

TABLE 4  
Results of reported microheterogeneous studies of AGP

No.	AGP used and method used	Results	Ref.
I	Neuraminidase-treated AGP; starch-gel electrophoresis, pH 4.8	Three types, each having two bands: Type I Slower moving main band and faster moving minor band Type II Faster moving main band and slower moving minor band Type III Two main bands at same position as main bands of types I and II	535
	Native AGP; starch-gel electrophoresis, pH 2.9	Three types with 7, 6, and 7 bands, respectively, and corresponding to the types I, II, and III, respectively, but after neuraminidase treatment of these three native types	535
II	Native AGP; starch-gel electrophoresis, pH 2.9	Four types with 5, 6, 7, and 8 bands, respectively, occurring at a relative incidence of 4, 36, 49, and 11%, respectively, and probably due to genetically determined types	470
III	Neuraminidase-treated AGP; starch-gel electrophoresis, pH 5.1	Three types, each with two bands; relative percentages of these three types differ between a white and a Japanese population; types genetically determined; different types due to differences in polypeptide moiety	478
IV	Neuraminidase-treated AGP; starch-gel electrophoresis, pH 5	Three types, each with two bands; types independent of stress (after surgery, during pregnancy, and after delivery), and genetically determined	534
V	Neuraminidase-treated AGP from plasma of patients with hysterectomy and irradiation; starch-gel electrophoresis, pH 5	Three types, each with two bands; types independent of disease and AGP plasma level; types genetically determined	591
VI	Neuraminidase-treated whole serum; agarose-gel electrophoresis, pH 5, and immunofixation	Three types, each with two bands, called SS, FE, and FS, corresponding with types I, II, and III, respectively; types genetically determined; types due to differences of amino acid composition of the peptide chain resulting in F and S bands with different electrophoretic mobilities	263
VII	Native AGP; isoelectric focusing	Two types with a relatively anodic and cathodic distribution of 6 to 8 bands, respectively; isoelectric points range from 2.90 to 3.30	52
	Neuraminidase-treated AGP; isoelectric focusing	Two types with one or two main bands, both exhibiting several minor components; isoelectric points, 4.55 and 4.70, respectively; pattern not correlated with those of native AGP; microheterogeneity due to amino acid replacements of polypeptide chain in combination with different linkages of sialic acid to carbohydrate residues in native AGP	52
VIII	Native AGP; isoelectric focusing and titration curves	At least seven bands with isoelectric points between 3.4 and 3.8; microheterogeneity very slight, between pH 6 and 8	23
	Neuraminidase-treated AGP; isoelectric focusing and titration curves	The same pattern as for native AGP, but with isoelectric points between 4.3 and 4.7; very slight microheterogeneity, between pH 6 and 8; microheterogeneity not due to differences in sialylation, but to other mechanisms	23

TABLE 4—Continued

No.	AGP used and method used	Results	Ref.
IX	Whole plasma of depressive patients; isoelectric focusing	Three types with 6, 7, and 8 bands, respectively, independent of total AGP level; isoelectric points range from 3.2 to 3.9; types due to genetically determined variants	530
X	Sera of cancer patients; crossed-immunoaffinity electrophoresis; binding to wheat germ agglutinin	Distribution of three bands changed in cancer disease; lower binding in cancer disease to wheat germ agglutinin possibly due to diminished content of sialic acids in outer part of carbohydrate moiety	65
XI	Crossed-immunoaffinity electrophoresis; influence of estrogen level	Pattern with three bands, changing to a pattern with two bands with more of the concanavalin A nonreactive bands after increase of sex hormone levels (during pregnancy and after estrogen therapy of prostatic cancer)	663
XII	Sera of healthy people, of cancer patients, and of women during pregnancy; crossed-immunoaffinity electrophoresis	Three bands in serum of normal subjects, but only two bands in serum of women during pregnancy and in serum of prostatic cancer patients treated with estrogen; increase of faster moving bands and disappearance of concanavalin A reactive band	427
XIII	Normal and inflammatory sera; crossed-immunoaffinity electrophoresis	Three bands with differences in pattern of distribution between normal and inflammatory sera; increase of concanavalin A reactive and concanavalin A weakly reactive bands during inflammation	873
	Concanavalin A affinity chromatography followed by isoelectric focusing	Only two bands, when separated by chromatography, namely a concanavalin A nonreactive band, which after isoelectrofocusing had 6 bands between pH 2.9 and 3.1 and 3 bands between pH 3.1 and 3.4, and a concanavalin A reactive band with 6 bands between pH 3.1 and 3.4 when followed by isoelectric focusing	374
	Concanavalin A affinity chromatography followed by crossed-immunoaffinity electrophoresis	Only two bands, when separated by chromatography, the concanavalin A nonreactive bands separated chromatographically contain, when followed by crossed-immunoaffinity electrophoresis, the nonreactive and the weakly reactive bands, whereas the A reactive component separated chromatographically contained, after crossed-immunoaffinity electrophoresis, a little weakly reactive band too; this band is also present in sera after crossed-immunoaffinity electrophoresis alone	374
XIV	AGP from sera of normals and patients with neoplastic disease; crossed-immunoaffinity electrophoresis followed by isoelectric focusing	In neoplastic disease additional bands between pH 3.7 and 4.4, compared with normals having bands between pH 3.2 and 3.8; due to differences in amino acid substitution and the presence of a non-covalently bound chromophoric group	508
XV	Crossed-immunoaffinity electrophoresis	Patterns with three bands, but with variable distribution of these bands; in severe disease and pregnancy these patterns change towards the less concanavalin A binding bands due to changes in the glycosylation of carbohydrate moiety of AGP, depending on severity of disease state	68, 89
XVI	Crossed-immunoaffinity electrophoresis	Three bands with a relative distribution of 44.5%, 40.4%, and 16.1%, respectively, being constant under nonpathological conditions	237

