Plasma 3S γ_1-Globulin: Identity with Erythrocyte Carbonic Anhydrase B

Abstract. *The 3S γ_1, 2S γ_2, and 0.5S γ_2 fractions of human plasma are heterogeneous in protein composition. Although each fraction contained a relatively small amount of protein antigenically related to the immunoglobulin light chains, most of the proteins were unrelated to immunoglobulin G or its light chains. Of the 3S γ_1-globulins the greater part was immunochemically identical to carbonic anhydrase B and had carbonic anhydrase activity. These findings explain earlier reports of an immunochemical similarity between 3S γ_1-globulins and immunoglobulin light chains in spite of marked differences in amino acid and peptide composition between the two. Apparently not all plasma γ-globulins are necessarily immunoglobulins.*

There are at least three different classes of γ-globulins in normal human plasma which have molecular weights of 25,000 or less; these have been designated 3*S* γ_1, 2*S* γ_2, and 0.5*S* γ_2, respectively, on the basis of their electrophoretic mobilities at *p*H 8.6 and their sedimentation rates (*1*). Although the 3*S* γ_1 class may contain proteins with antigenic determinants similar to those of the immunoglobulin light chains (*2*), the 3*S* γ_1-globulins and the immunoglobulin light chains are quite different from each other in peptide and amino acid composition (*1*). The nature of the 3*S* γ_1-globulins was explored further in our study.

The three classes of low-molecular-weight γ-globulins were isolated from pooled human plasma by a combination of ethanol fractionation, DEAE-cellulose (diethylaminoethyl) chromatography, and Sephadex G-100 filtration; the physicochemical properties of these preparations have been described (*1*). Human immunoglobulin G (γG) was isolated from pooled plasma by ethanol fractionation (*3*) and was used for the preparation of γG light chains (*4*). Antiserums against γG and against 3*S* γ_1 were prepared in rabbits. Portions of γG, γG light chains, and each of the low-molecular-weight γ-globulins were labeled with I^{131} by a nitrous acid method (*5*).

Table 1. Percentage of I^{131}-labeled protein precipitated by specific antiserum.

Labeled protein	Antiserum to	
	γG	0.3*S* γ_1
γG	98.6	<0.05
γG light chains	99.2	<0.05
3*S* γ_1	3.0	90.5
2*S* γ_2	8.7	19.0
0.5*S* γ_2	0.9	15.3

Mixtures of a given I^{131}-labeled protein, the same protein unlabeled as a carrier, and unlabeled γG were precipitated with antiserum to γG and antiserum to 3*S* γ_1 in the zone of antibody excess (37°C for 1 hour and overnight at 4°C); the precipitates were separated by centrifugation, and washed twice with 0.15*M* NaCl. Precipitates and supernatants were assayed for radioactivity with a well-type crystal (Table 1). Antiserum to γG precipitated more than 98 percent of the radioactivity of the labeled γG and γG light-chain preparations but only 3 percent of the labeled 3*S* γ_1-globulin; several different 3*S* γ_1-globulin preparations gave similar results. On the other hand, antiserum to 3*S* γ_1 precipitated 90.5 percent of the 3*S* γ_1-globulin but less than 0.05 percent of the labeled γG or γG light chains. Thus, the 3*S* γ_1 preparation contained several different proteins: (i) A protein which represented a small fraction of the total 3*S* γ_1-globulins had antigenic determinants which reacted with antiserum to γG; this reaction was not due simply to contamination of the 3*S* γ_1 preparation with γG, since it has been shown that the reacting protein lacks γG heavy-chain determinants (*2*). (ii) A protein which constituted the bulk of the 3*S* γ_1 preparation was precipitable with antiserum to 3*S* γ_1, and was antigenically different from γG; in support of this, the 3*S* γ_1 preparation gave a single precipitation line with antiserum to 3*S* γ_1 in Ouchterlony plates (*6*), and γG gave none (Fig. 1a). (iii) From Table 1, some of the 3*S* γ_1-globulins and most of the 2*S* γ_2- and 0.5*S* γ_1-globulins did not precipitate with either antiserum to γG or antiserum to 3*S* γ_1.

