A large concentration of charge carriers is a common structural feature of superionic conductors, and it will be interesting to examine other crystal structures for similar co-ionic compositions that may display nonlinear ionic transport. An attractive coordinate is the framework structure $M_{1+x}Zr_2Si_xP_{3-x}O_{12}$, where M = Li, Na, Ag, or K (*20*).

WALTER L. ROTH
GREGORY C. FARRINGTON
Research and Development Center, General Electric Company, Schenectady, New York 12301

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Radioimmunoassay for Abnormal Hemoglobins

Abstract. *A sensitive and specific radioimmunoassay has been developed for the identification or quantification of the human hemoglobin variants S, C, D-Los Angeles, E, G Philadelphia, Russ, O Arab, Beograd, J Paris I, G San Jose, Q Iran, Korle Bu, and F Malta I. In the immunoassay, monospecific antibody preparations are used which recognize the single amino acid substitution in the variant polypeptide chain and do not cross-react with normal hemoglobins or hemoglobin variants containing a different amino acid exchange at the same position.*

Hemoglobin (Hb) from normal individuals and those with various hemoglobinopathies has been the subject of extensive structural, functional, and genetic investigations. At the present time, more than 275 different hemoglobin variants have been discovered (*1*). In the majority of these variants, there is a single amino acid substitution in one of the two hemoglobin subunits. Generally these mutants are rare in the population and have no clinical consequences in heterozygous individuals, although four abnormal hemoglobins (S, C, D, and E) are frequently observed in certain populations and can be associated with pathological manifestations (*2*). Because the common method of detection and preliminary identification of variants is based on comparative electrophoretic differences between the abnormal and normal proteins, most of those reported have an amino acid exchange involving residues of unlike charge. However, since many different abnormal hemoglobins have similar or identical mobilities, conclusive identification requires structural analyses.

We have developed a radioimmunoassay (RIA) system with monospecific antiserums capable of recognizing and, if necessary, quantifying hemoglobin variants in red cell hemolyzates. Included in our study are the four most prevalent abnormal hemoglobins (S, C, D, and E), as as well as G Philadelphia, which is probably the most common α-chain variant in the black population in the United States (*3*). These and other hemoglobin variants for which RIA's have been developed are listed in Table 1.

Blood samples for isolation of the hemoglobin variants were obtained from patients whose abnormal hemoglobin had been structurally characterized. Methods of isolation included column chromatography on diethylaminoethyl (DEAE)-Sephadex, DEAE-cellulose, carboxymethyl (CM)-cellulose, and CM-Sephadex, with developers and conditions as described (*4*). Each preparation was analyzed for purity by starch-gel electrophoresis at *p*H 9.0 (*5*). All isolated hemoglobins migrated as single bands free of hemoglobins A, F, or A_2, except for HbE and HbO Arab which could not be completely separated from HbA_2.

Antiserums to the variants were prepared in white New Zealand rabbits with Freund's complete adjuvant according to the dose, route, and schedule outlined (*6*). Antiserum to HbE was produced in chickens by a similar immunization schedule (*7*). Antibody preparations were made monospecific for the variants by absorption with HbA conjugated to AH-Sepharose activated with glutaraldehyde (*8*); antiserum to HbF Malta I was absorbed with HbF ($^G\gamma$). In addition, antiserums to HbE and HbO Arab were absorbed with HbA_2. After absorption, the preparations of antiserums were concentrated approximately tenfold by negative-pressure dialysis.

The antibody titers were measured by determining the extent of binding of the absorbed antiserums with the corresponding ^{125}I-labeled variant hemoglobin; similar titrations were also made with ^{125}I-labeled normal hemoglobins (A, A_2, and F) to confirm the absence of cross-reactivity. The principle of the RIA is based on the separation of free and bound ^{125}I-labeled antigen by the addition of ammonium sulfate solution. Details of this method have been described (*6*). After absorption, each variant antiserum precipitated only the ^{125}I-labeled hemoglobin variant and did not react with labeled HbA, HbA_2, or HbF, in the case of antiserum to HbF Malta I.