TABLE 4—Continued

No.	AGP used and method used	Results	Ref.
XVII	Crossed-immunoaffinity electrophoresis	Four bands being differently distributed in normal health, inflammatory lung disease, and cancer of the lung; benign sera contain more of the concanavalin A nonreactive bands whereas cancer sera contain more of the concanavalin A reactive band; aid in diagnosis of cancer	214, 216

macological, or physical-chemical studies, although they are mentioned briefly in a few more fundamental physical studies (35, 97, 211-213, 274, 388, 507, 508). Presumably, most researchers assume that AGP is a very stable plasma protein. However, it should be noted that a temperature-dependent polymerization of AGP has been described, yielding two kinds of polymers differing in their biological activity (35) and in their drug-binding behavior (508). Halsall and Kirley (211) observed a temperature-dependent denaturation of AGP which is influenced by the degree of defatting and desialylation. From these observations, it can be concluded that sterilization of AGP by heating can induce denaturation of AGP.

Halsall et al. (212) reported (a) that aggregates of AGP are formed as a result of lyophilization or ultrafiltration; (b) that the acid-charcoal defatting procedure of Chen (109) induced polymer formation of AGP; (c) that lyophilization, especially of defatted AGP, induced polymerization, whereas the extent of polymerization proved to be dependent on the medium, the number of lyophilization cycles, and the protein concentration (increasing polymerization with decreasing protein concentration). They suggested therefore that care must be taken with defatted AGP and that lyophilization must be performed after extensive dialysis of an aqueous solution. Halsall et al. (212) observed further that AGP could be stored for 1 wk at 4°C in phosphate-buffered saline solution without the occurrence of polymerization, and that repeated freezing of an AGP solution and subsequent thawing did not result in polymerization. Recently, Busby and Ingham (97) reported that the thermal stability of AGP (as determined with fluorescent probes) is enhanced by lipids, propranolol, ethanol, and probably other organic solvents.

These studies demonstrate that different isolation and purification procedures result in AGP preparations with different physical-chemical properties.

#### B. Molecular Weight of Alpha-1-acid Glycoprotein

The molecular weights reported for AGP (see table 3) range from 37,000 to 54,000. These values depend on the methods of determination (53, 268), on the isolation procedure (97, 104, 152, 180, 205, 484), on whether the AGP is native or desialylated (e.g., as a result of neuraminidase treatment; 53, 268), and on the origin of the AGP samples (from plasma, urine, or membranes of normals or patients; 104, 180, 213, 215, 323).

The molecular weight generally assumed for AGP is 40,000, which is about the mean value of the molecular weights reported for native AGP isolated from plasma (reported values, 38,800 to 48,000; table 3). Higher molecular weights for AGP have been reported by Schultz et al. (484; table 3, no. II), Easton et al. (152), Hardwick and de Vaux St. Cyr (219), and Gahmberg and Andersson (180; table 3, no. VIII). Gahmberg and Andersson (180; table 3, no. VIII) reported, however, that the AGP with a molecular weight of 52,000 was a membrane-bound form of AGP, synthesized by the lymphocytes, but subsequently cleaved and released as the soluble serum form of AGP with a molecular weight of 41,000. Hardwick and de Vaux St. Cyr (219) and Easton et al. (152) reported the isolation from urine and serum of two AGP variants, with a molecular weight of 40,000 and 54,000, respectively. From table 3 it follows that desialylated AGP has a mean molecular weight of about 38,000 (reported values between 34,100 and 41,600; table 3).

Recently the amino acid sequence of human AGP has been inferred from the cDNA sequence using the molecular cloning technique (64). The molecular weight of the polypeptide moiety studied can be easily calculated from this sequence. Board et al. (64) remarked, however, that clones with different sequences can not yet be excluded. In order to find the total molecular weight of human AGP, the molecular weight of the five glycan chains should be added to that of the polypeptide chain.

#### C. Structural and Physical-Chemical Properties of the Polypeptide and Carbohydrate Moiety of Alpha-1-acid Glycoprotein

The chemical properties of AGP have been reviewed by Jeanloz (256, 257) and Schmid (469). AGP contains carbohydrate residues chemically bound to the protein. Therefore it can be catalogued among the groups of the glycoproteins (279, 487, 512), the mucoproteins (512, 560), the seroglobulins (298), and the alpha-1-globulins (285, 484).

AGP is composed of a single polypeptide chain and five carbohydrate moieties. Recently it has been shown that the polypeptide chain consists of 183 amino acids (64) [in contrast to the number of 181 reported earlier (469)] and contains two disulfide bonds (469, 471). The complete amino acid sequence, the multiple amino acid substitutions (21 of the 181 residues), and the homology with the immunoglobulins (about 80%) have been re-