The concentration of the globulin precipitable by antiserum to 3*S* γ_1 in 74 normal adult serums, as determined by radial immunodiffusion (*7*), ranged from less than 0.014 mg per 100 ml of serum, the lower limit of detection, to approximately 0.56 mg. However, serums with detectable hemolysis gave extraordinarily high values, and the concentration of this protein in erythrocytes averaged 300 mg per 100 ml of blood in 13 adults, 23 mg in 26 newborns, and less than 3 mg in three fetuses of 3 to 5 months' gestation. Since the change in erythrocyte concentration of the protein with age resembled the pattern of development of erythrocyte carbonic anhydrase, the low-molecular-weight γ-globulins were tested for carbonic anhydrase activity by an electrometric method (*8*) (Table 2). The carbonic anhydrase activity of the 3*S* γ_1-globulins was approximately 44 percent of that exhibited by erythrocyte lysates containing equivalent concentrations of protein precipitable with antiserum to 3*S* γ_1; this is compatible with the fact that erythrocytes have several carbonic anhydrases (*9*). The carbonic anhydrase activities of the 2*S* γ_2 and 0.5*S* γ_2 preparations were 22 and 3.7 percent, respectively, of that for the 3*S* γ_1 preparation; 19 and 15 percent of the total proteins in 2*S* γ_2 and 0.5*S* γ_2 preparations, respectively, were precipitable with antiserum to 3*S* γ_1. Hence the 0.5*S* γ_2-globulin precipitable with antiserum to 3*S* γ_1 must have had little carbonic anhydrase activity, and may represent degradation products of the antigenically related 3*S* γ_1-globulin. The γG preparations had no detectable carbonic anhydrase activity.

In Ouchterlony plates, the precipitation line formed between antiserum to

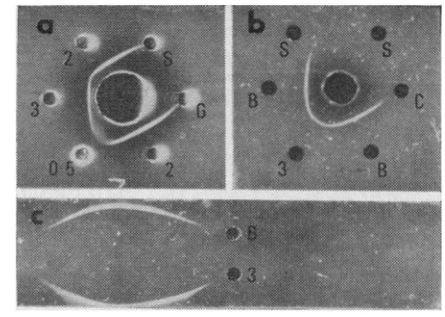

Fig. 1. (a) Ouchterlony plate; antiserum to 3*S* γ_1 in central well. S indicates 0.1*M* NaCl; G, γG; 3, 3*S* γ_1; 2, 2*S* γ_2; and 0.5, 0.5*S* γ_2. (b) Ouchterlony plate; antiserum to 3*S* γ_1 in central well. S indicates 0.1*M* NaCl; C, erythrocyte catalase; B, carbonic anhydrase B; 3, 3*S* γ_1. (c) Immunoelectrophoresis of carbonic anhydrase B (b) and 3*S* γ_1 (3); antiserum to 3*S* γ_1 diffused into agar from above and below the figure. Anode was to the right.

Table 2. Carbonic anhydrase activities per microgram of protein.

Preparation	Enzyme units (mean ± S.D.)
3*S* γ_1	1.87 ± 0.21
2*S* γ_2	0.414 ± 0.067
0.5*S* γ_2	0.069 ± 0.010
Lysate of adult erythrocytes	4.25 ± 0.05*

* Per microgram of protein precipitable with antiserum to 3*S* γ_1.

3S γ_1 and the 3S γ_1-globulins fused completely with that formed between antiserum and purified erythrocyte carbonic anhydrase B (Fig. 1b). On immunoelectrophoresis (*10*) with antiserum to 3S γ_1, the mobility of the reacting 3S γ_1-globulin was identical to that for carbonic anhydrase B (Fig. 1c).

Thus, although the plasma 3S γ_1-globulins included a small amount of a protein antigenically related to the immunoglobulins as reported earlier (*2*), most of the γ-globulin in this class of proteins was not related to γG or to γG light chains but was identical or very similar to carbonic anhydrase B, thus explaining the difference in peptide composition noted (*2*) between 3S γ_1 plasma fractions and the immunoglobulins as well as the similarity between an amino-terminal tetrapeptide obtained from the 3S γ_1-globulins and that of carbonic anhydrase B (*11*). In accord with this, the amino acid composition of whole 3S γ_1-globulin preparations is quite unlike that of the immunoglobulin light chains (*1*), but is identical, within the limits of error of the methods, with that reported for carbonic anhydrase B by others (*9*).