In order to further ensure the specificity of the antiserum for each particular mutant, standard RIA inhibition assays were performed. In these tests, the tubes contained a constant volume of antiserum, which was diluted to yield approximately 35 percent precipitation of the ^{125}I-labeled hemoglobin variant. Increments of unlabeled normal or variant hemoglobins were then added to the tubes. After incubation for 1 hour at room temperature, a constant quantity (0.1 μg) of the labeled hemoglobin variant was added to all tubes. Under these conditions, a competition reaction is established between the labeled and unlabeled hemoglobin for the available antibody-combining sites. If a sufficient amount of the homologous unlabeled Hb antigen is present, the antibody-combining sites become saturated, and the ^{125}I-labeled antigen is located in the supernatant fraction after the addition of ammonium sulfate solution. The representative inhibition curve (Fig. 1a) shows that the homologous antigen (HbS) competitively blocked the binding between antiserum to HbS and ^{125}I-labeled HbS in quantities ranging from 0.1 to 10.0 μg, while HbA, HbF, and HbA_2 did not inhibit the reaction at any of these concentrations.

The feasibility of using the RIA to identify and quantify abnormal hemoglobins in red blood cell hemolyzates was evaluated with the use of antiserums to

HbS and HbC. Standard dose-response curves were established for both HbS and HbC by determining the percentage of inhibition produced by known concentrations of each variant. A typical curve (Fig. 2) illustrates that the immunoassay can measure quantities as low as 1.0 percent of the variant (0.05 μg). Red cell hemolyzates containing HbS or HbC obtained from blood samples from the newborn could be distinguished from those lacking these variant hemoglobins. The average amount of HbS in samples from six newborn HbS heterozygotes was 7.5 percent and the average value for HbC in three heterozygote (AC) newborns was 8.3 percent. These values were comparable to average values of HbS and HbC in hemolyzates from the newborn, as determined by column chromatography (*9*). This RIA could be adapted for the identification and quantitation of these and other variants in adult, newborn, and possibly even in amniotic fluid erythrocytes (*10*). An immunochemical method for the identification and quantitation of abnormal hemoglobins offers certain advantages over current methodology. Although other techniques—such as electrophoresis, chromatography, or isoelectric focusing—are often helpful as an aid in the preliminary identification of a hemoglobin variant, conclusive identification can only be achieved by structural studies. Radioimmunoassays for various mutant hemoglobins would simplify the task since the time needed for an assay is only 1 to 2 days. In addition, microgram quantities of an unfractionated hemolyzate are used for RIA, as compared to large quantities of purified globin required for structural analyses. These features appear to make the RIA of considerable importance in the analyses of hemoglobin variants.

Table 1. Human hemoglobin variants for which radioimmunoassays have been developed. Abbreviations: Glu, glutamic acid; Val, valine; Lys, lysine; Gln, glutamine; Ala, alanine; Asp, aspartic acid; Arg, arginine; Asn, asparagine; His, histidine. β^6, for example, means the sixth residue of the beta chain.

Hemoglobin	Position in chain	Substitution
S	β^6	Glu → Val
C	β^6	Glu → Lys
G San Jose	β^7	Glu → Gly
E	β^{26}	Glu → Lys
Korle Bu	β^{73}	Asp → Asn
D Los Angeles	β^{121}	Glu → Gln
O Arab	β^{121}	Glu → Lys
Beograd	β^{121}	Glu → Val
J Paris I	α^{12}	Ala → Asp
Russ	α^{51}	Gly → Arg
G Philadelphia	α^{68}	Asn → Lys
Q Iran	α^{75}	Asp → His
F Malta I	γ^{117}	His → Arg

The importance of the unique amino acid side chain in determining immunological specificity was then examined. The following three sets of hemoglobin variants, each containing a different substitution at the same position, were analyzed for cross-reactivity, namely, β^6: HbS (Glu → Val) and HbC (Glu → Lys); β^{121}: HbD Los Angeles (Glu → Gln), HbO Arab (Glu → Lys), and Hb Beograd (Glu → Val); and α^{68}: HbG Philadelphia (Asn → Lys) and Hb Ube II (Asn → Asp). Each monospecific antiserum was tested with the homologous and heterologous hemoglobins in each group, except Hb Ube II to which specific antibody has not yet been obtained. The inhibition curve for HbO Arab, Hb Beograd and HbD Los Angeles with an antiserum to HbD Los Angeles (Fig. 1b) is representative of the results obtained with the other sets of variants. In each case, only the homologous antigen was effective in inhibiting the antigen-antibody reaction, thus emphasizing the dominant role of a single amino acid side chain in an antigenic determinant in combining with antibody. Reichlin has also immunized rabbits with mutant hemoglobins and shown that antiserums to nine variants were capable of

