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Functional Aspects of Hemoglobin Evolution in the Mammals

A. F. SCOTT[1], H. F. Bunn[2] and A. H. BRUSH[3]

[1]Division of Medical Genetics, Department of Medicine,
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
[2]Hematology Division, Department of Medicine, Peter Bent Brigham Hospital,
Boston, Massachusetts 02115
[3]Biological Sciences Group U-42, University of Connecticut,
Storrs, Connecticut 06268
USA

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Summary. Comparative studies of red cell 2,3 Diphosphoglycerate (DPG) and its effect on hemoglobin oxygen affinity from a taxonomically diverse set of mammals indicate two anomalous groups: members of the superfamilies Bovoidea (Actiodactyla) and Feloidea (Carnivora). In both taxa all of the individuals assayed had very low or unmeasurable quantities of DPG and red cell lysates with little, if any, DPG effect as measured by the change in oxygen affinity in the absence and presence of the phosphate. However, in both groups compensatory changes have occurred in hemoglobin structure and function so as to reduce the native oxygen affinity and thus cause them to resemble the hemoglobins of DPG-utilizing mammals as they occur in the setting of the red cell. We conclude that this parallelism of function is the result of convergent evolution.

Key words: Hemoglobin/2,3 Diphosphoglycerate (DPG)/Mammals/Molecular Evolution

Understanding the mechanisms by which proteins evolve is a central problem in modern biology. Whether molecular changes occur primarily through random processes or directional selection is currently controversial. Evidence for selectively neutral mutations has been inferred from a presumed constant amino acid substitution rate derived from sequences of homologous proteins from different species (Kimura & Ohta, 1971). An implicit underlying assumption is that these homologous are functionally equivalent, or if dissimilar, do not differ in selectively appropriate ways. Although for most proteins there is insufficient knowledge of their functional role in the micro-

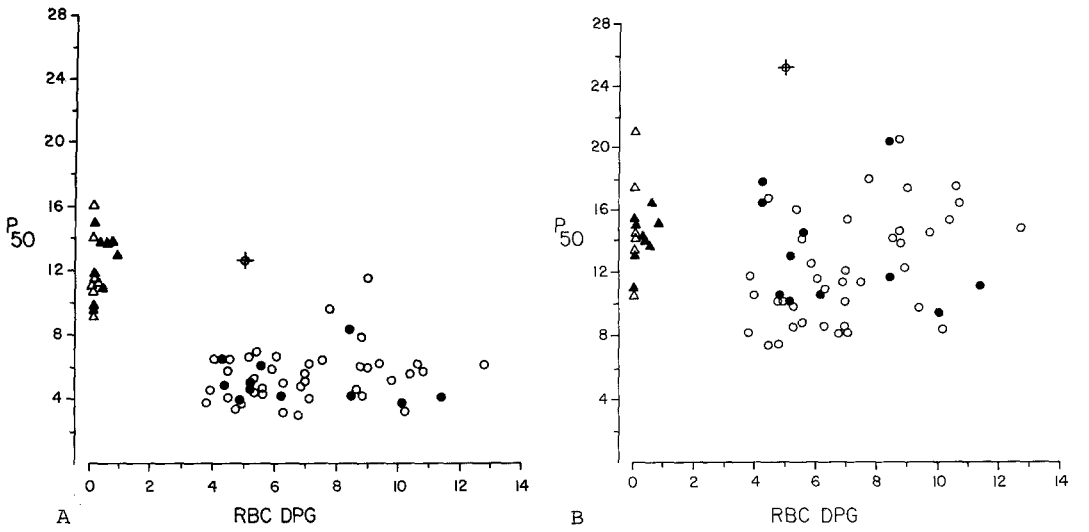

Fig.1A and B
The distribution of species according to their red cell DPG concentration plotted against the P_{50} of their hemoglobin (0.1 mM tetramer in 0.5 mM bis-Tris HCl, pH 7.2 at 20 °C, 0.1M NaCl) without DPG (A) and with 1.0 mM DPG (B). Details of experimental methods are cited in Bunn et al. (1974); complete sets of data are cited in Scott et al. (1976). Ruminants △, feloids ▲, primates ●, opossum ⊕, other DPG synthesizing mammals ○

environment of the cell to assess what might be selectively relevant properties in vitro, this difficulty is not insurmountable as Blundell & Wood (1975) demonstrated for insulin and as we hope to show for at least certain properties of hemoglobin.

Most mammals (28 of 33 families studied) synthesize red cell 2,3 Diphosphoglycerate (DPG) in molar concentrations nearly equivalent to those of hemoglobin (Bunn et al., 1974). This metabolite stabilizes the deoxygenated hemoglobin tetramer by forming salt bridges between the amino termini of the beta chains, reduces the affinity of the protein for oxygen and raises the partial pressure of oxygen at which the blood is half saturated (P_{50}) (Perutz, 1970). In humans, the oxygen affinity of the blood can be controlled through the modulation of DPG synthesis so that sufficient oxygen is delivered during periods of physiological stress, as in anemia or hypoxia (Benesch & Benesch, 1974).

