retina and rapidly secreted into the medium. Secretion is blocked by 10-5M monensin. No significant IRBP synthesis was observed in the pigment-epithelium-choroid complex. IRBP is thus the major component synthesized and secreted by the neural retina into the interphotoreceptor space. This, and its affinity for retinoid makes it a prime candidate for an extracellular retinoid transport vehicle.

Vitamin A (retinol) must be transported from storage depots in the pigment epithelium (PE) to the retinal photoreceptors and back again following photoreceptor bleaching. The primary route for such transport is probably via the interphotoreceptor space (IPS), the compartment between the neural retina and the PE which was first defined by Feeney (1).

Because free retinol is both potentially toxic and unstable in an aqueous environment, it is reasonable to expect that its transport between tissue compartments might be mediated by a specific binding protein. In studies of washes of the IPS of rabbit eyes (2), we identified a soluble 7S retinoid-binding protein (Interphotoreceptor Retinoid-Binding Protein, IRBP as a component of the interphotoreceptor matrix (IPM). Further studies using monkey eyes (3) showed the IRBP to be the major soluble protein and the only retinoid-binding protein present in the IPM. It is a glycoprotein of approximately 146,000 M_r as assessed by SDS-polyacrylamide

Abbreviations: IRBP: Interphotoreceptor Retinoid-Binding Protein; PE: pigment epithelium; IPS: interphotoreceptor space; IPM: interphotoreceptor matrix; SDS: sodium dodecylsulfate

0006-291X/84 $1.50

gel electrophoresis (3). Adler and Klucznik (4) have reported a 140,000 M_r glycoprotein to be a major component of bovine IPM and Liou et al (5) have isolated a 140-145K retinol-binding glycoprotein from bovine IPM.

In the present study, we have incubated whole monkey retinas and PE-choroid in short term organ culture with radiolabeled amino acids or glucosamine in order to determine the tissue of origin of the 146K IRBP. We have found IRBP to be synthesized by monkey retinas and secreted into the culture medium as would be expected of a protein present in the extracellular matrix, in this case the IPM. The secretion of IRBP was blocked by the addition of 10^{-5}M monensin to the culture medium. This proton ionophore is known to inhibit the secretion of a variety of proteins from different cells at the level of the Golgi Complex (6-9).

MATERIALS AND METHODS

Rhesus and cynomolgus monkeys (Macaca Mulatta and M. Fasicularis) were killed by exsanguination while under deep barbiturate anesthesia. Shortly after death, the eyes were enucleated and stored on ice. Following removal of the anterior portion of the eye and most of the vitreous, the neural retina was dissected away from the PE and the PE-choroid was then removed as a unit. There was usually some adherence of vitreous to the retina, and as much as possible was removed without causing damage to retinal tissue. There was also some adherence of PE to the retina.

One retina or PE-choroid unit was placed in 5ml RPMI 1640 medium (without leucine or glucose). For amino acid incubations, 100 ul 10% glucose, 100 ul 1.5M HEPES buffer, pH 7.4, 50ul Pen-Strep and 100 uCi 35S methionine (New England Nuclear, 1221.5 Ci/mmole) or 75 uCi 3H-L-amino acid mixture (New England Nuclear) plus 25uCi 3H-L-leucine (New England Nuclear, 52.3 Ci/mmole) were added to the medium. For sugar incubations, 10ul L-leucine (5 mg/ml) 50 ul Pen-Strep, 100 ul 1.5M HEPES buffer, pH 7.4 and 50 uCi ^{14}C-glucosamine (New England Nuclear, 325.5 mCi/mmole) were added to the medium.

Tissues were incubated for 3 hours with gentle agitation in a 37°C water bath under a 95% O_2, 5% CO_2 atmosphere. In some experiments, tissues were pre-incubated with or without 10^{-5}M monensin or Tunicamycin (Sigma Chem. Co., St. Louis, MO) for 15 minutes prior to the addition of radiolabeled precursors. Following incubation, the tissues were separated from the culture medium by low speed centrifugation. The culture medium was then centrifuged for 30 minutes at 110,000 g and the supernatant was concentrated approximately 10-fold in an Amicon stirred cell containing a YM-5 membrane. Tissues were homogenized in approximately 500 ul Tris buffer pH 7.6 and centrifuged for 1 hour at 110,000g to obtain the cytosol fraction. Total cytosol protein was determined by the dye-binding assay of Bradford (10) using a Bio-Rad kit. Aliquots (30ul) of the concentrated culture media and tissue cytosol samples were then subjected to SDS-polyacrylamide slab gel electrophoresis using the method of Laemmli (11) with a 3% stacking gel and an 8% running gel at 50V for 16-18 hours. High molecular weight standards (Bio Rad) were run concurrently, the gels were stained with 0.25% Coomassie Blue R-250 in 45% methanol, 9.2% acetic acid for 16 hours at room temperature and destained with glacial acetic

acid/methanol/water (1:3:7). Fluorography using EN3HANCE (New England Nuclear) was carried out according to directions using Kodak X-OMAT AR film. Exposure time was 2 weeks for ^{35}S-methionine and 4 weeks for ^{3}H-amino acids and ^{14}C-glucosamine.

