Ice Nuclei from Direct Reaction of Iodine Vapor with Vapors from Leaded Gasoline

Abstract. *Large numbers of ice nuclei, active at −15°C or colder, can be generated by mixing vapors from leaded gasoline with iodine vapor. This reaction will produce 10^6 Aitken nuclei per cubic centimeter in a closed system with a volume of 2 liters. When unleaded gasoline is substituted, no nuclei of either type are detectable.*

Schaefer (*1*) has shown that ice nuclei can be generated by exposing products of automobile exhausts to iodine vapor. My experiments show that ice nuclei, active at −15°C or colder, can also be generated by direct reaction of iodine vapor with vapors resulting from the evaporation of leaded gasoline. Whereas gaseous reactions forming condensation nuclei are quite well known, this is the first case in which the mixing of two gases has been shown to generate freezing nuclei.

Vapors from leaded gasoline, introduced to the cold chamber of an NCAR-Bollay (*2*) ice-nucleus counter, after passing over the surface of a solution (1.5 g per liter) of iodine in mineral oil at room temperature, produce a large number of ice crystals in about 90 seconds. Activity is significant at −15°C and rises sharply at −19° to −20°C. It was not possible to evaluate activity at temperatures warmer than −15°C with the equipment available at the field laboratory.

A significant background of iodine-activated nuclei, even in the rural environment of our field laboratory, necessitated the use of a filtered input to guarantee that the increased number of ice crystals detected was due to the addition of gasoline vapors. Admission of 1 liter of a mixture of air-leaded gasoline vapor with iodine vapor produced about 10^4 ice crystals at −20°C over a 15-minute period. Repetition of this experiment with "white," unleaded gasoline did not produce ice crystals at a temperature of −20°C.

A smaller, but still significant, number of ice crystals can be generated by placing a container of leaded gasoline near the entrance to the filter when iodine vapor is present in the system. The ability of these vapors to penetrate a filter and still form ice nuclei requires a thorough investigation to determine what methods can be used to eliminate the influence of these vapor-induced nuclei from future experiments with a cloud chamber.

Substitution of an Aitken nucleus counter for the ice-nucleus counter yields an impressive result, as more than 10^6 nuclei per cubic centimeter, active at 1.2 expansion, can be detected in the reaction bottle. A bottle with a capacity of at least 2 liters must be used because a short residence time is required for the reaction to take place and for the particles to grow to a size sufficiently large to be detected by the Aitken counter. When unleaded gasoline is substituted for leaded gasoline, no particles can be detected.

These experiments provide additional evidence that not only products from automobile exhausts, but also those from evaporation of leaded gasoline, are effective ice nuclei in the presence of iodine vapor. Because evaporative losses from the carburetor and gas tank can amount to as much as 8 percent of total automotive emission, this can be a significant source of atmospheric lead and freezing nuclei.

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Polymerization of Hemoglobins of Mouse and Man: Structural Basis

Abstract. *Human hemoglobin Pôrto Alegre and mouse hemoglobin (BALB/cJ) polymerize by forming intermolecular disulfide bridges. Both hemoglobins have externally oriented, reactive cysteinyl residues in the A-helix of the beta chain. Hemoglobin Pôrto Alegre can be designated as $\alpha_2\ \beta_2^{9\ Ser \rightarrow Cys}$. The cysteinyl residue of the mouse hemoglobin is at position 13 in the beta chain.*

Hemoglobins that polymerize occur frequently in amphibians and reptiles (*1*); they also occur in certain mice (*2*), the macaque (*3*), and in one Caucasian family (hemoglobin Pôrto Alegre) (*4*). Polymerization of amphibian, reptilian, and mouse hemoglobins occurs by formation of intermolecular disulfide bridges, usually after hemolysis (*5*). Measurements of oxygen equlibria in hemoglobins from the bullfrog, snapping turtle, and certain mice indicate that oxygenation is not changed by polymerization (*6*).

We have now determined the positions of the cysteinyl residues responsible for the polymerization of both human hemoglobin Pôrto Alegre and BALB/cJ mouse hemoglobin. Each hemoglobin contains a reactive cysteinyl residue near the NH_2-terminus of its β-chain, β-9 in Pôrto Alegre and β-13 in BALB/cJ. Residues at both positions are at the surface and are oriented outward according to the tentative atomic model of hemoglobin (*7*). Hemoglobin Pôrto Alegre is one of five known human hemoglobin variants which have amino acid substitutions that do not change the number of ionizable groups. The others are hemoglobins Köln (β-98 Val → Met), (*8*), Genova (β-10 Leu → Pro), Kansas (β-102 Asn → Thr), and Freiburg (β-23 Val → 0) (*9*). Each of these was discovered because the change affected its physiological properties, stability, or electrophoretic mobility. All other known variants (about 50) involve substitutions which alter the number of ionizable groups (*10*). We assume that many neutral variants must exist, but appropriate screening techniques for their detection are lacking.

