A yellow component associated with human transthyretin has properties like a pterin derivative, 7,8-dihydropterin-6-carboxaldehyde

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Abstract Transthyretin (TTR) in plasma is associated with yellow compounds. Their properties differ, and in the chicken protein a major yellow compound has recently been identified as a carotenoid, lutein, also called xanthophyll. We now show that the major yellow component extracted from human TTR has properties like a pterin derivative, 7,8-dihydropterin-6-carboxyaldehyde (2-amino-4-hydroxy-6-formyl-7,8-dihydropteridine). The human TTR derivative has chromatographic and spectral properties identical to a yellow photochemical degradation product of biopterin and a spectrum like that of the pterin aldehyde.

Key words: Transthyretin; Prealbumin; Carrier protein; Pterin; Folate; Thyroxine; Human

1. Introduction

Transthyretin (TTR) purified from different species is yellowish in colour and associated with several compounds [1–4]. The yellow compound has been elusive to determine, but can be isolated by methanol extraction. The extracted material constitutes a substantial part of the original protein, 2–6% [3]. It also contains fatty acids in appreciable amounts [4]. Recently, the yellow component extracted from chicken TTR was identified as a carotenoid, lutein or xanthophyll [3]. It was then also noted that the major chicken compound was stable, in contrast to the unstable yellow component associated with human TTR. The absorbance spectra of the two yellow compounds were also quite different. We here provide evidence for a pterin nature of the major human yellow compound and suggest it to be 7,8-dihydropterin-6-carboxaldehyde (2-amino-4-hydroxy-6-formyl-7,8-dihydropteridine).

2. Materials and methods

2.1. Materials

Serum was used as the source of human TTR. CNBr-activated Sepharose 4B, Sephadex G-25 and Sephacryl S-200 were purchased from Pharmacia. Pterin, pterin-6-carboxylic acid and xanthopterin monohydrate were purchased from Aldrich, and biopterin, thyroxine (T4) and triodothyronine (T3) from Sigma. Solvents for the FPLC and HPLC systems were of HPLC grade from Merck.

2.2. Protein purification

Sepharose coupled with human retinol-binding protein was prepared as described and used for isolation of human TTR [5]. For exclusion of immunoglobulin contamination, the protein from this affinity chromatography step was submitted to molecular exclusion chromatography on Sephacryl S-200 in 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.4.

2.3. Methanol extraction

The protein was desalted on Sephadex G-25 fine and lyophilized. The purified TTR (30 mg) was boiled for 5–10 min in 15 ml acidic methanol. The suspended protein was centrifuged and the supernatant containing the yellow component collected. The extract was yellow and stored in methanol at −70°C.

2.4. HPLC and FPLC

The HPLC system utilized LKB 2248 pumps and a Pharmacia LKB controller. The methanol extract was fractionated on a semi-preparative reverse-phase C_{18} column (Nucleosil 5, 100 Å, 250 × 10 mm; Scand. Gene Tech), using a 50–100% methanol gradient. The eluent was monitored at 280 and 430 nm and analyzed with a Schimadzu C-R5-A integrator.

The material corresponding to the peaks obtained from the HPLC system was submitted to FPLC on a reverse-phase column Pep RPC 5/5 HR (Pharmacia) with monitoring at 214 and 280 nm, utilizing two monitors UV-M. A gradient of 0–100% acetonitrile in 0.1% TFA over 20 min at a flow rate of 0.5 ml/min was used.

A second HPLC system (Waters 625 LC), with a 991 Photodiode Array Detector (200–600 nm), allowing direct spectrophotometric analysis of material corresponding to individual chromatographic peaks, was used for analysis of the purified yellow compound.

