N-terminal sequence homologies in interstitial retinol-binding proteins from 10 vertebrate species

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We report here the first comprehensive comparative NH_2-terminal sequence studies of interstitial retinol-binding protein (IRBPs) from nine mammals (including cattle) and one amphibian. This study has revealed that in many species the N-terminus of IRBP includes a 3–6 amino acid extension. IRBP possessing this leader sequence is sometimes mixed with IRBP from which this sequence has been excised.

Interstitial retinol-binding protein *N-terminal sequence*

1. INTRODUCTION

Interstitial retinol-binding (IRBP) is an extracellular matrix glycoprotein synthesized and secreted by the retinal photoreceptors [1]. It is believed to be involved in transporting retinol and possibly other ligands such as α-tocopherol and fatty acids between the cells of the neural retina and retinal pigment epithelium [2–11]. IRBPs have been found in the pineal organ of several species [12] and in the eyes of animals from all vertebrate classes. In teleosts, IRBP has an average apparent M_r of 67 000 on SDS-polyacrylamide gels, but in mammals, birds, amphibians, reptiles and elasmobranchs its apparent M_r is double this value, namely 134 000 [13–15]. Bovine IRBP has been investigated in terms of its cDNA, N-terminal and tryptic peptide structures [8,13,16–18], but its full amino acid sequence remains to be determined.

2. MATERIALS AND METHODS

2.1. *Preparative procedures*

Interphotoreceptor matrix was prepared essentially as described [19]. The matrix adhering to excised retinas was obtained by gently stirring them in phosphate-buffered saline containing phenylmethylsulfonyl fluoride as a protease inhibitor.

2.2. *Electroblotting and microsequencing*

Aliquots of interphotoreceptor matrix (equivalent to 2–6 eyes) were subjected to electrophoresis on 7.5% SDS-polyacrylamide gels [3,20]. Electroblotting to glass fiber sheets activated within 3-aminopropyltriethoxysilane (Pierce, Aldrich) was according to Aebersold et al. [21]. After staining with the fluorescent dye 3,3′-dipentyloxacarbocyanine iodide, the IRBP band was visualized by exposure to short wavelength UV radiation, cut out and placed directly in the cartridge of an Applied Biosystems gas-phase sequenator. The repetitive yield for 100 pmol or less of protein was typically 92–94%. As estimated from the yield of amino acid in step 1, the quantities of IRBP sequenced by this technique ranged from 15–60 pmol. PTH amino acids were identified by high-performance liquid chromatography on a Waters system or the Applied Biosystems 120A in-line PTH analyzer.

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2.3. *Antisera and immunoblotting*

SDS-gel electrophoresis of interphotoreceptor matrix, electrophoretic transfer to nitrocellulose and immunological visualization of IRBP with rabbit antibovine IRBP antibodies were as described [1].

3. RESULTS AND DISCUSSION

SDS-polyacrylamide gel patterns of cattle, human and frog interphotoreceptor matrix preparations used in the present study are depicted in fig.1. Apparent M_r of the three IRBPs (identified immunologically after blotting replicate gels to nitrocellulose membranes) were 144 000 (cattle), 135 000 (human) and 125 000 (frog). The IRBP band was clearly separated from contaminating proteins, and these preparations were usually used for electroblotting to activated glass fiber without further purification.

Fig.2 displays the amino terminal sequences for IRBP from ten different species (one amphibian, nine mammals). There is extensive homology, as might be expected from their antigenic similarities [14,15]. Of the 24 positions available for comparison, 19 (80%) are identical in cattle, sheep and pig. Not all the interchanges are conservative; e.g. Gln/Lys at position 11 in cattle and sheep. Conservative exchanges are Glu/Asp at position 8 and Val/Ileu at position 12. The sequences for other species could only be aligned if they were shifted by 3–6 amino acids relative to each other, i.e., the rabbit, guinea pig, hamster, human, bush baby, rhesus monkey and frog proteins had a 3–6 amino acid 'leader' peptide at the amino-terminal. In hamster IRBP, this leader consisted of a (His)-Pro-Ile-Gln-Asn-Leu- hexapeptide preceding a Phe-Gln-Pro-Ser-Leu-Val-(Leu)-(Asp)-Met sequence that is identical with that found in sheep, rabbit, guinea pig, rhesus monkey and human IRBPs. IRBP from the frog, which belongs to a vertebrate class that diverged earlier than the other 9 mammals, had an Asn-Pro-Val tripeptide preceding a sequence of 20 amino acids that displays 3 differences from cattle IRBP.

