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Pteridine-binding α_1-Acid Glycoprotein from Blood of Patients with Neoplastic Diseases[1]

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ABSTRACT

A glycoprotein was selectively enriched in the supernatant (Fraction b) obtained by alcohol and trichloroacetic acid fractionation of digitonin extracts from blood of patients with neoplastic diseases and of control subjects. Subsequent chromatography with concanavalin A:Sepharose separated a concanavalin A-reactive fraction from a concanavalin A-nonreactive one. In sodium dodecyl sulfate gel electrophoresis, the fractions from both malignant origin as well as control subjects appeared as single bands showing the same mobility. They were identical with the band obtained from commercial α_1-acid glycoprotein.

In Fraction b of malignant origin, greatly increased amounts of the α_1-acid glycoprotein from malignant cases (AGP_M) were found as compared to α_1-acid glycoprotein from controls (AGP_C). Furthermore, AGP_C had a higher glycine content than did AGP_M. The electrofocusing pattern of AGP_M showed additional bands between pH 3.7 and 4.4, whereas AGP_C and commercial α_1-acid glycoprotein focused between pH 3.2 and 3.8. In contrast to AGP_C and to a commercial α_1-acid glycoprotein, AGP_M is characterized by a chromophoric group with maximal absorbance at 400 nm. It could be detached by treatment with 6 M guanidine hydrochloride, thus indicating a noncovalent binding. The spectral data of the separated chromophore at pH 0.5 agreed with that of a 6,7-substituted pteridine. After detachment with reducing agents, a pteridine in its 7,8-dihydro form was indicated by spectral analysis.

INTRODUCTION

By injection of deuterated phenylalanine and tyrosine, a decrease in the rate of phenylalanine hydroxylation and of tyrosine catabolism was found *in vivo*, parallel with the growth of Yoshida ascites hepatomas and with Jensen sarcoma in rats (9, 35). These results had prompted investigations into blood levels of amino acids in both free and protein-bound form. In a supernatant fraction (Fraction b), obtained after digitonin:H_2O extraction and subsequent alcohol and trichloroacetic acid precipitation, levels of total tyrosine 3 times greater than normal were found in rats with experimental tumors and also in clinical cases (35). This was due presumably to protein-bound tyrosine which is detected by the color reaction with 1-nitroso-2-naphthol as is the free form (1, 9, 10).

Further investigations were resumed for mainly 2 reasons. On the one hand, the nature of the causative factor, presumably a protein, had remained unknown; on the other hand, only a restricted number of cases had been investigated, and controls had only covered healthy subjects. Especially, a discrimination against other soluble tumor markers (*e.g.*, carcinoembryonic antigen; *cf.* Ref. 14) and acid-soluble acute-phase reactants (*cf.* Ref. 13) was challenging. This was attempted via 2 different approaches: (*a*) by the isolation and biochemical characterization of the causative factor, described below; and (*b*) by an extended screening of clinical cases, which will be reported in a following paper (8).

During the previous investigation (35), in correlation with the increased amounts of protein in Fraction b, tetrahydrobiopterin had also been found in increased amounts during cancers. As shown recently by means of the *Crithidia* assay, high biopterin concentrations are characteristic of immature blood cells (36) and especially of blasts, isolated from peripheral blood during leukemias (34). Increased urinary levels of neopterin and biopterin during cancers have frequently, but not consistently, been found (11, 22, 30). The transitory increases in blood levels during some cases of solid tumors and the correlation between biopterin and AGP_M[3] levels (34) during leukemic cases prompted us to pay special attention to a possible link between the protein under investigation and the metabolism of pteridines, supposed previously to be an indicator of cell proliferation (11, 22, 30, 34, 36).

MATERIALS AND METHODS

Chemicals. SDS, guanidine hydrochloride, digitonin, acrylamide *N,N'*-methylene bisacrylamide, Coomassie Brillant Blue 250, and bovine serum albumin were obtained from Serva (Heidelberg, Germany); Sephadex G-25 and Con A:Sepharose were from Deutsche Pharmacia (Freiburg, Germany); and Ampholine was from LKB (Bromma, Sweden). The calibration proteins for SDS:gel electrophoresis (Combithek) were obtained from Boehringer (Mannheim, Germany), and the Bio-Rad protein assay was from Bioanalytical Systems, Inc. (West Lafayette, Ind.). Human AGP (purified from Cohn Fraction VI) and α-methyl-D-mannoside were obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals of analytical grade were obtained from Merck (Darmstadt, Germany).