RESULTS

When whole monkey retinas were incubated for 3 hours in organ culture in the presence of ^{35}S-methionine, a 146K band of newly synthesized protein was clearly present in the culture medium and in the retina cytosol (Fig. 1, A and B). A similar result was obtained when incubations were performed in the presence of an ^{3}H-amino acid mixture (Fig. 2, A and B). PE-choroid did not secrete a significant amount of 146K protein into the culture medium (Fig. 1 and Fig. 2). Incubations with ^{14}C-glucosamine as prescursor clearly demonstrated that the 146K protein was a major soluble glycoprotein synthesized by monkey retina during the 3 hour incubation as well as the only newly synthesized glycoprotein detected in

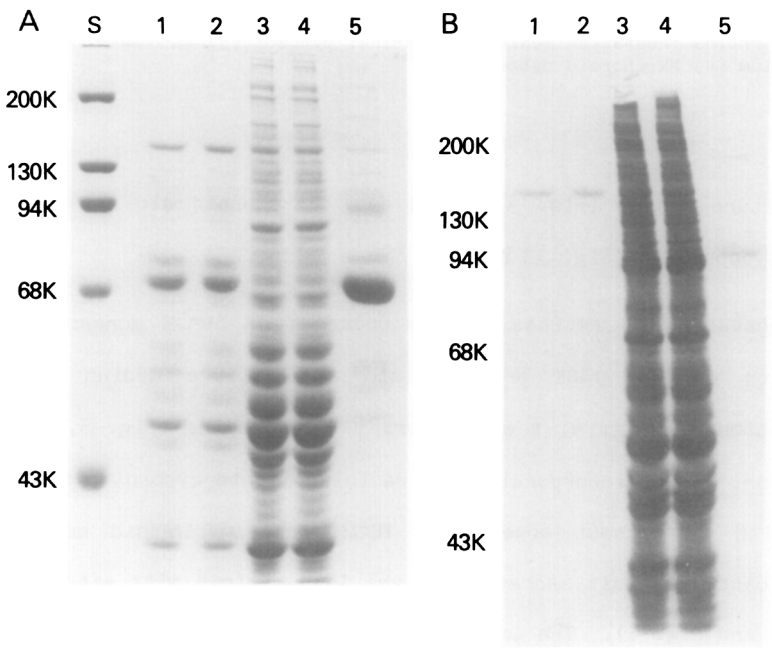

Figure 1. SDS-polyacrylamide gel electrophoresis of concentrated incubation medium or tissue cytosol following incubation of monkey retina or PE-choroid with ^{35}S-methionine. A. Coomassie blue-stained gel, (S) high molecular weight standards B. Fluorograph of gel shown in A. (1,2) retina incubation medium, (3,4) retinal cytosol (5) PE-choroid medium.

