Purification and properties of avian cellular retinol-binding protein

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Two intracellular proteins that bind retinol (vitamin A) and analogues are present in the cytosol of many animal tissues (Ong & Chytil, 1980). Cellular retinol-binding protein (cRBP) as isolated from rat tissues is a single polypeptide of 14600 daltons and binds 1 mol of retinol per mol of protein (Ong & Chytil, 1978). A different protein with the same molecular mass binds retinoic acid (cellular retinoic acid-binding protein) specifically (Ong & Chytil, 1975). Both may function as receptor and transport proteins for retinol and retinoic acid respectively like steroid hormone receptors. More detailed fractionation of the cRBP preparation from rat testis by isoelectricfocusing indicated that at least two forms of the protein with slightly different pI values were present (Ross *et al.*, 1978). It is of interest to determine if this heterogeneity in cRBP persists in another animal species and to gain more information about the different forms. It was decided to examine cRBP from chick liver since only cRBP has been purified from embryo-skin in that species hitherto (Sani & Banerjee, 1978).

The protein was isolated essentially by the procedure of Ong & Chytil (1975) for rat liver with additional ion-exchange chromatography on DEAE-cellulose at pH 6.0 and final fractionation of the cRBP by absorption chromatography on calcium phosphate gel (hydroxylapatite) column with 100mM-imidazole/HCl buffer at pH 6.8 containing a gradient of 0–50mM-sodium phosphate buffer at same pH. At each step of purification the presence of holo cRBP was monitored by its characteristic yellow–green fluorescence with maximum at 485nm and by u.v. absorption having maxima at 348nm and 283nm. The homogeneity of the cRBP preparations was examined by electrophoresis on 5% polyacrylamide disc-gels. The molecular mass was estimated by SDS/gel electrophoresis (Laemmli, 1970) and by gel filtration on Sephadex G-75. The pI was determined by chromatofocusing on polybuffer exchanger (PBE 94) (pH 7–4).

The final chromatography separates the cRBP preparation into two fractions (peaks 1 and 2) (Fig. 1), each of which contains protein of 14600 daltons by SDS/gel electrophoresis. Each fraction was also examined by disc-gel electrophoresis at pH 8.9 and the first (peak 1) to be eluted separated into two fluorescent zones before staining. The second (peak 2) showed only one fluorescent zone, which had the same electrophoretic mobility as the faster moving fluorescent component of peak 1 (Fig. 1 inset). After staining there were three protein zones in the gel from peak 1, the fastest moving component of which was non-fluorescent and was considered to be the apoform of cRBP. The middle component corresponding to the more advanced fluorescent zone from peak 1 had the same mobility as the single protein from peak 2. The

Abbreviations used: cRBP, cellular retinol-binding protein; SDS, sodium dodecyl sulphate.

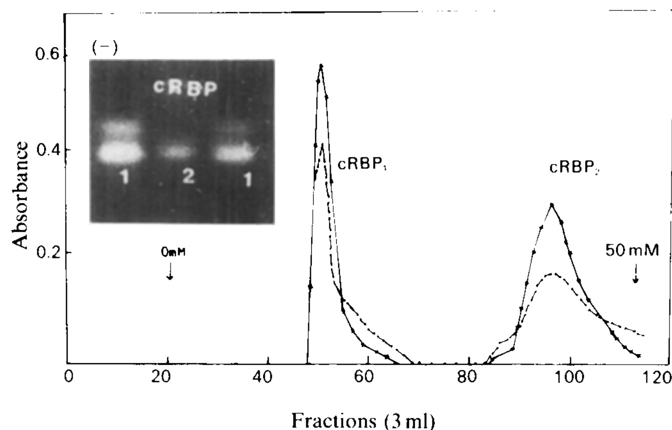

Fig. 1. *Separation of cellular retinol-binding isoproteins by chromatography on calcium phosphate gel (hydroxylapatite) column and by electrophoresis*

Gradient elution chromatography was performed with sodium phosphate (0–50mM) 0.1 M-imidazole/HCl buffer. Absorbance: x——x, 350nm; o——o, 280nm. Insert: polyacrylamide-gel electrophoresis at pH 8.9 of CRBP in peaks 1 and 2 from the column; photographed under u.v.-light at 350nm.

ratio of the u.v. absorption at 348nm/283nm for peak 2 material was found to be 1.78 whereas that for peak 1 was only 1.40, reflecting the presence of apo material in that fraction. The $E^{1\%}_{1cm}$ values for the purer peak 2 material were 19 at 283nm and 34 at 348nm. The slower moving but fluorescent component in the peak 1 fraction may be a partially denatured form of holo cRBP. The electrophoretic mobility of holo cRBP from peak 2 is higher than that of chick plasma retinol-binding protein by a factor of 1.15 and it has a higher pI value (4.7 as opposed to 4.5).

Chick cRBP was found to have the same molecular mass of 14600 daltons, as that of the rat and similar ratio of 1.78 for the u.v. absorption maxima at 348nm and 283nm. The liver preparation contained both unsaturated and saturated forms of the protein and a portion of the latter may be partially denatured holo cRBP. Furthermore the ligand in peak 1 material was noted to be less stable than that in peak 2. The absence of any cross reaction between cellular and plasma retinol-binding proteins and their respective specific antisera raised in the rabbit indicated a lack of homology between the proteins.

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