Blood Sampling, Extraction, and Preparation of Fraction b. Venous blood was drawn between 8 and 9 a.m. and anticoagulated with 3.8% (w/v) sodium citrate. The samples (2 to 5 ml) were extracted with 2.4 volumes of 0.1% (w/v) digitonin:H_2O at 4° for 10 min, and the bulk of the protein was subsequently precipitated by stirring with 3 volumes of methanol for 20 min. After centrifugation at 4000 × *g* (20 min), the alcohol was removed using a rotary evaporator, and the samples were lyophilized. The residues were redissolved in 0.06 M phosphate buffer, pH 7.2, to the volume of the original blood sample

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[3] The abbreviations used are: AGP_M, α_1-acid glycoprotein from malignant cases, SDS, sodium dodecyl sulfate; Con A, concanavalin A; AGP, α_1-acid glycoprotein; AGP_C, α_1-acid glycoprotein from control subjects; P-AGP_M, pteridine-binding α_1-acid glycoprotein from malignant cases.

and centrifuged for 10 min at 1300 × *g*. An equal volume of 12% trichloroacetic acid was added to the supernatant. After centrifugation at 4000 × *g* (10 min), the supernatant, designated as Fraction b, was used for further isolation and also for the clinical screening (8).

Isolation of AGP_M and AGP_C. For the isolation procedure, 5 to 10 samples of Fraction b were pooled to yield 15 to 30 ml, which were processed in separate procedures. From samples of malignant origin, only those were accepted for purification showing AGP levels >200 μg/ml by previous colorimetric assay (see Ref. 8). After dialysis against 10 mM Tris-HCl buffer, pH 7.4, containing 0.5 M NaCl (4 changes) and subsequent reduction of the volume by lyophilization, the batch was desalted on a Sephadex G-25 column (1.6 x 30 cm). The lyophilized fraction, containing the bulk of protein, was dissolved in 4 ml starting buffer (20 mM Tris-HCl buffer (pH 7.4):0.5 M NaCl) and applied to a column of Con A:Sepharose (1.6 × 30 cm). After extensive washing with the starting buffer, desorption of the bound proteins was achieved with the starting buffer, containing 0.5 M α-methyl-D-mannoside. The volume of each fraction was 1.8 ml. Desalting was performed using a Sephadex G-25 column (1.6 x 30 cm) with 0.1 M ammonium acetate, pH 5.5, as elution buffer from which the product was freed by repeated lyophilization. The eluant profiles were followed by monitoring the extinction at 280 nm.

Protein Determination. Due to the high tyrosine content of the glycoprotein, determinations according to the method of Lowry *et al.* indicated a 50% overestimation. Therefore, the Coomassie dye-binding method (4), using the Bio-Rad assay, was applied with bovine serum albumin as standard. The results obtained by this method closely agreed with those calculated from the amino acid analysis of the 24-hr hydrolysates.

Amino Acid Analysis. Samples of Fraction b (200 μl) containing 50 to 100 μg of protein were hydrolyzed with 6.0 N HCl for 24 hr at 105°, dried by flushing with air, redissolved in 200 μl H_2O, and applied in 50- to 100-μl portions to the amino acid analyzer. For analysis of isolated glycoproteins, portions of 50 μl of the desalted samples were dried, redissolved in 0.5 ml of 5.7 N HCl, sealed under vacuum, and subjected to hydrolysis at 105° for 24, 48, 72, and 96 hr, respectively. The hydrolysates were analyzed with a Biotronik LC 6000 amino acid analyzer according to the method of Spackman *et al.* (28). Half-cystine was determined as cysteic acid after oxidation with performic acid and subsequent hydrolysis for 24 hr (16).

Carbohydrate Analysis. Amino sugars were determined after hydrolysis with 4 N HCl at 100° for 3 hr with the amino acid analyzer (29). Neutral carbohydrates were estimated with the phenol:sulfuric acid method (7) using glucose as standard.

Polyacrylamide Gel Electrophoresis and Determination of Molecular Weight. The preparation of cylindrical gels (10 x 0.5 cm), stacking gels, fixation, and staining with Coomassie Brillant Blue R 250 as detection of glycoprotein by the periodic acid-Schiff reagent are described elsewhere (15). Using a 75 mM Tris:glycine buffer, pH 8.3, for electrophoresis, a current of 7 mA was applied for each tube. The molecular weight determinations by SDS:polyacrylamide gel electrophoresis were performed by the method optimized for glycoproteins (26) using 7.5, 10, 12.5, and 15% gels. The samples were prepared by incubations with 1 and 2% SDS in the presence of 20 mM dithiothreitol or 0.5 M mercaptoethanol for 1 hr at 37°, 20 min at 60°, and 10 min at 95°, respectively, with identical results. The molecular weights of the marker proteins were: trypsin inhibitor from soybean, 21,500; RNA polymerase α chain, 39,000; bovine serum albumin, 68,000; RNA polymerase β chain, 155,000; and RNA polymerase β′ chain, 165,000. Gels, stained with Coomassie Brillant Blue R 250, were scanned at 540 nm using a Kontron Uvikon 820 spectrophotometer equipped with a linear transport mechanism.