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Dihydroorotic Acid Dehydrogenase Activity of Human Diploid Cell Strains

Abstract. *A gene affecting the final two enzymes of uridylic acid biosynthesis does not affect a third, metabolically adjacent enzyme. Similarly, compounds that increase cellular activity for the affected enzymes do not increase activity for the third enzyme. The pyrimidine pathway can be subdivided into groups of concurrently responding enzymes. These groups may be smaller in human cells than they are in microbial cells.*

Dihydroorotic acid dehydrogenase (DAD, E.C. 1.3.3.1), one of the enzymes in the uridine biosynthetic pathway, catalyzes the reversible conversion of dihydroorotic acid to orotic acid (Fig. 1). This enzyme is present in circulating human leukocytes but absent from red blood cells (*1*). Both erythrocytes (*2–4*) and cultured diploid cell strains (*5, 6*) from patients with orotic aciduria, a rare autosomal recessive disorder, are deficient in two sequentially acting enzymes in the biosynthetic pathway (Fig. 1) leading to uridine-5′-monophosphate (UMP). These enzymes are orotidine-5′-monophosphate (OMP) pyrophosphorylase (E.C. 2.4.2.10) and OMP decarboxylase (E.C. 4.1.1.23); diploid cell strains from mutant homozygous donors show 1 percent of normal activity for both enzymes. When mutant homozygous cultures are grown in a medium containing either 5-azaorotic acid (a competitive inhibitor of OMP pyrophosphorylase) or 6-azauridine (whose ribotide is a competitive inhibitor of OMP decarboxylase), the cells develop nearly normal activity for both enzymes (*7, 8*). Barbituric acid, which is not an inhibitor of either enzyme, also causes mutant homozygous cells to develop nearly normal activity (*8*). The response of normal and heterozygous cells to these agents is similar to that of the mutant homozygous strains, but in both enzymes the proportionate increase is smaller. The other two enzymes peculiar to this pathway (Fig. 1), aspartate transcarbamylase (E.C. 2.1.3.2) and dihydroorotase (E.C. 3.5.2.3), which catalyze consecutive reactions immediately preceding the one which DAD catalyzes, are not deficient in mutant cells (*2, 3, 6*). Moreover, cellular dihydroorotase activity does not rise in response to at least one of the agents which increases OMP pyrophosphorylase and OMP decarboxylase activity (*8*). The effect of the gene for orotic aciduria, or of the compounds mentioned above, on the activity of DAD has not been reported. We have therefore sought answers to the following questions. (i) Does the gene for orotic aciduria affect DAD activity? (ii) Do human cells increase their DAD activity in response to the compounds that

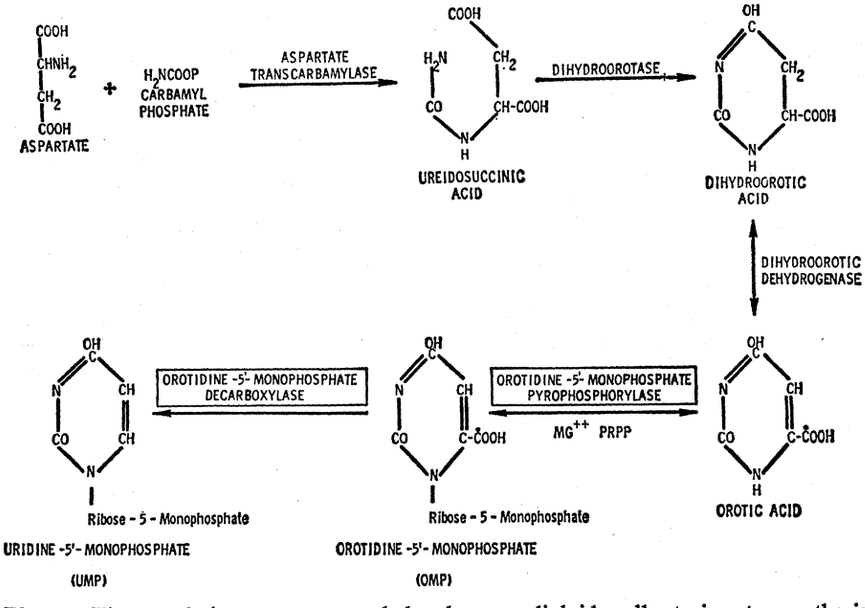

Fig. 1. The catalytic sequence used by human diploid cell strains to synthesize uridine-5′-monophosphate.