A Pôrto Alegre hemolyzate (*11*), dialyzed against carbon-monoxide–saturated 0.2*M* NaCl at 4°C overnight, was applied to a column (2.8 by 70 cm) of Sephadex G-100 (*5*). The polymeric fraction (Fig. 1) had a sedimentation coefficient, $S_{20,w}$, of 9.5. The polypeptide chains were separated in 8*M* urea on carboxymethylcellulose (*12*). Vertical starch-gel electrophoresis of the globin at *p*H 1.8 (*13*) showed that the β-chain was absent from its normal position, while the α-chain appeared unchanged (Fig. 1, inset). Polymerization of BALB/cJ hemoglobin was accelerated by incubation

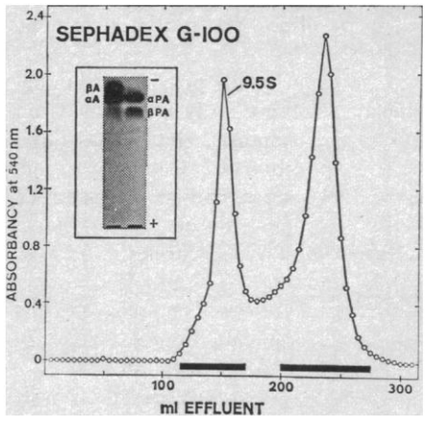

Fig. 1. Isolation of polymeric hemoglobin Pôrto Alegre from columns of Sephadex G-100 with 0.2*M* NaCl. Inset shows the starch-gel electrophoresis at *p*H 1.8 of globin A (left) and globin from 9.5*S* Pôrto Alegre hemoglobin.

of a solution in 0.2*M* NaCl for 72 hours at 30°C under an atmosphere of O_2. Polypeptide chains from polymerized BALB/cJ hemoglobin (*14*) were prepared and analyzed by the same procedures. The cysteinyl residues responsible for polymerization of BALB/cJ mouse hemoglobin were shown to be in the β-chains (*5*). The amino acid composition of the α-chains of both Pôrto Alegre and BALB/cJ hemoglobins showed only a single cysteinyl residue. Amino acid analysis of the β-chains of hemoglobin Pôrto Alegre after oxidation with performic acid (*15*) showed 3.25 cysteic acids; the normal human β-chain has two. Both Pôrto Alegre and BALB/cJ β-chains were aminoethylated (*16*) and digested with trypsin. Tryptic peptides were chromatographed (Fig. 2, a and c) on a column (0.9 by 16 cm) of Spinco PA-35 resin (*9*). Zones I and II containing the βT-2 peptides of BALB/cJ (Fig. 2c) were rechromatographed (Fig. 2d). Quantitative amino acid analysis after hydrolysis in evacuated tubes in 6*N* HCl for 24 hours gave the composition of the peptides (Table 1). These results show that βT-2 of Pôrto Alegre lacks serine and contains cysteine. The amino acid sequence of this peptide, determined by the subtractive Edman procedure (*17*), was Cys-Ala-Val-Thr-Ala-Leu-Try-Gly-Lys (*8*). We can therefore designate hemoglobin Pôrto Alegre as $\alpha_2 \beta_2^{9\ Ser \rightarrow Cys}$.

The βT-2 peptide of BALB/cJ was split into two parts, *a* and *b*, by the original tryptic digestion (Fig. 2c). The rechromatography (Fig. 2d) showed that βT-2a was homogeneous. The adjacent peak is that of ammonia and a contaminating peptide. The Edman procedure gave the sequence Ala-Ala-Val-Ser-Cys. We emphasize the hazard of inferring a sequence by comparison with homologous human peptides. Had we relied on homology we would have inferred the *incorrect* sequence Ser-Ala-Val-Ala-Cys, corresponding to the known human sequence Ser-Ala-Val-Thr-Ala. We also isolated the preceding peptide βT-1 and determined its sequence to be Val-His-Leu-Thr-Asx-Glx-Ala-Lys. The rechromatography of βT-2b showed the presence of two homologous peptides, βT-2b$_1$ (17 percent of the total) and βT-2b$_2$ (83 percent of the total). From their compositions we *infer* their probable sequences: Leu-Try-Ala-Lys (b_1) and Leu-Try-Gly-Lys (b_2).