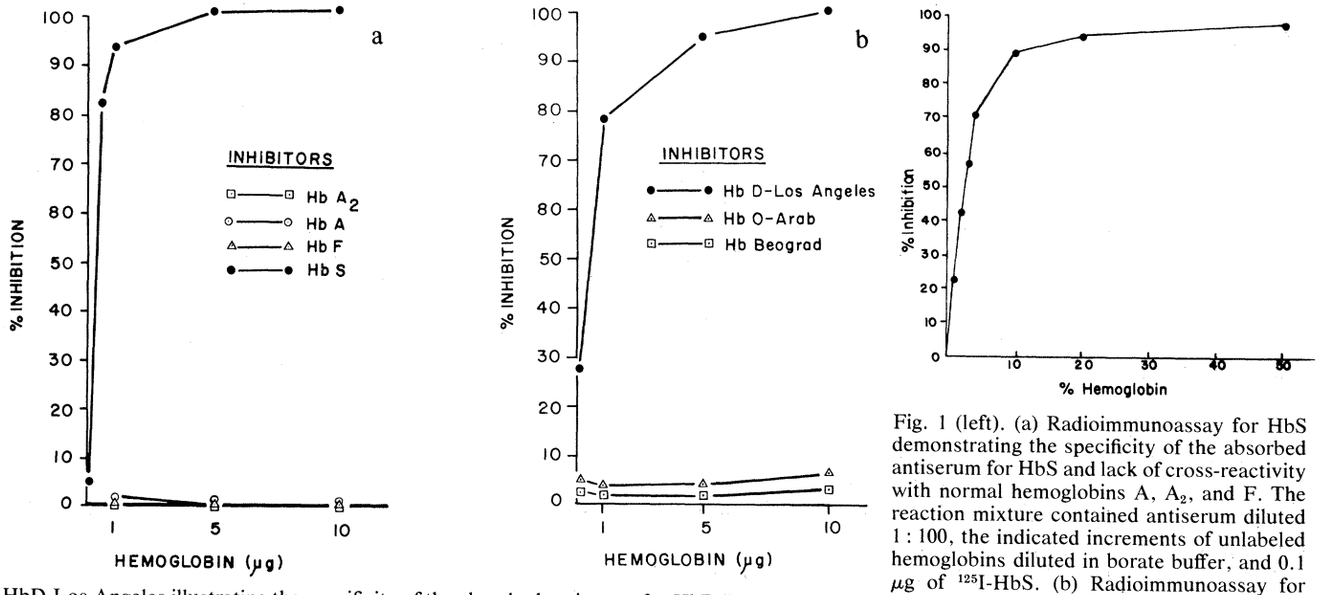

Fig. 1 (left). (a) Radioimmunoassay for HbS demonstrating the specificity of the absorbed antiserum for HbS and lack of cross-reactivity with normal hemoglobins A, A_2, and F. The reaction mixture contained antiserum diluted 1 : 100, the indicated increments of unlabeled hemoglobins diluted in borate buffer, and 0.1 μg of ^{125}I-HbS. (b) Radioimmunoassay for HbD-Los Angeles illustrating the specificity of the absorbed antiserum for HbD Los Angeles and absence of cross-reactivity with HbO Arab and HbL Beograd. The tubes contained antiserum to HbD Los Angeles diluted 1 : 20, specified increments of unlabeled hemoglobins, and 0.1 μg of ^{125}I-labeled HbD Los Angeles. Fig. 2 (right). Representative standard curve for quantification of HbS. The percent inhibition of the binding between absorbed antiserum to HbS and ^{125}I-labeled HbS by various concentrations of unlabeled HbS was measured. The HbS was mixed with HbA in the indicated percentages at a final hemoglobin concentration of 50 μg/ml, and 5 μg of each was added. The concentrated antiserum was diluted to 1 : 100, and 0.1 μg of labeled HbS was used.

distinguishing the mutant from HbA by either complement fixation or precipitin tests (*11*), although attempts to actually isolate a mutant-specific antibody population were only reported with goat antibodies to HbS (*12*).

