THE TRANSPORT OF RETINOL IN HUMAN PLASMA

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1. Introduction

Evidence in support of the view that tryptophan-rich prealbumin [1] may serve as the specific transport protein for retinol in human plasma has previously been reported by Alvsaker, Haugli and Laland [2]. Since thyroxine is transported partly by this protein [3], thyroxine and retinol would then share the same transport protein. In order to investigate this further, the retinol-containing protein in human plasma was re-examined.

The results obtained show that retinol in human plasma is not linked to tryptophan-rich prealbumin as previously suggested [2] but to an immunologically related protein with an $S_{20,w}$ value of 1.85 (ultracentrifugation) as compared to 4.6 of tryptophan-rich prealbumin. In disc electrophoresis the retinol-binding protein was localized to the alpha$_1$ globulin position. In vitro experiments further revealed that it did not bind thyroxine in contrast to tryptophan-rich prealbumin.

2. Materials and methods

2.1. *Sera*

Blood from a healthy male, aged 25, was used. Serum was obtained as previously described [2]. Horse immune serum against human serum was purchased from the Centraal Laboratorium van de Bloedtransfusiedienst van het Nederlandsche Roode Kruis, Amsterdam, The Netherlands. Rabbit immune serum against human tryptophan-rich prealbumin was obtained from Behringwerke A.G., Marburg-Lahn, Germany.

2.2. *Gel columns*

Sephadex G-50 (course grade) and DEAE Sephadex A-50 were purchased from A/B Pharmacia, Uppsala, Sweden.

2.3. *Tryptophan-rich prealbumin*

Material isolated by the method of Schultze et al. [1] was kindly supplied by Dr. Schwick of Behringwerke A.G., Germany.

2.4. *Radioactive material*

[^{131}I]-L-thyroxine (11 600 mC/mmole) was purchased from Institute for Atomic Energy, Kjeller, Norway.

2.5. *Electrophoresis*

Paper electrophoresis was carried out on Whatman No. 3 MM paper (4 cm X 40 cm) in 0.2 M glycine-0.13 M sodium acetate buffer pH 8.6 in the apparatus of LKB Produkter A.B., Stockholm, Sweden. A voltage of 3 V/cm was applied for 18 hr at 4°C. Immunoelectrophoresis was carried out as previously described [2]. Analytical disc electrophoresis was carried out at a running pH of 9.3 in the discontinuous buffer system described by Ornstein and Davis [4]. The temperature-regulated equipment of Buchler Instruments, Inc., New Jersey, USA, was employed. Unstained protein zones in the polyacrylamide gel were cut out and transferred to 1% (w/v) of Difco Special Agar Noble in sodium barbitalbuffer (pH 8.6, $I = 0.05$) or physiological saline solution (0.9% w/v sodium chloride in distilled water) for immuno-electrophoresis and immunodiffusion, respectively.

2.6. *Immunodiffusion*

This was carried out as previously described [2].

2.7. *Measurement of radioactivity*

The strip counter equipment of Frieseke and Hoepfner, Erlangen-Bruck, Germany, was used for the recording of [^{131}I]-L-thyroxine on paper strips.

2.8. *Fluorescence measurements*

These were carried out as described in detail in a previous report [2].

2.9. *Extraction and identification of retinol*

Extraction of retinol was performed by ethanol/cyclohexane as described by Kahan [5]. The identification of retinol was obtained by the Carr-Price test and anhydrovitamin A test as previously described [2]. Retinol was also identified by thin-layer chromatography on silica gel with cyclohexane/ethylether (80:20) as the solvent. All-trans vitamin A alcohol prepared from the acetate (Hoffman-La Roche & Co., Basel, Switzerland) was used as reference substance.

2.10. *Ultracentrifugation*

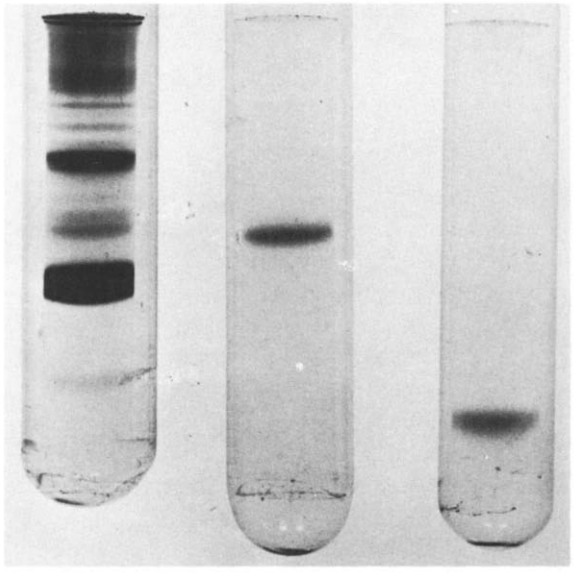
The sedimentation coefficient ($S_{20,w}$) was measured using a Spinco model E analytical ultracentrifuge with an AN-D rotor. Centrifugation was conducted at 3.3°C at 60 000 rpm on a solution of the protein (optical density 0.58 at 280 mμ) in 0.005 M phosphate buffer, pH 7.35, containing 0.5% NaCl and photographs were taken with absorption optics at 280 mμ. We are indebted to Dr. T.Christensen of this department for carrying out the analysis.