The βT-2 peptides of both Pôrto

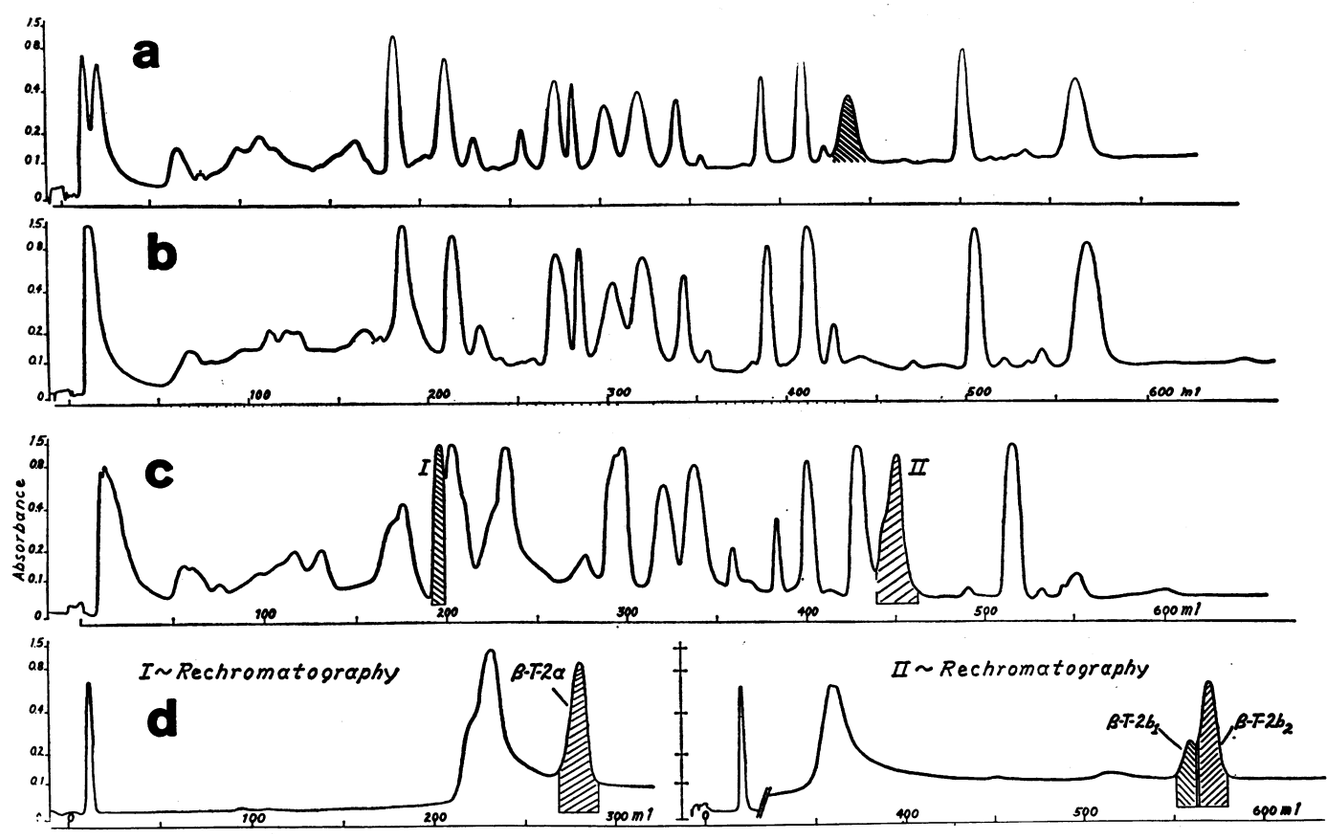

Fig. 2. Comparison of tryptic peptide patterns of aminoethylated β-chains. (a) Pôrto Alegre; (b) normal human A; (c) BALB/cJ mouse. The shaded zone in (a) contains the βT-2 peptide of hemoglobin Pôrto Alegre. Zones I and II in (c) contain βT-2a and βT-2b peptides, respectively. (d) The rechromatography of zones I and II on columns (0.9 by 50 cm) of BioRad AG-50-X2 resin. Cysteic acid was added as a marker to each sample; it emerges as the first peak in each chromatogram.