A particularly interesting observation was that IRBPs lacking a leader peptide were also detected in interphotoreceptor matrix preparations from rabbit, human and rhesus monkey. In the rabbit, the two forms were present in comparable

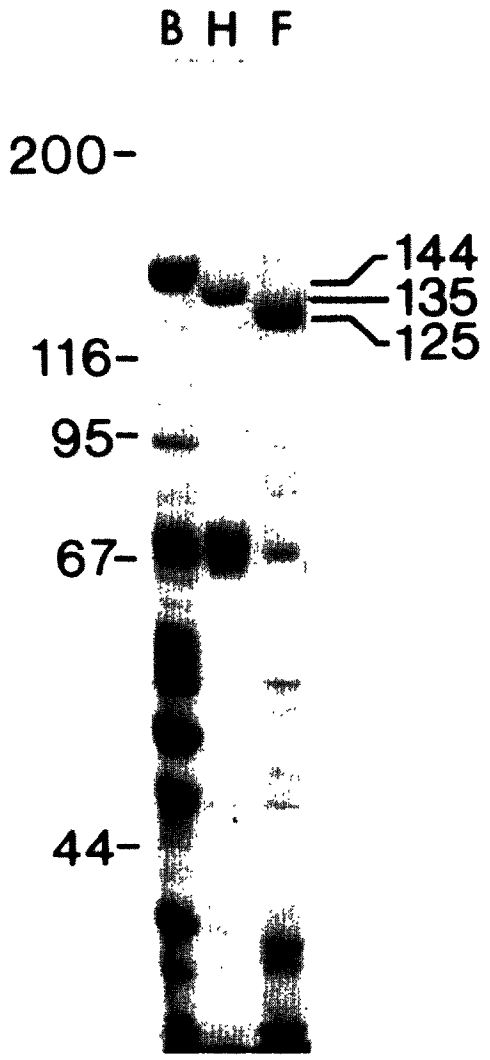

Fig.1. Coomassie blue-stained SDS-7.5% polyacrylamide gel of aliquots of cattle, human and frog (*R. pipiens*) interphotoreceptor matrix preparations used for electroblotting to activated glass fiber and microsequencing. Apparent $M_r \times 10^{-3}$ of corresponding IRBPs shown on the right, positions of M_r markers shown on the left.

amounts, while in the rhesus monkey the IRBP that lacked a leader peptide constituted about one-third of the mixture. Individual variation does not account for this finding, because only a single rhesus monkey was used. Two IRBPs were also seen in an interphotoreceptor matrix preparation from the paired eyes of a single rabbit. Human and