3. Results

3.1. HPLC and FPLC of the methanol extract of TTR

The yellow material extracted from human TTR by acidic methanol was separated by reverse-phase chromatography. The first HPLC run (Fig. 1a) resulted in a number of fractions, the major fraction (A) being yellow. The preceding fraction, eluting after 18.9 ml, was rechromatographed in the same system, and separated into three fractions, A1–A3 (Fig. 1b), A3 having a retention volume corresponding to that of A in the previous chromatography. Fractions A1 and A3 both contained yellow component(s) as apparent by absorbance maxima at 430 nm. Material eluting later than A in the first HPLC separation was pooled from several preparations and rechromatographed in the same system (Fig. 1c), resulting in a number of fractions (A–F). The collected material, corresponding to the different peaks, was fractionated further in an FPLC system on reverse-phase Pep 5/5 (Fig. 2). The results show that fractions A1 and A3 both contain the same yellow compound (retention volume 19.7 ml), associated with hydrophobic material with retention volumes of 17.6 ml (fraction A1) and 15.4 ml (fraction A3). The latter material was identified as thyroxine. The yellow component was finally analyzed by the second HPLC system (Fig. 3).

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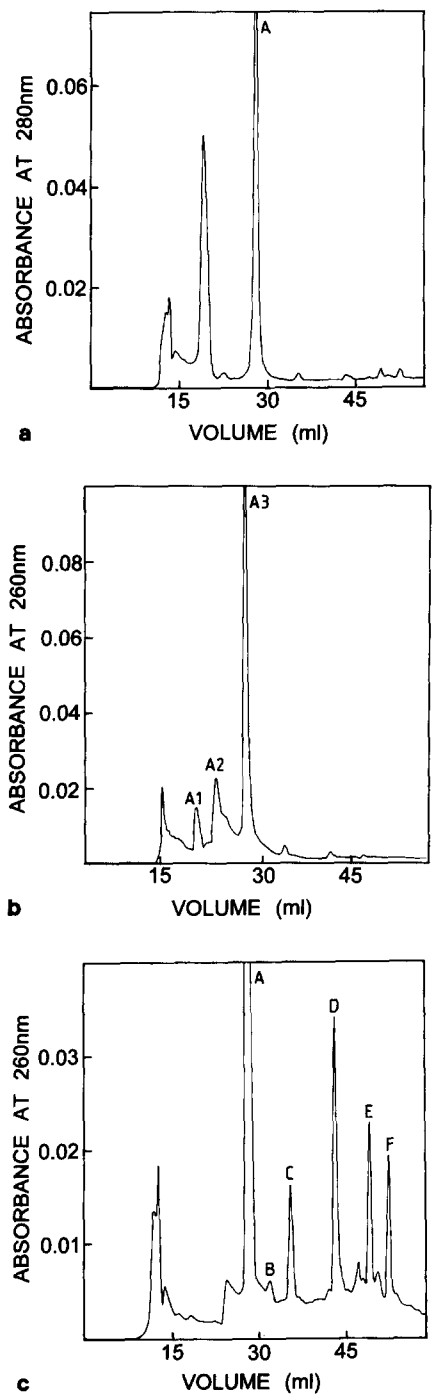

Fig. 1. Chromatographies of methanol extracts of human TTR. (a) HPLC on C_{18} (Nucleosil 5, 100 Å). Elution with a gradient of 80–100% methanol, 0–10 min, then 100% methanol, 3 ml/min. The yellow material is recovered from fraction A as judged from the absorption spectrum. (b) Material corresponding to the peak eluting at 18.9 ml in (a) was rechromatographed in the same system and a separation obtained (peak A1, A2 and A3). (c) Material eluting still later than fraction A in (a) was pooled from several chromatographies and rechromatographed with a separation into peaks A–F.

3.2. Identification of thyroxine

In fractions A2 and B, major components were found to be thyroxine and triiodothyronine, respectively (Fig. 2). The minor material associated with the yellow component in fraction A3 was also identified as thyroxine. The identification was based on the characteristic spectra of the thyroid hormones at acidic and alkaline pH and on the chromatographic properties of commercial preparations of thyroxine and triiodothyronine being identical to those of the TTR-derived compounds.

Fig. 2. FPLC on Pep 5/5 HR of different fractions (A1, A2, A3, B, C, D) from the HPLC system in Fig. 1. Isocratic elution with 6 ml 0.1% TFA in water, followed by a 0–100% acetonitrile gradient in 0.1% TFA, 6 to 26 ml. (A1) Major fraction with retention volume 19.7 ml (indicated by Y), identified as the yellow compound by its absorbance maximum above 400 nm. The minor fraction at 17.6 ml is not identified, λ_{max} 254, 281 nm at pH 1; 262, 285, 328 nm at pH 9. (A2) Major fraction identified as thyroxine (T4). (A3) Major fraction at 19.7 ml (Y) is the yellow compound, the minor fraction at 15.4 ml is thyroxine (T4). (B) Major peak at 20.8 ml, not identified. The minor fraction at 14.7 ml is identified as triiodothyronine (T3). (C) Major peak at 21.8 ml, not identified. (D) Major peak at 24.0 ml, not identified.