Electrofocusing. Isoelectric focusing was performed on a gradient ranging from pH 2.5 to 4.5, using LKB Ampholine 1818-106 with 1 M H_3PO_4, pH 1.0, as the anode buffer and 0.4 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 5.5, as the cathode buffer. The polyacrylamide gels (12.5 x 0.2 cm) were 9.2% (w/w) with respect to acrylamide and had been polymerized by ammonium persulfate according to LKB Application Note 250. The runs were carried out in an LKB 2117-301 Multiphor for 3 hr at 25 watts of constant power. The pH gradient was determined by elution of 1.0-cm gel sections cut along the migration axis. Coomassie Brillant Blue R 250 staining was performed according to the procedures described for glycoproteins (26).

Spectral Data. Absorption spectra were obtained using a Kontron Uvikon 820 spectrophotometer and monitored by a Uvicord 21.

Analysis of the Pteridine Group. The reduction of P-AGP_M was achieved by addition of solid sodium dithionite or sodium borohydride up to a final concentration of about 0.5 M, followed by anaerobic incubation for 30 to 60 min at room temperature. For detachment under reductive conditions by 1% SDS in the presence of 20 mM dithiothreitol and under nonreductive conditions by 6 M guanidine-HCl, respectively, a concentrated solution of the denaturating reagents was added to give the final concentration indicated. The separation of the AGP moiety from the detached low-molecular-weight pteridine was achieved by a Sephadex G-25 column (1.0 x 10 cm), equilibrated with the respective incubation solution. Only after SDS treatment, elution was performed with H_2O. For determination of biopterin activity, the protozoological assay with *C. fasciculata* was used, as outlined by Baker *et al.* (2). For pteridine release, 100-μl samples (83 μg glycoprotein) of Con A-reactive P-AGP_M were autoclaved for 5 min with 1 ml 50 mM aconitate buffer, pH 4.5, or incubated with 20 μl 0.1 N HCl (20 min at 100°) or with 20 μl 0.1 N NaOH (20 min at 100°), respectively, and subsequently neutralized.

RESULTS

Isolation of AGP_M and AGP_C. Fraction b, as described in "Materials and Methods," was used as the starting material for the further purification of AGP_M and AGP_C. Polyacrylamide gel electrophoresis of the neutralized and desalted samples revealed that, in Fraction b, a rather homogeneous protein had been selectively enriched (Chart 1). Two bands, Band I and Band II, comprised more than 90 to 95% of the total protein in Fraction b. Both bands could be stained by the periodic acid-Schiff reagent, indicating a glycoprotein. Consequently, affinity chromatography on Con A:Sepharose was used for further purification. The pooled samples of Fraction b from malignant sources were separated into a Con A-nonreactive and a Con A-reactive component (Chart 2). The very minor Con A-nonreactive Fractions 9 to 11 were discarded. From 1 ml of blood of malignant origin, 70 to 110 μg of Con A-nonreactive AGP_M and 200 to 300 μg of Con A-reactive AGP_M were obtained. Performing the purification procedure with blood from control subjects, both Con A-reactive and Con A-nonreactive AGP_C appeared at the same elution volumes as did the proteins from malignant origin. The yields, however, amounted to only about 10% of that of AGP_M.

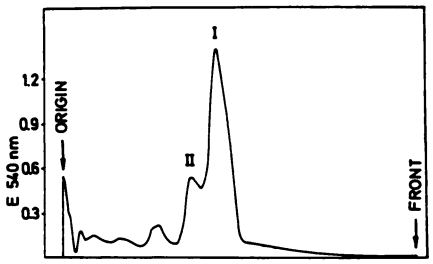

Chart 1. Densitometric scan after disc gel electrophoresis of Fraction b. Prior to electrophoresis, the sample had been neutralized and desalted. *E*, optical density at 540 nm.

Homogeneity and Molecular Weight. After SDS gel electrophoresis with 7.5, 10, 12.5, and 15% polyacrylamide gels, respectively, both Con A-nonreactive and Con A-reactive AGP_M appeared as single bands (Fig. 1*A*). Their migration was identical with that of Con A-reactive AGP_C and of commercial, crystalline AGP (Fig. 1*B*). The relative migration with respect to the marker proteins was independent of the gel concentration, and the molecular weight was found to be 42,000 to 44,000.