3. Results and discussion

Serum (100 ml) was gel-filtered on a Sephadex G-50 (course grade) column (5.5 × 5 cm) equilibrated with 0.02 M sodium phosphate buffer pH 7.35. The macromolecular fraction was transferred to a DEAE-Sephadex A-50 column (3 × 9.5 cm) equilibrated with the same buffer. The column was washed with this buffer containing 0.15 M sodium chloride until no more protein appeared. The column was then eluted with the buffer containing 0.25 M NaCl. This brought out essentially all the green fluorescent protein in serum. This fraction which is designated fraction II gave an excitation maximum of 330 mμ and a fluorescence maximum of 470 mμ in accordance with the data for retinol reported by Duggan et al. [6]. Carr-Price and anhydrovitamin A tests were positive with this material.

When protein fraction II was studied by immunoelectrophoresis, a distinct precipitation arc corresponding to tryptophan-rich prealbumin was found. Fraction II was subjected to paper electrophoresis at pH 8.6 followed by inspection of the dried paper strips in the ultraviolet light. No significant fluorescence could be detected corresponding to the position for tryptophan-rich prealbumin whereas a distinct slower moving green fluorescent zone was found. By adding tracer amounts of [^{131}I]-L-thyroxine (0.004 μg thyroxine in 5 μl 50% (w/v) propylen glycol) to the protein fraction II (50 μl 5% (w/v) protein fraction II) prior to paper electrophoresis radioactivity was recovered in the tryptophan-rich prealbumin position whereas no significant amounts of radioactivity could be recorded in the fluorescent zone. In the control run thyroxine did not move towards the anode. The tryptophan-rich prealbumin zone obtained after paper electrophoresis was eluted from the paper strips and investigated by analytical disc electrophoresis. Only one protein band could be detected. This band occupied the same position as reference tryptophan-rich prealbumin. Hence the tryptophan-rich prealbumin binds thyroxine whereas the fluorescent protein fraction does not.

When the fluorescent zone obtained after paper electrophoresis was eluted and subsequently examined by analytical disc electrophoresis, three different protein bands were detected. The slowest moving band localized in the alpha$_1$ globulin position exhibited a distinct green fluorescence. In addition there was a weak band corresponding to tryptophan-rich prealbumin and an even fainter band corresponding to albumin. The fluorescent band was eluted from the polyacrylamide gel and subjected to repeated disc electrophoresis. Only one protein band was then obtained. The fluorescent protein and the tryptophan-rich prealbumin were purified once in disc electrophoresis. The purity of the fluorescent protein and the tryptophan-rich prealbumin obtained is seen from fig. 1. For comparison, the result of disc electrophoresis of serum is recorded in fig. 1. In this run the greenish fluorescent protein was only just visible. The



(A) (B) (C)

Fig. 1. Analytical disc electrophoresis (anode at the bottom). (A) Serum. (B) Purified retinol carrying protein. (C) Purified tryptophan-rich prealbumin.

tryptophan-rich prealbumin and the fluorescent protein were studied by immunodiffusion. Both proteins reacted with specific antiserum against tryptophan-rich prealbumin whereas no precipitation occurred when horse antiserum against human serum was used. Together with reference tryptophan-rich prealbumin these two proteins in immunodiffusion formed a precipitation of complete identity. The fluorescent protein had excitation and fluorescent maxima corresponding to those of retinol [6]. Retinol was extracted from the protein and studied by thin-layer chromatography. The R_f value of the spot obtained corresponded to that of reference retinol and gave a positive Carr-Price test. Ultracentrifugation of the tryptophan-rich prealbumin isolated in the present work gave a $S_{20,w}$ value of 4.6 as previously reported for this protein [3] whereas the corresponding value for the retinol-carrying protein was 1.85.

From these observations it is concluded that retinol in human plasma is not linked directly to tryptophan-rich prealbumin as previously suggested [2] but to a protein with lower molecular weight which seems to be immunologically related to tryptophan-rich prealbumin. This protein is localized in the $alpha_1$ globuline position in analytical disc electrophoresis.

When this work was completed, Kanai, Raz and Goodman [7] reported observations indicating that retinol in human plasma is transported linked to an $alpha_1$ globulin of lower molecular weight which forms an aggregate with tryptophan-rich prealbumin. No data concerning thyroxine-binding properties are reported by these authors. Other data presented, however, strongly indicate that this $alpha_1$ globulin may be identical to the retinol-binding protein isolated in the present work. One discrepancy remains to be elucidated. In contrast to our findings, Kanai et al. [7] found no immunological relationship between tryptophan-rich prealbumin and their retinol-binding $alpha_1$ globulin.

References

[1] H.E.Schultze, M.Schönenberger and G.Schwick, Biochem. Z. 328 (1956) 267.
[2] J.O.Alvsaker, F.B.Haugli and S.G.Laland, Biochem. J. 102 (1967) 362.
[3] J.H.Oppenheimer, M.I.Surks and M.Bernstein, J. Biol. Chem. 240 (1965) 173.
[4] B.I.Davis and L.Ornstein, Ann. N.Y. Acad. Sci. 121 (1964) 404.
[5] J.Kahan, Scand. J. Clin. Lab. Invest. 18 (1966) 679.
[6] D.E.Duggan, S.L.Udenfriend, R.Bowman and B.B.Brodie, Arch. Biochem. Biophys. 68 (1957) 1.
[7] M.Kanai, A.Raz and De Witt S. Goodman, J. Clin. Invest. 47 (1968) 2025.