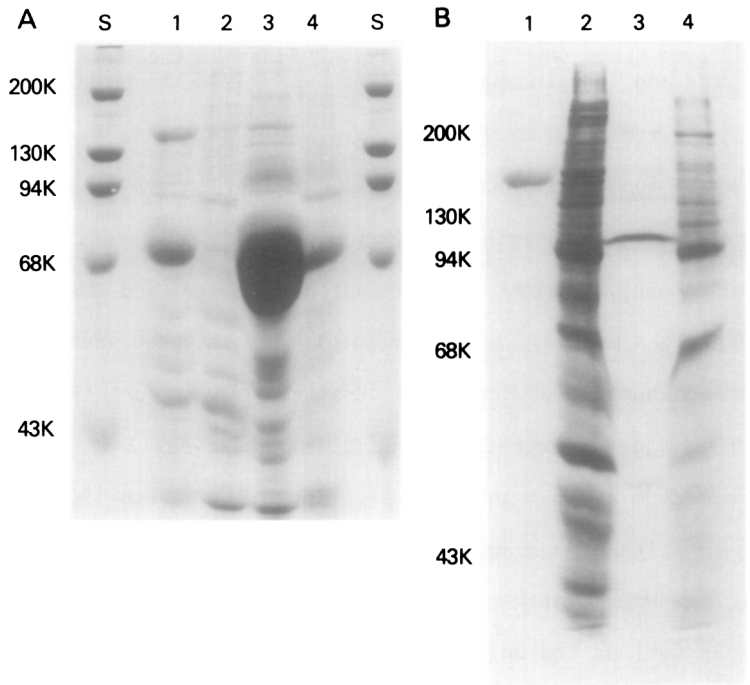

Figure 2. SDS-polyacrylamide gel electrophoresis of concentrated incubation medium or tissue cytosol following incubation of monkey retina or PE-choroid with ^{3}H-amino acid mixture. A. Coomassie blue-stained gel (S) high molecular weight standards. B. Fluorograph of gel shown in A. (1) retina incubation medium (2) retinal cytosol (3) PE-choroid incubation medium (4) PE-choroid cytosol.

the culture medium (Fig. 3, A and B). PE-choroid did not synthesize a 146K glycoprotein (Fig. 3A and B).

Incubation of retinas in the presence of $10^{-5}M$ monensin inhibited secretion of the 146K protein into the culture medium with either ^{35}S-methionine (Fig. 4, A and B) or ^{14}C-glucosamine (Fig. 5, A and B) as precursor. Total incorporation of radiolabel into cytosol protein was the same with or without monensin. $10^{-5}M$ tunicamycin did not appear to significantly inhibit secretion of the 146K protein with either precursor (Fig. 4 and Fig. 5). The amount of newly synthesized IRBP varied somewhat from one experiment to another when ^{14}C-glucosamine was used as the precursor and probably reflected variable dilution with endogenous carbohydrate pools.

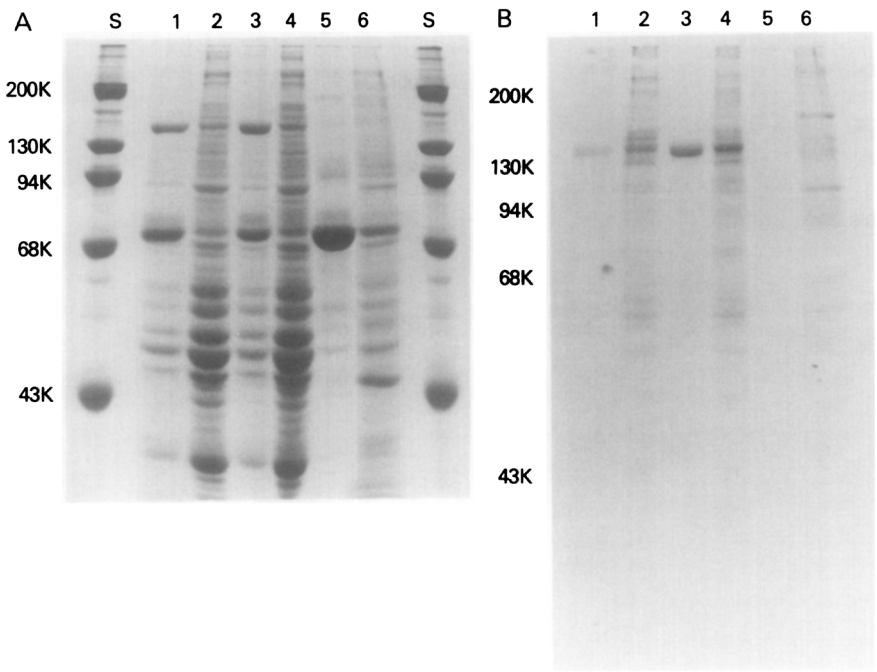

Figure 3. SDS-polyacrylamide gel electrophoresis of concentrated incubation medium or tissue cytosol following incubation of monkey retina or PE-choroid with ^{14}C-glucosamine. A. Coomassie blue-stained gel (S) high molecular weight standards. B. Fluorograph of the same gel. (1,3) retina incubation medium (2,4) retinal cytosol (5) PE-choroid incubation medium (6) PE-choroid cytosol.

DISCUSSION

Our earlier studies have indicated that IRBP is a soluble, peripheral protein which is loosely associated with retinal photoreceptors in the interphotoreceptor matrix (2,12,13). It binds retinol endogenously and differentially binds ^{3}H-retinol in light and in dark (2,14). Bunt-Milam and Saari have recently presented immunocytochemical evidence for the localization of IRBP in the IPS (15). Thus, although IRBP does appear to be a specific vitamin A-binding protein present in the extracellular space between the neural retina and pigment epithelium, either tissue could be the source of this protein.