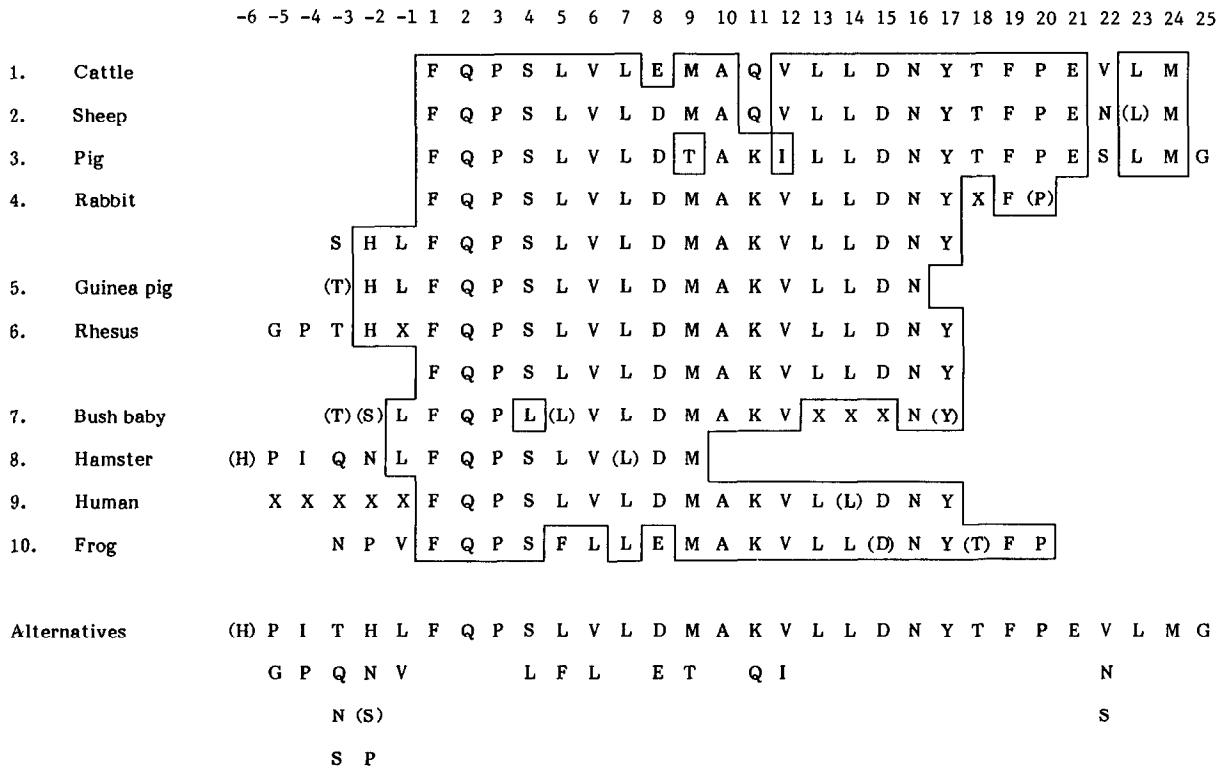

Fig.2. Comparison of IRBP N-terminal sequences from 10 species. Identical residues are enclosed in boxes. Parentheses denote tentative assignments. The cattle sequence is from Saari et al. [8]: identical sequences extending over the first 14–15 residues have been reported by others [13,16]. The rhesus monkey sequence substantially extends that previously published [16] and additionally identifies a second form lacking the −1 to −5 leader sequence. Redmond et al. [16] reported Ala instead of His at position −2; the Leu at position −1 in their sequence could not be identified with certainty in the present work.

bush baby IRBPs also appeared to be heterogeneous, showing that in these species there had been partial cleavage of the leader peptide, in some cases at a Leu-Phe bond. Whether this was an extracellular or intracellular event is not clear. In contrast, no trace of leader peptide could be found in cattle, sheep and pig IRBPs. Conversely, there was no indication of leaderless IRBP in guinea pig, hamster and frog. Human IRBP is complex. Fig.3 shows that there may be at least 2 different leader sequences, but further work is necessary to clarify this observation.

The leader peptide of IRBP may be analogous to that found in a pro-protein such as proalbumin, which retains a hexapeptide NH_2-terminal extension after the signal peptide of preproalbumin has been cleaved [22–26]. In albumin, this extension is normally excised intracellularly, but this is not obligatory for secretion [27,28]. Since cleavage of the leader sequence does not always occur in IRBP, it is possible that this hydrophobic amino-terminal region may play a role in interactions with cell surface membranes. The strong sequence homologies at the N-terminus of the IRBPs studied

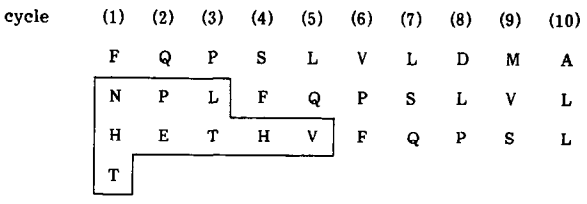

Fig.3. Complexity of a human IRBP preparation from 3 pairs of eyes. Amino acids in the first 10 sequenator cycles are shown: presumptive leader sequences are boxed.

in the present work suggest that their different apparent molecular masses on SDS-polyacrylamide gels (e.g. fig.1) may arise from C-terminal processing.

