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Hemoglobin Solubility and α-Chain Structure in Crosses between Two Inbred Mouse Strains

Abstract. *In genetic crosses between mouse strains with low and high solubilities of hemoglobin, solubility segregated as a Mendelian unit and appeared to be determined by the alleles controlling α-chain structure. New data eliminate close linkage between the α-chain locus and genetic markers in linkage groups III, IV, XI, XIII, and XVI. Simplified methods are presented for screening differences in α-chain peptides and solubility.*

Hemoglobins of mice from various inbred strains differ in the primary structure of their α- and β-chains (*1*) and in their solubility in buffered salt solutions (*2*). Alleles at the *Hb*-locus (linked to albinism) affect β-chain structure and hemoglobin solubility (*3*). Each mouse classified as Hb^sHb^s contains one major hemoglobin of either intermediate or low solubility (*3*, *4*). Mice classified as Hb^dHb^d carry unequal amounts of two major hemoglobins whose non-α-chains differ from each other and from the β-chain of all Hb^s hemoglobins which have been studied (*1*). These Hb^d hemoglobin complexes tend to be highly soluble over a wide range of experimental conditions (*3*, *4*).

Popp has suggested that all heritable solubility differences among hemoglobins from Hb^sHb^s mice are controlled by alleles at a single *Sol* locus (*5*) and has named four alleles in this series (*6*). He has also postulated that genes at the *Sol*-locus affect the primary structure of the α-chain of hemoglobin. In Hb^sHb^s segregants from a cross NB × BALB/c (defined by Popp as $Sol^3 \times Sol^2$), he demonstrated changes in the two-dimensional electrophoretic and chromatographic patterns of tryptic peptides associated with differences in hemoglobin solubility (*7*). However, differences in α-chain-peptide pattern, hereafter called "fingerprint" pattern, are not always demonstrable in Hb^sHb^s mice differing in hemoglobin solubility. For example, no α-chain-peptide difference has been found between Popp's prototype for Sol^1 (C57BL) and his prototype for Sol^2 (SEC), between C57BL (Sol^1) and BALB/c (Sol^2), or between C57BL (Sol^1) and HBS (Sol^2) (*1*, *8*). These findings are subject to two different interpretations: there might be differences in α-chain structure which do not affect "fingerprint" patterns; or the difference in solubility between C57BL, SEC, and HBS could be controlled by alleles at a locus distinct from that determining α-chain structure in NB and BALB/c hemoglobins. We have tested these two possibilities by studying segregations for hemoglobin solubility and for α-chain structure in mice produced in crosses between the chinchilla-colored ($c^{ch}c^{ch}$) SEC/1Re-*Se* inbred strain (hereafter called SEC), and the albino (*cc*) SWR/J inbred strain (hereafter called SWR), both of which are homozygous for Hb^s (that is, they contain only a single β-chain).

We have limited our crosses to strains which have only one hemoglobin, since the presence, even in heterozygotes, of extra hemoglobins with differing non-α-chains introduces large solubility differences which modify and obscure effects of α-chain substitutions. The hemoglobin of our strain SEC mice is very insoluble (Sol^2, optical density of standard preparations, 0.1 to 0.2 in our test system), while that of SWR has a higher solubility, similar to but not identical with that of C57BL/6J (*4*, *9*). The α-chain of SEC contains peptide α4, also found in C57BL/6J, while that of SWR contains α4H, staining for histidine (*10*), also found in strain FL/Re (*1*). Previously reported techniques for studying α-chain inheritance by classification of the peptide composition of mouse hemoglobins (*11*) required two-dimensional "fingerprinting" to identify α4H (from FL/Re); however, we find that one-dimensional chromatography of crude hemoglobin digests is quite adequate for distinguishing the presence or absence of a single dose of α4H (*12*).