In the present study, incubation of monkey neural retina and PE-choroid in organ culture in the presence of radiolabeled amino acids or glucosamine showed that IRBP, identified as a glycoprotein of approximately 146K M_r on SDS-polyacrylamide gel electrophoresis, was synthesized

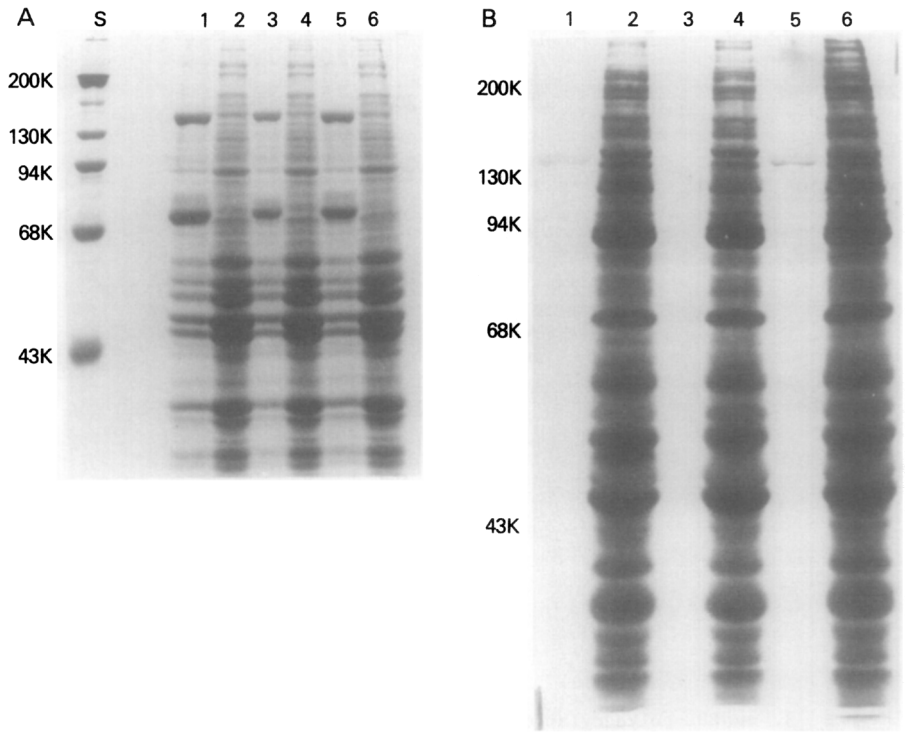

Figure 4. SDS-polyacrylamide gel electrophoresis of concentrated medium or tissue cytosol following incubation of monkey retina cytosol with ^{35}S-methionine in the presence of 10^{-5}M monensin or 10^{-5}M tunicamycin. A. Coomassie blue-stained gel (S) high molecular weight standards B. Fluorograph of the same gel (1) control retina medium (2) control retina cytosol (3) retina medium with monensin (4) retina cytosol with monensin (5) retina medium with tunicamycin (6) retina cytosol with tunicamycin.

by the neural retina, not by the pigment epithelium. After a 3 hour incubation period, IRBP was a major soluble glycoprotein synthesized by monkey retina and virtually the only detectable newly synthesized protein present in the culture medium. This latter observation is consistent with our earlier work which showed that in monkey eyes, IRBP comprised about 70% of the total soluble protein in the IPM isolated by a gentle cannulation technique (3).

Monensin, a monovalent carboxylic acid ionophore produced by Streptomyces cinnamonensis, has been shown in the ultrastructural studies of Tartakoff and Vassalli (6,7) to cause a dilation of the Golgi apparatus and thus to impede the transport of proteins from the Golgi to the extracellular space. The fact that newly synthesized IRBP was not present