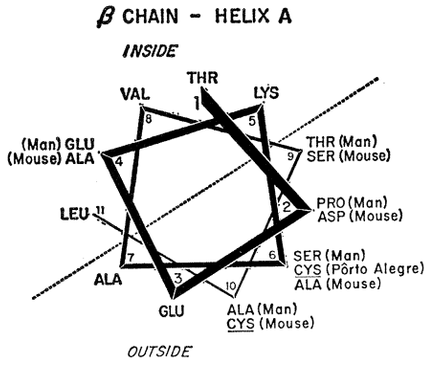

Fig. 3. Diagram of the approximate arrangement of amino acids in helix A of the β-chain, showing the positions of the cysteinyl residues responsible for polymer formation. Residues in positions 2 and 3 in mouse hemoglobin are uncertain and could be either asparagine or aspartic acid, or glutamine or glutamic acid, respectively.

Alegre and mouse BALB/cJ hemoglobins form part of the A-helix near the NH_2-terminus (*7*). The approximate arrangement of the first 11 residues is shown diagrammatically (*18*) in Fig. 3 which summarizes the data for the A-helix of the β-chain of mouse and human hemoglobins. The cysteinyl residues in this part of the β-chain of both Pôrto Alegre and BALB/cJ hemoglobins are similarly oriented outward. This arrangement explains the ease of polymer formation. These results may explain why Rucknagel *et al.* (*19*) observed the loss of a tryptophan-positive peptide, βT-2, among the soluble tryptic peptides of hemoglobin Pôrto Alegre. Hutton *et al.* (*20*) observed the loss of the same peptide in digests of AKR and FL mouse hemoglobin. Presumably two βT-2 peptides joined by disulfide linkage were rendered insoluble.

Table 1. Molar ratios of amino acids in the βT-2 peptides from hemoglobins Pôrto Alegre and BALB/cJ.

Amino acid	Pôrto Alegre	BALB/cJ		
	βT-2	βT-2a	βT-2b$_1$	βT-2b$_2$
Lysine	0.9		1.14	0.99
Histidine	0.06			
AE-cysteine	0.5	0.97		
Aspartic acid	0.05			
Threonine	0.98			
Serine	0.04	0.99		
Glutamic acid	0.08			
Glycine	1.02	0.02	0.29	1.09
Alanine	1.95	2.00	1.02	0.02
Valine	0.99	1.04		
Leucine	1.03		0.84	0.92
Phenylalanine	0.05			
Tryptophan*	+		+	+
Micromoles analyzed	0.03	0.07	0.04	0.10

* Presence detected either by Ehrlich's reagent (*26*) or by amino acid analysis of residual quantity remaining after acid hydrolysis.

Each polymerizing hemoglobin tetramer ($\alpha_2\beta_2$) has two extra cysteinyl residues, one on each β-chain. Each of these is capable of reacting with the corresponding residue of another $\alpha_2\beta_2$ unit. This explains why aggregates larger than octamers occur. Although polymerization to octamers occurs most readily, the reaction continues and components with sedimentation coefficients of approximately 8*S* to 11*S* are frequently observed (*6, 21*).

Much confusion has surrounded the interpretation of the electrophoretic patterns of mouse hemoglobins which have been designated either "single" or "diffuse" according to their appearance (*22*). Mouse hemoglobins with "diffuse" patterns appear to be heterogeneous (*20, 23*). If mixtures of two electrophoretically distinct hemoglobins give a diffuse pattern rather than two discrete bands, we infer that the components are interacting with each other. Treatment of fresh hemolyzates with iodoacetamide (IAA) prevented polymerization of certain mouse hemoglobins (*5*), but did not eliminate the diffuse appearance of the electrophoretic pattern. Only that part of the diffuse pattern associated with the polymeric species was absent. We mention this because these observations on polymerization and the electrophoretic patterns have been widely misinterpreted to mean that polymerization explains diffuseness (*24*), or that the minor components accompanying the major hemoglobin component are absent from IAA-treated hemolyzates (*23*).

We have not yet isolated the different components of BALB/cJ mouse hemoglobin. However, the presence of two βT-2b peptides (Table 1) indicates heterogeneity. Peptides βT-7, 11, and 14 are also heterogeneous. If we assume that the relative yields of the βT-2b peptides are equal, two component chains in the proportion of 1 to 5 would be present. We cannot know the number of unique β-chains until they are isolated. This heterogeneity may have arisen either by gene duplication followed by mutation or by translation errors, as discussed by others (*25*). Rifkin *et al.* (*25*) found that the α-chains of SEC mice were heterogeneous: half had threonine in position 68, the rest had serine.

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