Molecular Composition and Amino Acid Substitution. The Con A-reactive AGP_M agreed in all amino acid residues with the abnormal orosomucoid (24), with AGP described elsewhere (*cf.* Ref. 25), and with commercial crystalline AGP (Table 1). However, the Con A-reactive AGP_C showed a significantly increased glycine content if compared with Con A-reactive AGP_M and with the data known from the literature. Control analysis of the unhydrolyzed proteins confirmed the total absence of free glycine, which could contribute to the apparent differences between AGP_C and AGP_M. As shown by sequence analysis of AGP (25), amino acid substitution may occur at 21 different positions. Among these, only one glycine residue was found to be exchanged in normal AGP of different genetic origin. The marked difference in the glycine content between Con A-reactive AGP_M and AGP_C suggests a selective enrichment of an additional AGP variant in Fraction b, characterized by a higher degree of glycine substitution. The carbohydrate analysis (Table 2) indicated reduced amounts of neutral and, in particular, of amino sugars in the Con A-reactive AGP_M, as compared to AGP reported in the literature (5, 25). The striking difference between AGP_M and AGP_C with respect to pI heterogeneity (see below) needs a more detailed carbohydrate analysis, especially of the sialic acid content, which is presumably responsible for the marked pI polymorphism.

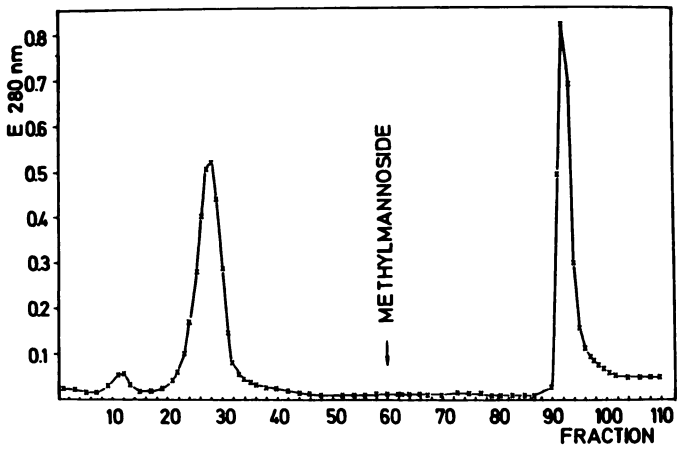

Chart 2. Separation of P-AGP_M on Con A:Sepharose. Fractions 22 to 32, Con A-nonreactive P-AGP_M; Fractions 90 to 96, Con A-reactive P-AGP_M. Elution buffer, 20 mM Tris-HCl (pH 7.4):0.5 M NaCl:0.5 M α-methyl-D-mannoside. *E*, optical density at 280 nm.

Amino Acid Analysis of Fraction b. Purified AGP_M showed a relatively low content in glycine and a relatively high content in tyrosine and phenylalanine if compared with the amino acid composition of Fraction b of control subjects. AGP_C is present there only in low concentrations, and AGP_M is absent (8). Thus, background protein and, especially, the free amino acids of the plasma and the corpusculate fractions are dominating, which

Table 1

Amino acid composition of Con A-reactive and Con A-nonreactive fractions of AGP_C and AGP_M

Comparison with commercial AGP and with the values given in the literature is made.

	Residues/100 amino acids (av.)[a]					
	Con A-reactive AGP			AGP		
Amino acid	Cancer[c]	Control[c]	Con A-nonreactive AGP_M[b, c]	Commercial[c]	Schmid (25)	Abnormal orosomucoid [Rudman *et al.* (24)]
Aspartic acid	11.7	11.1	10.5	11.3	11.6	11.5
Threonine	8.6	8.0	8.0	8.8	8.1	8.6
Serine	4.2	5.1	4.4	4.2	4.1	4.8
Glutamic acid	16.2	16.7	17.1	16.3	16.6	16.7
Proline	4.2	4.3	4.1	4.1	3.9	4.7
Glycine	4.3	7.8	7.2	4.4	4.1	3.9
Alanine	5.0	6.5	5.0	5.2	5.5	4.8
½ Cystine	2.5[d]	ND[e]	4.7[d]	2.2[d]	2.2	2.3
Valine	5.1	5.4	4.8	5.1	4.9	4.6
Methionine	0.6	0.6	0.6	0.6	0.7	0.7
Isoleucine	5.4	4.8	4.8	4.9	5.1	5.2
Leucine	8.1	7.7	7.1	8.0	8.1	7.8
Tyrosine	5.4	4.5	4.7	5.6	6.0	5.4
Phenylalanine	4.9	4.5	4.4	5.2	4.9	5.3
Lysine	7.6	7.1	7.1	7.7	7.2	7.5
Histidine	1.8	1.7	1.6	1.7	1.7	1.6
Arginine	4.5	4.5	4.0	4.7	5.3	4.4
Tryptophan	ND	ND	ND	ND	(1.7)[f]	(1.9)

[a] The molar percentages were calculated from the sum of residues excluding tryptophan, at AGP_C excluding ½ cystine and tryptophan.

[b] Due to the minimal amounts obtained from Fraction b, the amino acid composition of Con A-nonreactive AGP_C could not be established.