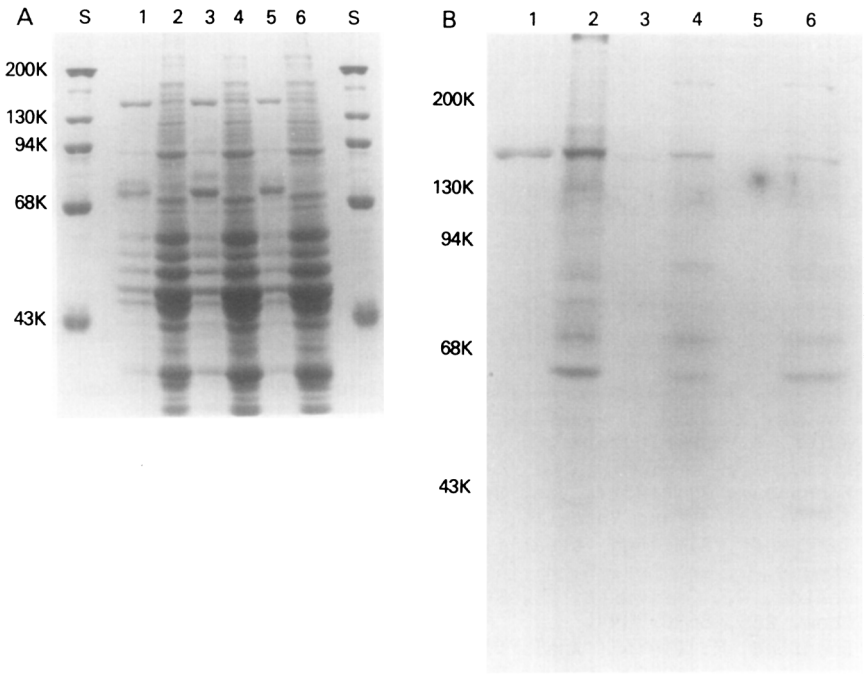

Figure 5. SDS-polyacrylamide gel electrophoresis of concentrated medium or tissue cytosol following incubation of monkey retina with ^{14}C-glucosamine in the presence of 10^{-5}M monensin or 10^{-5}M tunicamycin. A. Coomassie blue-stained gel (S) high molecular weight standards B. Fluorograph of the same gel (1) control retina incubation medium (2) control retinal cytosol (3) retina incubation medium with tunicamycin (4) retinal cytosol with tunicamycin (5) retina incubation medium with monensin (6) retinal cytosol with monensin.

in the culture medium following organ culture of monkey neural retina in the presence of monensin indicates that IRBP is secreted by the retina, probably via the Golgi, and does not merely leak out in a non-specific manner. The antibiotic tunicamycin which inhibits the initial events in glycosylation of asparagine residues (16,17) did not appear to affect the secretion of IRBP. Further studies will be necessary to determine whether this result was due to the failure of the tunicamycin to enter the retinal cells which synthesize and secret IRBP or to the synthetic pathway of IRBP being tunicamycin insensitive.

In any event, the substantial synthesis of IRBP by the neural retina, its rapid release into the medium coupled with the striking effects of monensin make this system of general interest in studying the biosynthesis of extracellular proteins and of particular interest in attempting

to define the role of this unique retinoid-binding protein in the visual process.

ACKNOWLEDGEMENT

The authors wish to thank Mr. John P. Cogan, Chief, In Vivo Vaccine Test Section, Office of Biologics, National Center for Drugs and Biologics, FDA for supplying the primate tissue used in this study.

REFERENCES

1. Feeney, L. (1973) Develop. Biol. 32, 101-114.
2. Lai, Y., Wiggert, B., Liu, Y. and Chader, G. (1982) Nature 298, 848-849.
3. Pfeffer, B., Wiggert, B., Lee, L., Zonnenberg, B., Newsome, D. and Chader, G. (1983) J. Cell. Physiol. (in press).
4. Adler, A. and Klucznik, K. (1982) Exp. Eye Res. 34, 423-434.
5. Liou, G., Bridges, C., Fong, S-L., Alvarez, R. and Gonzalez-Fernandez, F. (1982) Vis. Res. 22, 1457-1467.
6. Tartakoff, A. and Vassalli, P. (1977) J. Exp. Med. 146, 1332-1345.
7. Tartakoff, A.M. and Vassalli, P. (1978) J. Cell. Biol. 79, 694-707.
8. Strous, G. and Lodish, H. (1980) Cell 22, 709-717.
9. Uchida, N., Smilowitz, H., Ledger, P. and Tonizer, M. (1980) J. Biol. Chem. 255, 8638-8644.
10. Bradford, M. (1976) Anal. Biochem. 72, 248-254.
11. Laemmli, U. (1970) Nature 227, 680-685.
12. Bergsma, D., Wiggert, B., Funahashi, M., Kuwabara, T. and Chader, G. (1977) Nature 265, 66-67.
13. Wiggert, B., Mizukawa, A., Kuwabara T. and Chader, G. (1978) J. Neurochem. 30, 653-659.
14. Wiggert, B., Derr, J., Fitzpatrick, M. and Chader, G. (1979) Biochim. Biophys. Acta 582, 115-121.
15. Bunt-Milam, A. and Saari, J. (1983) J. Cell Biol. 97, 703-712.
16. Struck, D. and Lennarz, W. (1977) J. Biol. Chem. 252, 1007-1013.
17. Tabas, I., Schlesinger, S. and Kornfeld, S. (1978) J. Biol. Chem. 253, 716-722.