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3.3. Properties of the yellow compound

The crude methanol extract and the purified yellow compound were run analytically in a second HPLC system for analysis of the spectrum of the material corresponding to individual chromatographic peaks (Fig. 3). The yellow compound had a spectrum with absorbance maxima at 225, 271 and 436 nm (100% acetonitrile, 0.1% TFA, pH 1; Fig. 3, bottom). In the FPLC system with Pep 5/5 (Fig. 2), the yellow compound was eluted with 69% acetonitrile. To elute the compound from the HPLC reverse-phase C_{18} column, 100% acetonitrile was required (gradient 50–100% acetonitrile, 0.1% TFA; Fig. 3), indicating that the yellow compound has a highly hydrophobic character.

The purified yellow compound was sensitive to air and was not regained after lyophilization, but could be stored in 100% methanol at −70°C for more than 1 year. It was also possible to concentrate the yellow compound by evaporation of solvent with a blast of nitrogen.

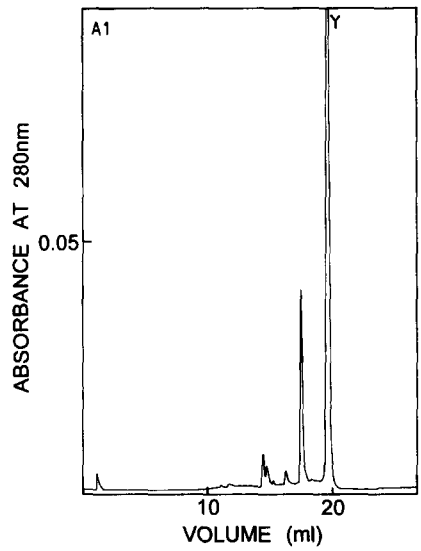
3.4. A pterin nature of the yellow component

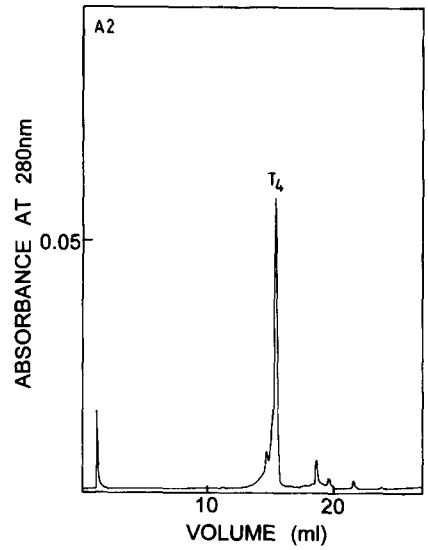
The absorbance spectrum of the yellow compound corresponds to published data for the spectrum of an unstable hydrophobic dihydropterin, 7,8-dihydropterin-6-carboxaldehyde. The material in different chromatographic fractions derived from our methanol extract was therefore lyophilized, resolved and oxidized with hydroperoxide. The resulting material was again lyophilized, resolved and rechromatographed in the FPLC system and tested for the presence of xanthopterin and pterin-6-carboxylic acid. In contrast to the very unstable 7,8-dihydro-pterin-6-carboxaldehyde, these compounds are stable and commercially available for comparison. The covalently hydrated form of xanthopterin was indeed chromatographically detected after oxidation of some of the non-yellow material, indicating the possible presence of pterins other than the yellow component.

3.5. Yellow degradation product of biopterin and dihydrofolate

The yellow 7,8-dihydropterin-6-carboxaldehyde is known to be formed by photochemical degradation of biopterin and neopterin under anaerobic conditions and by oxidative degradation of dihydrofolate [6,7].