[c] Amino acid analyses were carried out with 2 different preparations, respectively. The proteins were subjected to the following hydrolysis times: 24, 48, 72, and 96 hr for the Con A-reactive and Con A-nonreactive AGP_M; 24 and 72 hr for the Con A-reactive AGP_C; 24, 48, and 96 hr for commercial AGP.

[d] Determined as cysteic acid.

[e] ND, not determined.

[f] Numbers in parentheses, extrapolated values.

are characterized by double the amount of glycine as compared to phenylalanine and tyrosine (see Ref. 6). Consequently, increased amounts of AGP_M found in Fraction b of malignant origin (see Ref. 8) may be expected to change drastically the percentage of distribution of these amino acids in this fraction. As demonstrated in Chart 3, the percentage of increase of total phenylalanine and tyrosine, which is paralleled by an increase of AGP_M, gives a good correlation with the percentage of decrease of total glycine in Fraction b. This negative correlation illustrates the extent to which AGP_M exceeds background protein and possibly, to some degree, the replacement of AGP_C and AGP_M in Fraction b of malignant origin.

Chromophoric Group. The absorption spectra of both Con A-reactive and nonreactive P-AGP_M revealed the presence of a chromophoric group, which could be subjected to reversible reduction (Chart 4). In eight preparations derived from samples of malignant origin, the ratio of 280:400 nm absorbance amounted to 3.9 to 4.2. It is apparent that the chromophoric group essentially contributes to the absorbance in the 270 to 280 nm region. This would explain the 2- to 3-fold overesti-

Table 2

Carbohydrate content of the Con A-reactive fraction of AGP_M

Comparison with the values given in the literature is made.

	% (w/w) referred to the protein content		
AGP	Sialic acid	Hexosamine	Neutral sugars
Con A-reactive AGP_M	ND[a]	17.8 ± 1.0	20.4 ± 1.0[b]
AGP, as calculated from Charlwood *et al.* (5)	20.0	25.7	29.4
AGP, as calculated from Schmid (25)	19.3	25.8	23.8

[a] ND, not determined.
[b] Mean ± S.D.

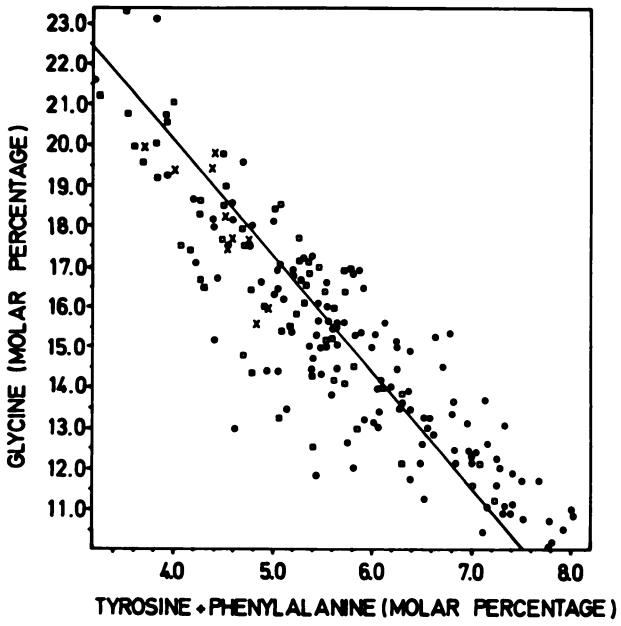

Chart 3. Correlation between molar percentages of free and bound glycine and those of free and bound tyrosine + phenylalanine in individual samples of Fraction b, as obtained during the screening of clinical cases (see Ref. 8), with a correlation index of −0.85 and $p = <0.05$. ×, control subjects; □, nonmalignant cases; ○, malignant cases without cytostatic treatment; ●, malignant cases with cytostatic treatment.