Table 1. Segregation of differences in hemoglobin solubility, α-chain structure, and coat color in 24 mice produced by backcrossing (SEC × SWR) F_1 × SEC, demonstrating complete correspondence between high hemoglobin solubility [measured as optical density (O.D.) of hemoglobin remaining in solution, values above 0.30] and positive histidine stain in peptide α4 on chromatograms, versus low hemoglobin solubility (optical density below 0.20) and absence of histidine stain in peptide α4. Coat-color difference assorts independently of both hemoglobin characteristics. Twelve mice in each experiment.

O.D. Hb. solub. (mean ± S.E.)	Histidine peptide α4	Color genotype	
		$c^{ch}c^{ch}$	$c^{ch}c$
0.13 ± .01 (0.09 − 0.17)	All negative	4	8
0.38 ± .01 (0.30 − 0.45)	All positive	4	8

Twenty-four progeny from backcrosses of (SEC × SWR)F_1 mice to the SEC inbred strain were classified (at the Jackson Laboratory) according to albino alleles carried ($c^{ch}c^{ch}$ or $c^{ch}c$ by progeny test) and according to solubility of hemoglobin (*13*, *14*). The mice were individually identified by ear punch and shipped to the University of Kentucky where their hemoglobins were classified according to whether they contained α4 or α4H. Prior knowledge of the solubility was withheld from the investigators at the University of Kentucky. Results of this experiment are given in Table 1. Exact agreement was obtained between hemoglobin solubility and the nature of the α-chain. However, it is likely that this difference is not due to the presence or absence of histidine in peptide α4, since strain SEC hemoglobin has α4 (no histidine) but is much less soluble than either C57BL (α4) or SWR (α4H), both of which have similar solubilities. The low solubility characteristics of SEC hemoglobin and the Sol^2 allele previously reported to segregate independently of the β-chain is shown here to be due to an undefined feature of the α-chain of SEC mice, which has not been visualized in "fingerprint" studies reported to date. Our data also confirm independent assortment (*5*) between genes at the α-chain and albino loci (Table 1).

Popp has reported breeding tests eliminating for several *Sol*-alleles both sex-linkage and close linkage with a variety of genes on several different autosomes (*6*, *9*). Because of the great advantage which would accrue from finding a close genetic linkage between the α-chain structural locus and a marker gene with clear-cut expression, we have employed the solubility method to extend the series of *Sol*-linkage tests, using efficient methods of backcrossing as follows: for dominant markers (for

example, *Sl*), (C57BL/6-*Sl* × SEC)F_1 × C57BL/6; for recessive markers (for example, *ln*), (C57BL/6-*lnln* × SEC)F_1 × C57BL/6-*lnln*. In each case, the indicated marker gene and the Sol^1 allele of C57BL/6 were contributed by the same parent. Recombination percentages indicate absence of linkage with marker genes belonging to linkage groups III (Patch, *Ph*, 53 ± 8.0), XI (White, Mi^{w^h}, 68.2 ± 9.9), XIII (leaden, *ln*, 66.7 ± 10.3, and fuzzy, *fz*, 61.9 ± 10.6), and XVI (Varitint-waddler, *Va*, 52.4 ± 10.9). Results obtained for crosses involving *Sl*, in linkage group IV, suggest but do not prove loose linkage (36.6 ± 7.7) between the *Sol* locus and the *Sl* locus. Matings have been set up involving other genetic markers in linkage group IV, in order to test this possibility.

Particular peptide markers for both α-chains [from our work and that of Popp (*7*)] and β-chains (*11*) of certain mouse hemoglobins are now available to use for rapid screening of large numbers of mice, greatly facilitating definitive linkage experiments. Wherever it is applicable, the rapid one-dimensional chromatographic method for distinguishing hemoglobins with peptides α4 and α4H is preferable in linkage experiments to the currently used solubility test for two reasons. Several variables in technique, including concentration of hemoglobin in solution, temperature and time of incubation, *p*H (*13*), and molarity (*2*) of salt solution, alter the solubility of many mouse hemoglobins tested, so that within experiments which depend upon solubility for identification of hemoglobins, conditions must be carefully selected and controlled (*13*), and quantitative comparisons between systems may be misleading. Also, the chromatographic method may be applied reliably to hemoglobins from mice carrying the Hb^dallele, whose presence complicates identification of α-chain solubility effects.