All the variants eliciting monospecific antibody (Table 1) contained substitutions exposed to the exterior of the macromolecular surface, a finding consistent with observations made with other globular protein antigens (*13, 14*). Since the amino acid substitution in each of the variant hemoglobins is responsible for inducing a specific antibody response, it must be located in or near an antigenic determinant area. Therefore, either these areas exist as natural determinants on normal hemoglobin, or the substitutions transformed immunogenically silent areas into new reactive sites. To examine these alternatives we absorbed antiserum to HbA with HbG Philadelphia and obtained antibody reactive with HbA but not with HbG Philadelphia, suggesting that the asparaginyl residue at α^{68} of HbA is part of a normal antigenic determinant. In contrast, absorption of antiserum to HbA with Hb Beograd removed all antibody to HbA, suggesting that the glutamyl residue at β^{121} is not a part of a determinant in normal HbA. Reichlin has also identified the involvement of the asparaginyl residue at α^{68} in an antigenic determinant site of HbA (*15*), whereas a substitution at β^{121} had no effect on the antigenicity and was presumably not located in an antigenic area (*14*). Similarly, absorption of antiserum to HbA with HbS or HbC completely eliminated all antibody activity to HbA, indicating that the glutamyl residue at β^{6} is not generally immunogenic in rabbits and may explain the difficulty experienced by other investigators in preparing specific HbA [β^{6}(Glu)] antiserums. Another explanation advanced for the failure to obtain rabbit antibodies specific for the β^{6} (Glu) residue after absorption is that the antibody has comparable degrees of binding affinity for both HbA and HbS and is removed by immunoabsorption (*14*). Further absorption of antiserum to HbA with a variety of different variant hemoglobins should help clarify the structural basis of the immunogenicity and antigenicity of hemoglobin.

FRED A. GARVER, MARILYN B. BAKER
C. SIDNEY JONES, MARSHA GRAVELY
GULTEKIN ALTAY
TITUS H. J. HUISMAN
Department of Cell and Molecular Biology and *Comprehensive Sickle Cell Center, Medical College of Georgia, Augusta 30902*

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Feline Oncornavirus–Associated Cell Membrane Antigen: Expression in Transformed Nonproducer Mink Cells

Abstract. *The feline oncornavirus–associated cell membrane antigen (FOCMA) is a target for naturally occurring immunity that protects the cat against development of fibrosarcoma and leukemia. Feline sarcoma virus–transformed "nonproducer" mink cells express high levels of FOCMA, but not of the major viral structural proteins. Transformation of the same cells by murine sarcoma virus, or infection with feline leukemia virus, which is nontransforming for epithelial or fibroblastic cells, did not induce FOCMA. Thus, FOCMA expression in mink lung cells is specifically associated with transformation by feline sarcoma virus.*

Antibody to feline oncornavirus–associated cell membrane antigen (FOCMA) is the major factor in determining whether or not an animal successfully resists tumor development after infection with feline sarcoma virus (FeSV) (*1, 2*) and feline leukemia virus (FeLV) (*3*). In vivo, FOCMA is immunologically identical whether induced by FeLV or FeSV (*3, 4*). Healthy viremic cats often have significant titers of antibody to FOCMA, but such animals never have free antibody to the major virus envelope (gp70) and core (p30) proteins (*4, 5*). By a number of criteria, FOCMA appears to be distinct and separate from the viral structural proteins (*4–6*).

The availability of nonproducer FeSV-transformed mink cells (*7, 8*) has made it possible to test for FOCMA expression in cells of a heterologous species in the absence of virus production. Three independently isolated cell lines of FeSV-transformed mink lung cells (Mvl-Lu) were used; each had been transformed with the Gardner-Arnstein strain of FeSV (*9*). Our data indicate that FOCMA expression is dependent on transformation by FeSV, and independent of the presence of viral structural proteins. In association with previous results in vivo, FOCMA, therefore, appears to be induced by transformation events associated with the expression of either FeLV or FeSV. We believe this is the first report describing a tumor virus–associated antigen on nonproducer tumor cells that is known to be immunogenically effective under natural conditions.

The FeSV-transformed nonproducer mink cell cultures used are as follows: lines 64F1 and 64F2 which were uncloned mixed cultures of transformed and untransformed cells; F1 Cl 10, F1 Cl 13, and F1 Cl 16 that were derived from transformed single cell clones of 64F1; and 64F3 Cl 7 which was a clonal culture of transformed cells derived from a third mixed culture. The parent line (Mvl-Lu) was also nonproductively transformed with the Kirsten murine sarcoma virus (Ki-MSV) and designated