To obtain further evidence for the character of the TTR-


A1
Y
ABSORBANCE AT 280nm
0.05
10
20
VOLUME (ml)


A2
T_4
ABSORBANCE AT 280nm
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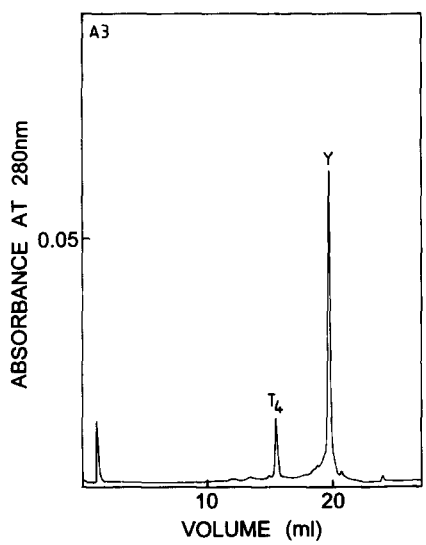
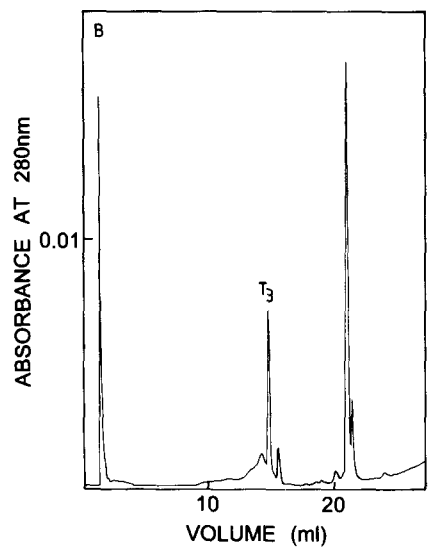
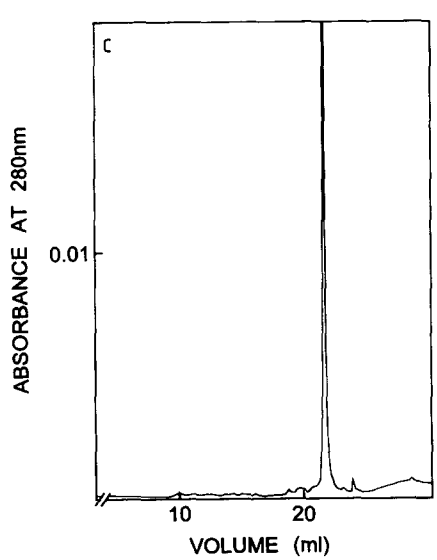
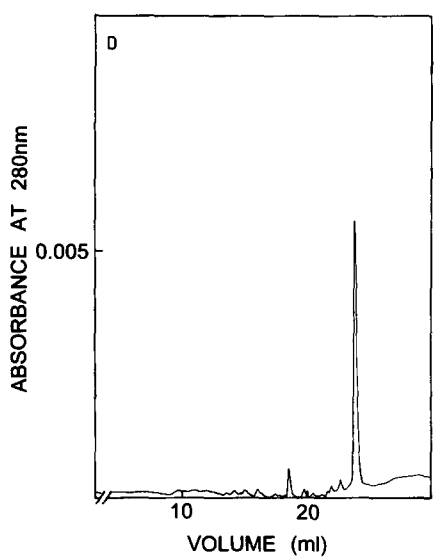
10
20
VOLUME (ml)


A3
Y
T_4
ABSORBANCE AT 280nm
0.05
10
20
VOLUME (ml)


B
T_3
ABSORBANCE AT 280nm
0.01
10
20
VOLUME (ml)


C
ABSORBANCE AT 280nm
0.01
10
20
VOLUME (ml)


D
ABSORBANCE AT 280nm
0.005
10
20
VOLUME (ml)

