Reports

The vitamin A transporting protein complex in human hereditary pigmentous retinal dystrophy. G. Maraini.

Highly purified preparations of prealbumin-retinal-binding protein complex (PA-RBP), prealbumin (PA), and retinol-binding protein (RBP) have been isolated from the plasma of patients with inherited retinitis pigmentosa and compared with the same proteins obtained from normal human plasma. On the basis of absorption and fluorescence characteristics, polarization fluorescence measurements at low and physiologic ionic strength, and chromatographic behavior it is concluded that no evidence has been obtained that retinitis pigmentosa RBP differs from normal RBP in its ability to interact with PA or in its capacity to act as a carrier of retinol.

Vitamin A alcohol is present in human serum bound to a specific transport protein called retinol-binding protein (RBP). 3 which has a molecular weight of approximately 21,000 daltons. Evidence has been presented that one molecule of RBP binds one molecule of retinol 3 which is the only molecular form of vitamin A transported by this specific carrier. The RBP level in serum is probably a function of the amount of available vitamin A, the protein being rapidly released from an existing pool in the liver into the bloodstream after chylomicron vitamin A injection. 4 It has been suggested that newly synthesized RBP requires retinol for its release from the hepatocytes. 5 The concentration of RBP in normal human serum is about 40 μg per milliliter. 6

Under physiologic conditions, RBP circulates in serum as a complex with a thyroxine-binding prealbumin (PA) (molecular weight, 64,000); the formation of this retinol-protein-protein complex (molecular weight, 85,000) probably has a remarkable importance in preventing its binding to PA; the absence of retinol per se does not explain the incapacity to bind PA since Rask, Vahlquist, and Peterson 8 demonstrated that RBP from which retinol has been extracted retains its affinity for PA. The retinol-containing and the retinol-free RBP give an identical reaction when examined by Ouchterlony immunodiffusion analysis, an immunologic difference between the two proteins becoming evident only when the much more sensitive quantitative precipitin technique is employed. 8 It has been suggested 6 that free RBP represents a transitory catabolic form of the protein which has fulfilled its physiologic role and is on the way of being eliminated or degraded.

This better knowledge of some of the molecular mechanisms regulating vitamin A transport and metabolism might be directly relevant to some of the diseases of ophthalmologic interest in which a disturbance of vitamin A utilization may be suggested. Dowling and Wald 9 demonstrated that a vitamin A-deficient diet in weanling rats causes a degeneration of the outer segments of photoreceptor cells which cannot be prevented by feeding these animals vitamin A acid supplements. The retinal histologic picture in this situation bears a striking similarity to that of the inherited retinal dystrophy observed in several animals. 10

Human pigmentary dystrophy of the retina is a disease of unknown etiology characterized by a progressive degeneration of photoreceptor cells; available data on the level of vitamin A in the blood of subjects affected by the disease are somewhat contradictory, normal 11 and subnormal 12 values having been reported. Campbell, Harrison, and Tonks 12 claimed improvement of the visual field and of dark adaptation after the administration of suitable doses of vitamin A. However, since, in these patients, there is no evidence of a general vitamin A deficiency, a possible defect has been suggested 24 in the supply or in the utilization of the vitamin, perhaps at the level of pigment and in the neuroepithelium of the retina.

The level of RBP in the serum of patients with retinitis pigmentosa has recently been found by Rahi 14 with an immunochemical method to be on the average 76 per cent of the mean normal value; this finding has been interpreted as of possible importance in causing poor retinol supply to the retina or an accumulation of free retinol in
serum with eventual damage to the lysosomes of retinal pigment epithelium.