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REFERENCES

[1] Gonzalez-Fernandez, F., Landers, R.A., Glazebrook, P.A., Fong, S.-L., Liou, G.I., Lam, D.M.K. and Bridges, C.D.B. (1984) J. Cell Biol. 99, 2092–2098.
[2] Liou, G.I., Bridges, C.D.B. and Fong, S.-L. (1982) Invest. Ophthal. Vis. Sci. 22, 65.
[3] Liou, G.I., Bridges, C.D.B., Fong, S.-L., Alvarez, R.A. and Gonzalez-Fernandez, F. (1982) Vision Res. 22, 1457–1468.
[4] Adler, A.J. and Martin, K.J. (1982) Biochem. Biophys. Res. Commun. 108, 1601–1608.
[5] Pfeffer, B., Wiggert, B., Lee, L., Zonnenberg, B., Newsome, D. and Chader, G. (1983) J. Cell Physiol. 117, 333–341.
[6] Bunt-Milam, A.H. and Saari, J.C. (1983) J. Cell Biol. 97, 703–712.
[7] Fong, S.-L., Liou, G.I., Landers, R.A., Alvarez, R.A. and Bridges, C.D.B. (1984) J. Biol. Chem. 259, 6534–6542.
[8] Saari, J.C., Teller, D.C., Crabb, J.W. and Bredberg, L. (1985) J. Biol. Chem. 260, 195–201.
[9] Adler, A.J., Evans, C.D. and Stafford, W.F. iii (1985) J. Biol. Chem. 260, 4850–4855.
[10] Johnson, A.T. and Kretzer, F.L. (1985) in: The Interphotoreceptor Matrix in Health and Disease (Bridges, C.D. and Adler, A.J. eds) pp. 251–277, New York.
[11] Bazan, N.G., Reddy, T.S., Redmond, T.M., Wiggert, B. and Chader, G.J. (1986) J. Biol. Chem. 260, 13677–13680.
[12] Bridges, C.D.B., Landers, R.A., Fong, S.-L. and Liou, G.I. (1986) in: Pineal and Retinal Relationships (O'Brien, P.J. and Klein, D.C. eds) Academic Press, New York, in press.
[13] Bridges, C.D.B., Alvarez, R.A., Fong, S.-L., Gonzalez-Fernandez, F., Lam, D.M.K. and Liou, G.I. (1984) Vision Res. 24, 1581–1594.
[14] Bridges, C.D.B., Peters, T., Smith, J.E., Goodman, D.S., Fong, S.-L., Griswold, M.D. and Musto, N.A. (1986) Fed. Proc., in press.
[15] Bridges, C.D.B., Liou, G.I., Alvarez, R.A., Landers, R.A., Landry, A.M. jr and Fong, S.-L. (1986) J. Exp. Zool., in press.
[16] Redmond, T.M., Wiggert, B., Robey, R.A., Nguyen, N.Y., Lewis, M.S., Lee, L. and Chader, G.J. (1985) Biochem. 24, 787–793.
[17] Barrett, D.J., Redmond, T.M., Wiggert, B., Oprian, D.D., Chader, G.J. and Nickerson, J.M. (1985) Biochem. Biophys. Res. Commun. 131, 1086–1093.
[18] Liou, G.I., Fong, S.-L., Beattie, W.G., Cook, R.G., Leone, J., Landers, R.A., Alvarez, R.A., Wang, C., Li, Y. and Bridges, C.D.B. (1986) Vision Res., in press.
[19] Fong, S.-L., Liou, G.I. and Bridges, C.D.B. (1986) Methods Enzymol. 123, 102–111.
[20] Laemmli, U.K. (1970) Nature 227, 680–685.
[21] Aebersold, R.H., Teplow, D.B., Hood, L.E. and Kent, S.B.H. (1986) J. Biol. Chem. 261, 4229–4238.
[22] Russell, J.H. and Geller, D.M. (1975) J. Biol. Chem. 250, 3409–3413.
[23] Quinn, P.S., Gamble, M. and Judah, J.D. (1975) Biochem. J. 146, 389–393.
[24] Yu, S. and Redman, C. (1977) Biochem. Biophys. Res. Commun. 76, 469–476.
[25] Strauss, A.W., Donohue, A.M., Bennett, C.D. and Alberts, A.W. (1977) Proc. Natl. Acad. Sci. USA 74, 1358–1362.
[26] Strauss, A.W., Bennett, C.D., Donohue, A.M., Rodkey, J.A. and Alberts, A.W. (1977) J. Biol. Chem. 250, 3409–3413.
[27] Brennan, S.O. and Carrell, R.W. (1978) Nature 274, 908–909.
[28] Redman, C.M., Avellino, G. and Yu. S. (1983) J. Biol. Chem. 258, 3446–3452.