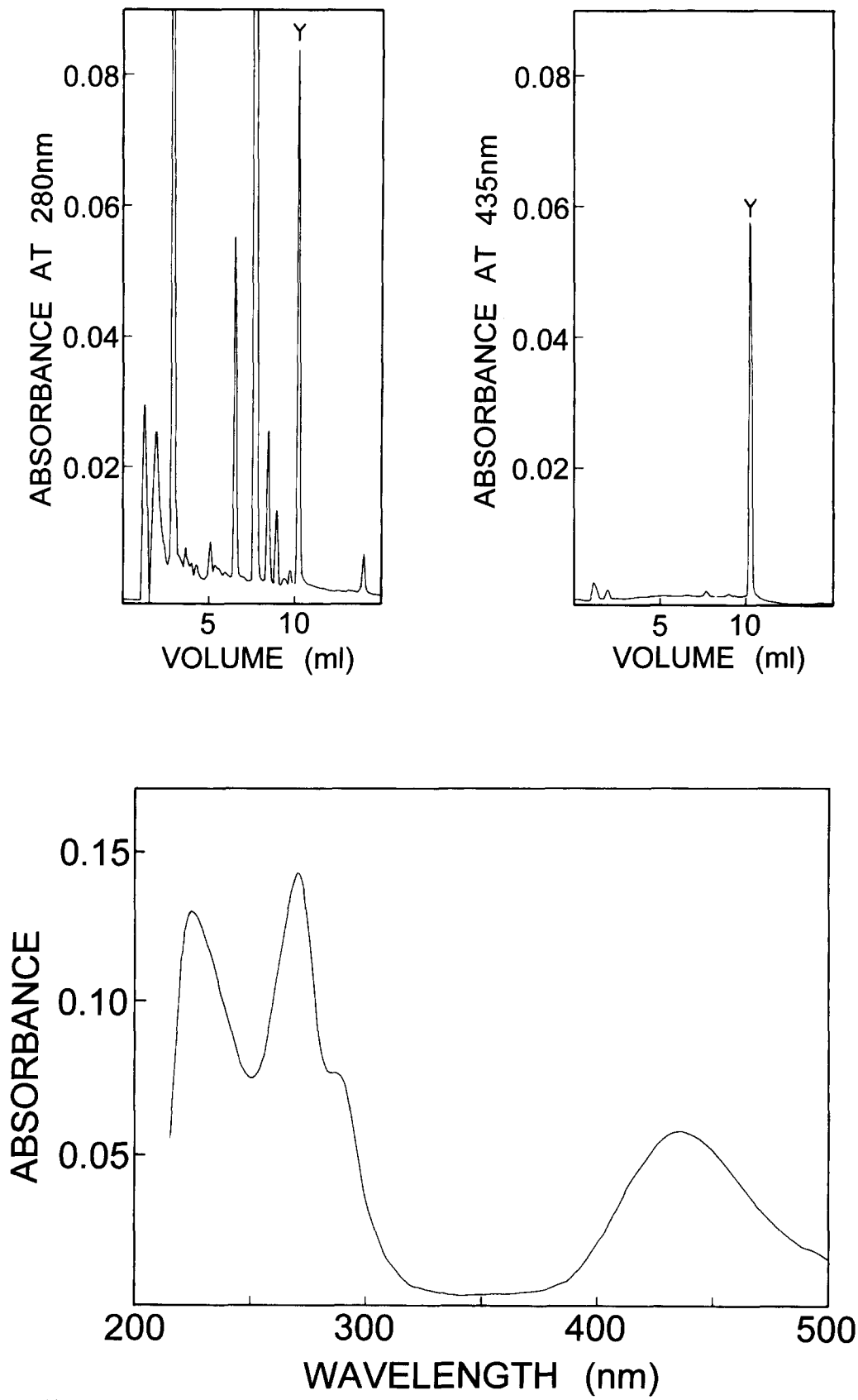


Fig. 3. Chromatographic analysis of the yellow methanol extract obtained from human TTR. Analysis by a Waters 625 Inert Gradient System with a 994 Photodiode Array Detector. Gradient 50–100% acetonitrile in 0.1% TFA for 0–10 min, flow rate 1 ml/min. The only peak (Y) with absorbance maximum above 400 nm eluted after 10.2 ml. Chromatograms monitored at 280 and 435 nm (top) and absorbance spectrum of the yellow compound (bottom) are shown.