Since a qualitative as well as a quantitative modification of RBP and/or of its complex with PA might result in a deficient transport of retinol in the plasma, I have purified the PA-RBP complex and RBP from the plasma of patients with retinitis pigmentosa and I have compared it with that obtained from normal subjects. About 200 ml. of pooled plasma from normal or diseased subjects were used for each experiment. Highly purified preparations of PA-RBP, PA, and RBP were isolated following the procedure described by Peterson\textsuperscript{15} by means of successive column chromatography on DEAE-Sephadex A-50, Sephadex G-100, DEAE-Sephadex, and finally Sephadex G-200. The elution position of RBP following chromatography was demonstrated both by measuring the absorbance of retinol at 330 nm. and by its fluorescence emission at 470 nm. upon excitation at 330 nm. For PA-RBP complex from both normal and pathologic plasma, dissociation was complete at an ionic strength of 0.002. Spectra in the ultraviolet region of the PA-RBP complex and pure RBP from retinitis pigmentosa patients were identical to those of the normal proteins; the 

\[ \text{E}_{280} : \text{E}_{330} \] ratio for normal and retinitis pigmentosa RBP was, respectively, 1.4 and 1.35, thus indicating that in both instances about 70 per cent of the RBP molecules contained one retinol molecule. Typical results are reported in Fig. 1.

Fluorescence measurements showed for both normal and retinitis pigmentosa PA-RBP and purified RBP an excitation maximum at 335 nm. and a maximal light emission at 460 nm. (Fig. 2). Fluorescence depolarization measurements also gave for retinitis pigmentosa PA-RBP and RBP results identical to the normal vitamin carrier; the higher fluorescence depolarization (70 to 74 per cent) for purified RBP with respect to the PA-RBP complex (57 to 61 per cent) suggests, as expected, a higher mobility of the smaller RBP molecule.

As for normal RBP, when RBP from retinitis pigmentosa plasma was extracted with a two-phase system consisting of water and heptane, all of the material absorbing at 330 nm. was present in the heptane phase and showed absorption and fluorescence typical for retinol.

Recombination of separated PA and RBP was investigated on a solution of the two proteins either by rechromatography at a higher ionic strength or by following fluorescence polarization changes.

If a solution of equimolar amounts of PA and RBP was dialyzed for 24 hours against 0.002 M Tris-HCl buffer (pH 8.0) containing 1 M NaCl, applied on a Sephadex G-100 column and eluted with the same buffer, all protein appeared in a single elution position corresponding to that of the PA-RBP complex.
dropped from 70 to 62 per cent immediately after increasing the ionic strength from 0.002 to 0.7; this shift from a fluorescence depolarization value typical for isolated RBP to that recovered in original PA-RBP complex was taken as evidence of the recombination of the protein complex under the experimental conditions used. No change in the quantum yield of retinol fluorescence was observed during the recombination of PA-RBP complex; accordingly the quantum yield of the 470 nm. fluorescence was practically identical for purified RBP and the original PA-RBP complex. This is in good agreement with the results of Futterman and Heller, while Peterson and Rask reported that the quantum yield of retinol for the protein complex is about 50 per cent higher than for RBP.

In conclusion, present investigations failed to demonstrate any appreciable qualitative difference between the proteins involved in the transport of retinol in plasma in pigmented dystrophy of the retina with respect to the physiologic situation and suggest a substantial identity between them. No evidence has been obtained that retinitis pigmentosa RBP differs from normal RBP in its ability to interact with PA or in its capacity to act as a carrier of vitamin A alcohol. The quantitative RBP defect present in the plasma of these patients does not seem to be associated with a qualitative change of the molecular mechanism which regulates the transport of retinol.

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REFERENCES


Autoradiographic studies of DNA-repair in the retina of rabbits, Ludwig Fichler, Heinrich Gumpel-Mayr.

DNA-repair in 60Co-γ-irradiated (60,000 rad) rabbit retinae has been investigated by autoradiography. A repair mechanism has been shown to exist at the same high degree in cells of the ganglion layer and inner nuclear layer, but, however, to a much lesser extent in the cells of the outer nuclear layer of these retinae.

Experimentally induced lesions in the retina and their repair have been described in many...