The symbol now in use for the α-chain template locus, *Sol*, relates to a property not limited to effects of α-chain substitutions. The particular complex of non-α-chains found in mice carrying the Hb^d allele, whose presence the β^s-chain of human sickle-cell hemoglobin. If suitable mouse β-chain mutants become available for testing, it is probable that some of these also will influence hemoglobin solubility. In view of the accepted concept of a polypeptide chain as the direct product of a cistron, and in view of increased understanding of mouse hemoglobin genetics, we urge that serious consideration be given to replacement of the current *Sol* and *Hb* genetic designations by more appropriate symbols clearly associating each template locus with its respective polypeptide chain.

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12. Mice were killed; hemoglobin was prepared as usual except that dialysis was omitted (*1*), and 40 mg of undialyzed hemoglobin was digested in a final volume of 2 ml in the usual way for "fingerprinting" (*1*). Spots (100 μl) of each of 12 or 13 digests were placed on the long dimension (near the edge) of a sheet (46.3 × 57.2 cm) of Whatman No. 3 chromatography paper. The papers were developed in the short direction for 22 hours by descending chromatography with the upper phase of *n*-butanol, acetic acid, and water (4:1:5 by volume). After drying overnight, the spotted sheets were sprayed with Pauly's reagent and the presence or absence of the histidine-containing peptide α4H was noted.
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14. Solubility of hemoglobin was determined by a procedure simplified from those described previously (*2*, *13*); 40 μl of blood was drawn directly from the orbital sinus of a nonanesthetized mouse into heparinized capillary tubes, and mixed with 2 volumes of distilled water in a small beaker. After lysis of all cells, the hemoglobin plus stroma were added to 3 ml of 2.8*M* KH_2PO_4—K_2HPO_4 buffer solution at *p*H 6.8. This was immediately filtered through No. 2 Whatman paper, to remove precipitated stroma and serum proteins, and the filtrate was incubated for a period of 7 hours at room temperature. Solutions were again filtered through No. 2 Whatman paper and the density of hemoglobin remaining in solution (optical density) was determined in a spectrophotometer at wavelength 540 mμ.
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Wheat: Reconstitution of the Tetraploid Component (AABB) of Hexaploids

Abstract. *The tetraploid AABB genomic component of two varieties of common hexaploid wheat (AABBDD; 2n = 42) was reconstituted by a simple backcrossing technique in which known phylogenetic relationships between the hexaploid and tetraploid groups of* Triticum *were used. The reconstituted tetraploids do not closely resemble commonly described varieties of the present-day group of tetraploid species. The plants are dwarfed, lack vigor, and are partially or completely self-sterile, depending on the variety of the hexaploid source. Chromosome pairing is similar to that observed in a variety of durum wheat (AABB; 2n = 28). Synthetic hexaploids derived from hybrids between one of the reconstituted tetraploids and several strains of* Aegilops squarrosa *(D genome) are of normal growth and vigor and are highly fertile.*

The phylogenetic relationships among species comprising the allopolyploid wheat genus *Triticum*, and the related genus *Aegilops*, are perhaps more clearly understood than those of any other polyploid group of plants. The wheats fall naturally into three groups of species: the diploids, tetraploids and hexaploids with 14, 28, and 42 somatic chromosomes, respectively. The chromosome set of the diploid species has been designated as the A genome. The tetraploid group has the full complement of diploid wheat chromosomes and, in addition, a second set of seven chromosomes derived from another diploid species, supposedly *Aegilops speltoides* Tausch. (*1*). This second genome has been designated B so that the tetraploids have the genomic formula AABB. Hexaploid wheats have the two sets of genomes possessed by the tetraploids and a third set, the D genome of the diploid species *Aegilops squarrosa* L. The hexaploids have, therefore, the genomic constitution AABBDD. This last group has evolved by amphiploidization in two steps. First, the tetraploid component originated as an amphiploid through hybridization of diploid wheat (A genome) and *A. speltoides* (B genome) or a