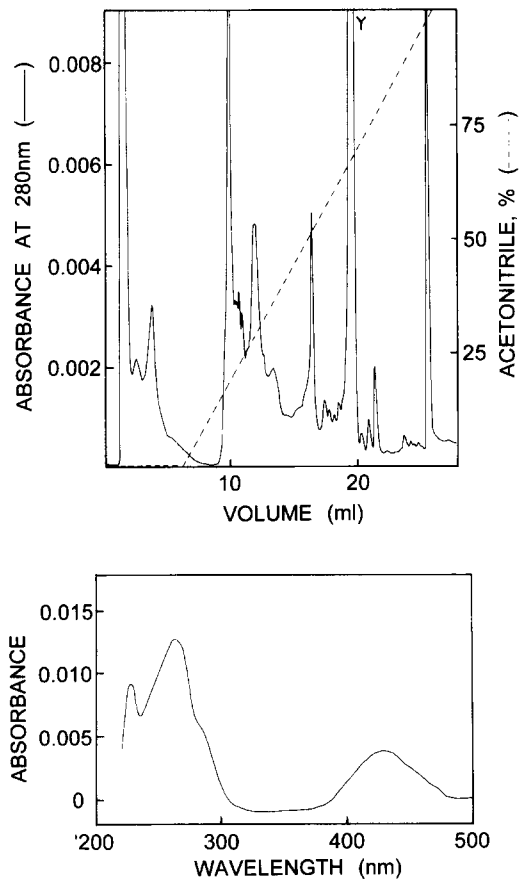


Fig. 4. FPLC of material resulting from exposure of biopterin in acidic methanol to light for 2 weeks under anaerobic conditions. Column: Pep 5/5 HR as in Fig. 2. In the chromatogram the first large peak represents intact biopterin, the peak at 16.4 ml probably xanthopterin in its covalent hydrated form, and the peak at 19.6 ml a yellow compound (Y), probably 7,8-dihydropterin-6-carboxaldehyde, known to be formed by photodegradation of biopterin. The yellow fraction was collected, immediately bubbled with nitrogen, and analyzed with a spectrophotometer for a rough spectrum (bottom).

associated yellow compound, commercial biopterin was dissolved in methanol acidified with hydrochloric acid, bubbled with nitrogen, closed, and left in a glass tube submitted to sunshine. This procedure repeatedly resulted in brown-red products, some of which precipitated in the tubes. The spectra of these products indicate that they may correspond to 5,8-dihydropterin-6-carboxaldehyde and 5,8-dihydropterin-7-carboxaldehyde [8,9], which on exposure to oxygen are spontaneously dehydrogenated to the corresponding pterin-aldehydes [10], further oxidation yielding pterin-carboxylic acids [9]. However, one of our photodegradation attempts, with bright sunshine for 2 weeks, resulted in a clear yellow colour of the originally uncoloured biopterin solution. This photodegraded biopterin was chromatographed and the yellow pterin identified in the FPLC and HPLC systems. The chromatographic behaviour and the spectrogram was identical to those of the yellow compound associated with human TTR (cf. chromatograms in Figs. 2C and 4 and spectra in Figs. 3 and 4). Of the different yellow pterin compounds, the spectrum is in best agreement with that of 7,8-dihydropterin-6-carboxaldehyde [11,12].

4. Discussion

4.1. Origin of the major yellow compound

Our results confirm that hydrophobic compounds extractable with methanol are associated with human TTR. In contrast to the situation with chicken TTR, no component with a spectrum with three strong absorbance maxima in the 400–550 nm region, chacteristic of most carotenoids, has been found. Also, in contrast to the yellow fraction associated with chicken TTR, the one extracted from human TTR is highly unstable in its chromatographically pure form and is rapidly degraded to uncoloured compounds when exposed to air. Comparison with the yellow product from photochemical degradation of commercial biopterin shows that the yellow compound associated with human TTR is a pterin, probably 7,8-dihydropterin-6-carboxaldehyde. This unstable aldehyde is apparently derived from a more stable pterin during the preparation of TTR from human serum. Native TTR is only slightly yellow and a strong colour does not appear until boiling in acidic methanol. It may also be noted that amyloid TTR is distinctly yellow and that the yellow compound in that case is extractable with neutral methanol without boiling, indicating that it is preformed and not the result of the preparative procedure. Spectra of material in individual chromatographic peaks were now obtained with a diode array detector on line, which is a great advantage with unstable compounds. A tentative conclusion is that human plasma TTR is associated with either a reduced pterin or a folate, which during preparation of TTR is degraded, resulting in the formation of the yellow compound 7,8-dihydropterin-6-carboxaldehyde.