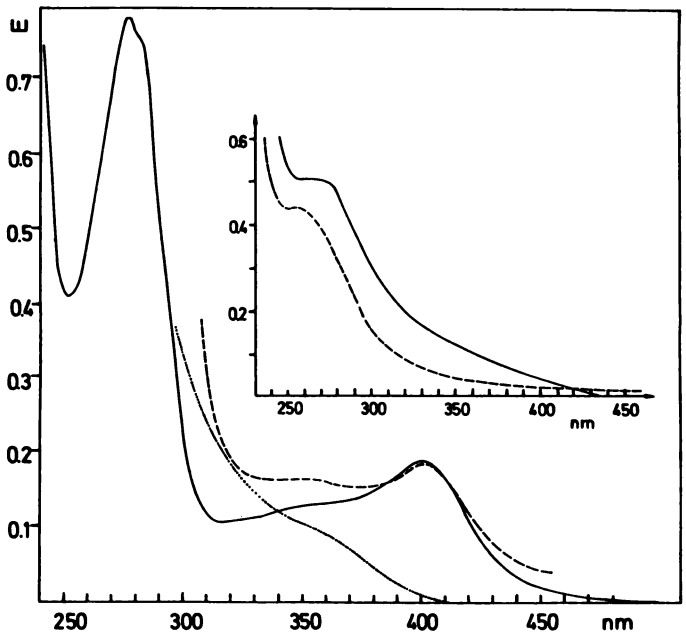

Chart 4. Absorption spectrum of Con A-reactive P-AGP_M and P-AGP_C, and the effect of reductive treatment. ——, Con A-reactive P-AGP_M (380 μg/ml):100 mM ammonium acetate (pH 6.7); – – – –, 1 min after addition of sodium dithionite (0.5 mM); ····, 4 min after addition of sodium dithionite (0.5 mM). *Inset*, ——, Con A-reactive AGP_C in 100 mM ammonium acetate, pH 6.7 (qualitatively); – – – –, Con A-reactive AGP_M after reduction of P-AGP_M with dithionite and subsequent separation by molecular sieving in the presence of 0.02 M dithionite (qualitatively). For spectral data, see Refs. 3 and 12. *E*, optical density.

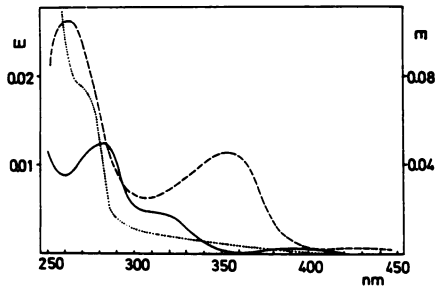

Chart 5. Absorption spectrum of detached pteridine moieties, separated from the AGP_M part by gel filtration. ——, detachment with 1% SDS and 20 mM dithiothreitol. The spectrum was run at pH 7.2 using 20 mM sodium phosphate buffer (qualitatively). – – – –, detachment with 6 M guanidine hydrochloride, spectrum at pH 1.0 (with HCl, qualitatively); ····, detachment with 6 M guanidine hydrochloride, spectrum at pH 13 (NaOH, qualitatively). For spectral data, see Refs. 3 and 12. *E*, optical density.

mation of the glycoprotein, if quantified by absorbance rather than by the dye-binding method (4) or by amino acid analysis. Apparently, treatment of P-AGP_M under reductive conditions detached the chromophoric group which could be separated by subsequent gel filtration from the protein moiety. The spectrum of the residual protein was largely identical with that of AGP_C (Chart 4, *inset*). The spectral data of the low molecular fraction indicated the presence of a 7,8-dihydropteridine (Chart 5).

After detachment under nonreductive conditions by 6 M guanidine hydrochloride and subsequent molecular sieving, AGP_M retained about 15% of its chromophoric group, as estimated by the ratio of 280:400 nm absorbance. Apparently, the pteridine is attached by noncovalent, rather than by covalent, binding. The spectral data at pH 1 of the low-molecular-weight

moiety (cationic form), detached under nonreductive conditions, agreed with those of a pteridine residue with a substitution at positions 6 and 7 as is the case, *e.g.*, in 7-methylxanthopterin. However, at pH 13, any absorbance at a wavelength > 320 nm slowly disappeared within 60 min. This may indicate a ring cleavage step rather than a substitution at N-8, which would also cause disappearance of absorbance in the long-wave UV range (19).

The spectral data had already largely excluded the persistence of the biopterin structure in P-AGP_M, the pterin originally found in the blood and accumulated, *e.g.*, in leukemic cells (34). This was further corroborated by the *Crithidia* assay after acid and alkaline treatment. Incubation under acid conditions left no biopterin activity at all. After alkaline treatment, a 10^4- to 10^5-fold amount of growth factor was needed for half-maximum growth as compared to free biopterin. This calculation is based on a molar ratio of 1:1 to 1:10 between biopterin and AGP_M. Such activities were found to be typical for pteridines, which had lost the hydroxyalkyl side chain and of which reuse as growth factor may need intermediary degradation, possibly to the pyrimidine level (20).

Microheterogeneity. SDS gel electrophoresis of AGP_M revealed a single band (Figs. 1*A* and 1*B*), whereas, in the absence of denaturating agents, 3 bands appeared (Fig. 2*A*). The minor Band III with a slower mobility may represent a polymeric (dimeric) form of AGP_M for 2 reasons. (*a*) In SDS gel electrophoresis, this band was not detectable. (*b*) The amino acid composition of AGP_M was completely identical with that described in the literature (24, 25), indicating the absence of proteinaceous impurities in the preparation. Band II disappeared if the pteridine moiety of P-AGP_M was detached under reducing or nonreducing conditions and removed by gel filtration prior to electrophoresis (Fig. 2*B*). Electrofocusing of AGP_C and of commercial AGP resulted in largely identical bands, focusing between pH 3.2 and 3.7. AGP_M, however, lacked the most acidic bands of AGP_C, whereas additional ones, especially in Con A-reactive AGP_M, appeared between pH 3.7 and 4.4 (Fig. 3). These additional polymorphic forms with higher pI values may be associated with Band II obtained by electrophoretic separation, which is absent in AGP_M after detachment of the chromophoric group as well as in AGP_C. In conclusion, amino acid substitution seems to differentiate Con A-reactive AGP_M from AGP_C. But additional polymorphism, to which at least in part the attachment of the chromophoric group contributes, substantially distinguishes AGP_M from AGP_C. The involvement of a decreased sialic acid content in the polymorphic forms of AGP_M with increased pI values has to be examined.

DISCUSSION

The characterization of P-AGP_M, responsible for the increases in protein-bound tyrosine in Fraction b during cancers, resolved some questions remaining after our first approach (35). Moreover, the results seem to develop the problem of abnormal orosomucoid, found in Fraction E during neoplastic diseases, as described by Rudman *et al.* (24). Fraction E is presumably identical with P-AGP_M. Besides molecular weight and amino acid composition, which do not differ from normal AGP, the reduced sugar content is common to Con A-reactive AGP_M and to abnormal orosomucoid. The difference in the electrophoretic pattern of normal and abnormal orosomucoid at pH 2.7, found by Rudman *et al.* (24), points to an additional heterogeneity in the abnormal one. These findings coincide with the appearance of polymorphic forms of AGP_M with more basic pI values, not present in normal AGP. Because of the generally observed pI polymorphism in AGP, which is especially pronounced in the Con A-reactive protein of malignant origin (Fig. 3), quantitative analysis of *N*-acetylneuraminic acid in AGP during cancers and acute phase is of great interest. The increase in the pI values of an AGP population, as described recently during inflammation in humans (17), seems to be much less drastic for acute-phase AGP than found for AGP_M.

From the total AGP present in blood, the isolation procedure enriches selectively variants of AGP, characterized by either a different amino acid content (Table 1) and/or a different pI pattern (Fig. 3). These findings agree with the fact that only 10 to 15% of the total AGP_C were found in controls (8), serum levels of which for AGP_C were estimated at 750 to 1000 μg/ml (13). The higher glycine content in the Con A-reactive AGP_C is caused presumably by amino acid substitution, unknown to such an extent from analysis of pooled AGP of normal subjects until now (see Ref. 25).

The most striking difference between AGP_M and AGP_C is the presence of a noncovalently bound chromophoric group in AGP_M. Recently, the binding of basic drugs, *e.g.*, methadone (23) and quinidine (18), has been demonstrated for AGP. The physiological significance of the pteridine binding to an AGP_M is still unknown and needs further investigation. Further analysis of the detached chromophore is required first of all to ascertain whether it represents a single compound. Therefore, the spectral data, indicative of a 6,7-substituted pteridine and its dihydroform, respectively, should not be interpreted in more detail. The absence of a side chain at position 6 is strongly indicated by the very low growth-promoting activity towards *C. fasciculata*. The detachment by guanidine hydrochloride suggested a noncovalent binding of the pteridine to the protein moiety. However, the nature of binding has to be studied in more detail. Even though a bathochromic shift is known to be caused by the attachment of pteridines, *e.g.*, to pigment carrier granules (*cf.* Ref. 32), the difference in wavelength of 30 nm between the absorption maximum of the detached pteridine and that in P-AGP_M needs more detailed investigation. The striking difference between the data of AGP_M and those reported for folate-binding proteins (31) largely excludes their identity as well as the possibility that the pteridine is in close relationship to the folate series.

Clinical data pointed to a close interdependence between increased biopterin concentrations in immature blood cells during leukemias and subsequently increased levels of P-AGP_M (8). Possibly, AGP_M may function as an acceptor of a biopterin metabolite. This would explain the most sporadic increases in blood biopterin levels during growth of solid tumors (8), which is in contrast to tetrahydrobiopterin (21) readily excreted in humans (33). Increased urinary levels, occurring frequently but not consistently during malignant growth (11, 22, 30), may represent the overspill of biopterin in blood. A chromophoric group in urinary α_1-microglycoprotein during a case of relapsed plasma cell leukemia has already been described but still remained uncharacterized (27).

The 280:400-nm ratio in absorbance amounted to 3.9 to 4.2 and was found to fluctuate within narrow limits among various preparations of P-AGP$_M$. Possible variations in pteridine saturation, however, have to be kept in mind. In this respect, detailed analyses of individual cases with cancers and comparison with acute phase are challenging.