4.2. Possible functional implications

Tetrahydrobiopterin has been suggested to be a cofactor in the deiodination of thyroxine, though shown not to be required as a cofactor to the cellular 5′-deiodinase [13]. It is more probable that reduced pterins may have a protective role against superoxide radicals, thus a role similar to that for the carotenoids [14].

4.3. Transthyretin and the choroid plexus

TTR is a major protein synthesized and secreted by the choroid plexus in reptiles, birds and mammals [15]. This TTR is relevant for the transport of thyroxine into the cerebrospinal fluid [16]. TTR might also be important as a carrier of folates into the brain after absorption of the folates through the action of the glycosylphosphatidylinositol-anchored folate receptor on the epithelial cells of the choroid plexus [17].

4.4. Transthyretin and hydrophobic compounds

Recent research has shown that different hydrophobic compounds, such as the yellow pterin derivative (this work), the carotenoid lutein [3], and also fatty acids [4], may be associated with TTR from different species. This suggests that TTR may have a hydrophobic cleft into which these compounds bind. However, some specificity must exist as chicken and human TTRs appear to be associated with different compounds, and in the chicken protein apparently with lutein only, but not with other carotenoids.

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References

[1] Pettersson, T., Carlström, A. and Jörnvall, H. (1987) Biochemistry 26, 4572–4583.
[2] Pettersson, T., Carlström, A., Ehrenberg, A. and Jörnvall, H. (1989) Biochem. Biophys. Res. Commun. 158, 341–347.
[3] Pettersson, T.M., Ernström, U., Sjövall, J., Griffith, B., Bergman, T. and Jörnvall, H. (1995) Eur. J. Biochem. (submitted).
[4] Hermansen, L.F., Bergman, T., Jörnvall, H., Husby, G., Ranløv, I. and Sletten, K. (1995) Eur. J. Biochem. 227, 772–779.
[5] Fex, G., Laurell, C.-B. and Thulin, E. (1977) Eur. J. Biochem. 75, 181–186.
[6] Hillcoat, B.L., Nixon, P.F. and Blakley, R.L. (1967) Anal. Biochem. 21, 178–189.
[7] Maharay, G., Selinsky, B.S., Appleman, J.R., Perlman, M., London, R.E. and Blakley, R.L. (1990) Biochemistry 29, 4554–4560.
[8] Mengel, R., Pfleiderer, W. and Knappe, W.R. (1977) Tetrahedron Lett. 32, 2817–2820.
[9] Pfleiderer, W. (1987) Biochemical and Clinical Aspects of Pteridines, vol. 5, pp. 3–21, de Gruyter, Berlin.
[10] Baur, R., Kappel, M., Mengel, R. and Pfleiderer, W. (1979) in: Chemistry and Biology of Pteridines. (Kisliuk, R.L. and Brown, G.M. eds.) pp. 13–17, Elsevier, Amsterdam.
[11] Whitely, J.M., Drais, J., Kirchner, J. and Huennekens, F.M. (1968) Arch. Biochem. Biophys. 126, 955–957.
[12] Chippel, D. and Scrimgeour, K.G. (1970) Can. J. Biochem. 48, 999–1009.
[13] Rokos, H. and Scheiffele, E. (1979) in: Chemistry and Biology of Pteridines (Kisliuk, R.L. and Brown, G.M. eds.) pp. 201–206, Elsevier, Amsterdam.
[14] Rousseau, E.J., Davidsson, A.J. and Dunn, B. (1992) Free Rad. Biol. Med. 13, 407–433.
[15] Harms, P.J., Tu, G.F., Richardson, S.J., Aldred, A.R., Jaworowski, A. and Schreiber, G. (1991) Comp. Biochem. Physiol. B 99, 239–249.
[16] Chanoine, J.P., Alex, S., Fang, S.L., Stone, S., Leonard, J.L., Körhle, J. and Braverman, L.E. (1992) Endocrinology 130, 933–938.
[17] Weitman, S.D., Lark, R.H., Coney, L.R., Fort, D.W., Frasca, V., Zurawski Jr, V.R. and Kamen, B.A. (1992) Cancer Res. 52, 3396–3401.