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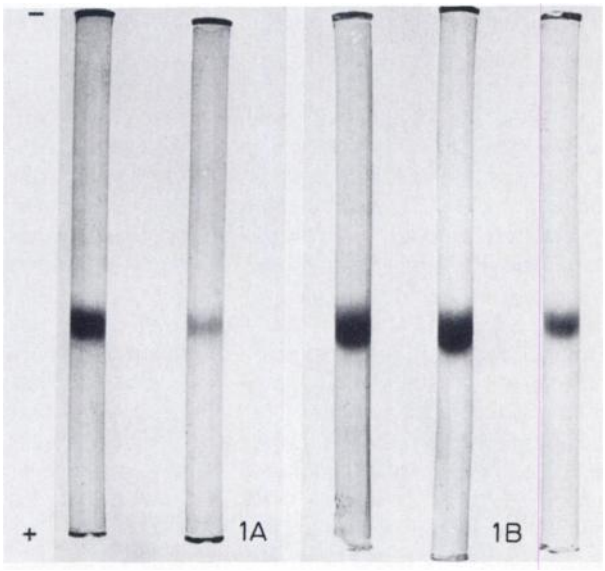

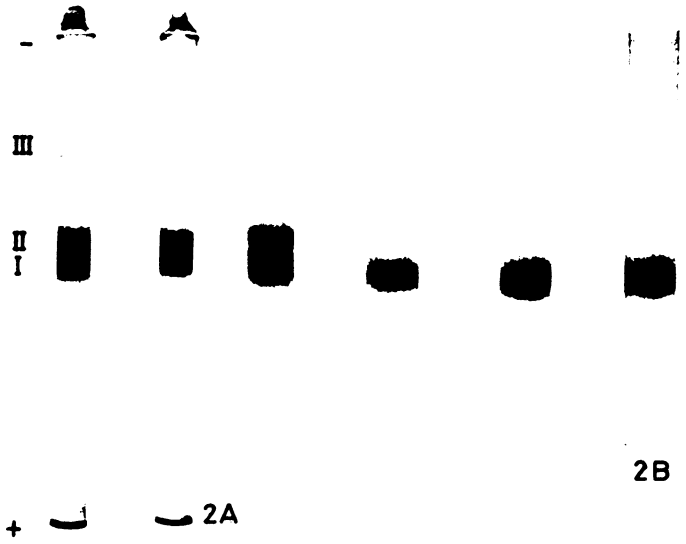

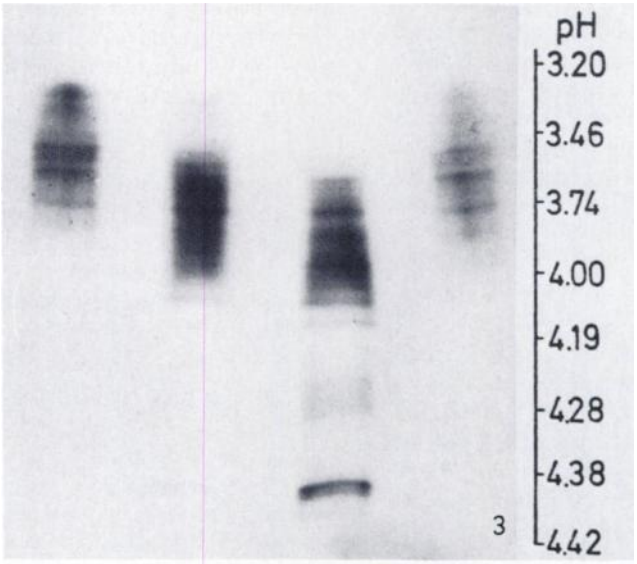

Fig. 1. SDS gel electrophoresis (10% polyacrylamide) of AGP. *A*, Con A-reactive P-AGP$_M$ (*left*) and Con A-nonreactive P-AGP$_M$ (*right*). *B*, Con A-reactive P-AGP$_M$ (*left*), AGP from commercial source (*middle*), and Con A-reactive AGP$_C$ (*right*).

Fig. 2. Gel electrophoresis (10% polyacrylamide) of AGP without SDS. *A*, Con A-reactive P-AGP$_M$ (*left*) and Con A-nonreactive P-AGP$_M$ (*right*). *B* (section enlarged), Con A-reactive P-AGP$_M$, Con A-reactive P-AGP$_M$ after detachment of the pteridine moiety, Con A-reactive AGP$_C$, and AGP from commercial source (*left* to *right*).

Fig. 3. Electrofocusing of Con A-reactive AGP$_C$, Con A nonreactive P-AGP$_M$, Con A-reactive P-AGP$_M$, and AGP from commercial source (*left* to *right*).

Pteridine-binding α_1-Acid Glycoprotein from Blood of Patients with Neoplastic Diseases

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