In contrast, DPG occurs in low concentrations in cat, viverrid and hyaenid (order Carnivora, superfamily Feloidea) red cells and in nearly undetectable amounts in the blood of adult cervids, bovids, and antelocaprids (superfamily Bovoidea) (Bunn et al., 1974; Rapoport & Guest, 1941; Dhindsa et al., 1974) and giraffes (superfamily Giraffoidea) (Charache et al., 1975) of the Artiodactyla. Further, hemoglobins of the feloids

and ruminants respond weakly to DPG as measured by the change in P_{50} in its absence and at nearly saturating concentrations (Fig.1A,B) (Bunn et al., 1974). Although hemoglobins of DPG-synthesizing mammals generally have an intrinsically high affinity for oxygen, the feloids and ruminants both have hemoglobins with a low affinity for oxygen. This example of functional parallelism represents convergent evolution at the molecular level.

The loss or reduction in DPG modulation of oxygen affinity may have presented both feloids and ruminants with physiological shortcomings that have been partially compensated by different mechanisms of regulation. Domestic cats are known to be peculiarly susceptible to hypoxia (Reeves et al., 1963) and when made anemic experimentally, modify oxygen delivery by synthesizing increased (although still low) amounts of DPG as well as the normally minor hemoglobin B that has a high oxygen affinity and is insensitive to phosphate (Mauk et al., 1974). In response to hypoxia or anemia, goats and some sheep turn on a normally unexpressed gene resulting in the synthesis of hemoglobin C, with presumably advantageous properties (Huisman et al., 1969).

The structural basis for the failure of DPG binding in ruminants is a deletion of the second residue in the beta chain. The loss of this amino acid reduced the length of the polypeptide by three Angstroms and, as a result, increases the distances between the N-termini of the two beta chains to about 22 Angstroms (Bunn, 1971). DPG cannot bridge this gap and form the normally stabilizing linkages. The small DPG effect of cat blood has been partially explained by unusual structural features of the feline hemoglobins. Type A hemoglobin, with a weak DPG effect, has an N-terminal glycine and a phenylalanine substituted for the beta 2 histidine (Taketa, 1973) which may interact with DPG (Arnone, 1972). Type B hemoglobin, with no DPG effect, has both a terminal serine which is blocked by an acetyl group as well as phenylalanine at the beta 2 position (Taketa, 1973). Thus, the functional similarities in DPG binding between feloid and ruminant hemoglobins are achieved by entirely different structural means.

One possible explanation for the evolution of functionally similar hemoglobins among the feloids and ruminants is that there was gradual selection in each group for molecules with a low affinity for oxygen accompanied secondarily by loss of the DPG effect. By this scheme, the opossum, whose hemoglobin has a low oxygen affinity but a normal DPG effect, may represent an intermediate step. An alternative explanation is that mutations occurred first in both feloid and ruminant ancestries which led to the sudden inability of their hemoglobins to bind phosphate and the affinity of their hemoglobins for oxygen changed subsequently. The mutation in the ruminants was almost

certainly a deletion, but in the feloids it may have been a base change leading to a substitution at the DPG binding site. If, like the majority of living mammals, the ancestral feloids and ruminants had hemoglobins with a high oxygen affinity, then the sudden loss of the DPG effect would have created a problem of unloading sufficient oxygen at the tissues. This would be analogous to human hemoglobinopathies that involve high oxygen affinity mutants or metabolic conditions such as hexokinase deficiency in which DPG synthesis is reduced (Delivoria-Papadopoulos et al., 1969).

There are several possible ways to explain how mutations with such presumably drastic physiological consequences could have survived to fixation in each group. First, the P_{50} of whole blood has a weak correlation to body weight (Schmidt-Nielsen & Larimer, 1958) and large mammals have relatively low values. If the early ruminants and feloids were large, then the loss of the DPG effect would have been of less consequence. However, supportive fossil evidence for this alternative is lacking. Second, as discussed above, feloids and ruminants may have been pre-adapted for the loss of DPG binding if they had evolved hemoglobins with low oxygen affinity. We discount this alternative because it implies gradual loss of a regulatory mechanism which would seem, from other evidence, to be important for successfull homeostasis. Third, as Coates (1975) has suggested, loss of DPG imparted ruminant hemoglobins with a greater heat exchange capability and thus may serve as an important mechanism to reduce evaporative water loss. Fourth, the ancestral forms from both groups may have had a lower metabolic rate than predicted from present metabolism-body weight allometric regressions, as do certain present-day mammals that are poor homeotherms (Schmidt-Nielsen, 1974). This would make reduced oxygen delivery compatible with their metabolic demands. This latter explanation may also account for the relatively high P_{50} observed for the opossum. Lastly, other physiological or behavioral characteristics, such as greater cardiac output or different hunting or grazing strategies, may have partly ameliorated the hypothesized shortcomings in oxygen delivery as they often do for human hemoglobin mutants (Parer, 1970).

If the sequence changes which distinguish homologous proteins represent selectively neutral mutations accumulating by stochastic processes, then the homologues should be identical or closely similar in their functional properties in vivo, if not in vitro (Kimura & Ohta, 1974). Feloid and ruminant hemoglobins clearly do not fit this pattern. By whatever mechanism the original mutations were tolerated, other than the pre-adaptation hypothesis which implies prior selection, natural selection has acted independently and in parallel in each group. The fact that the low oxygen affinities of both rumi-

nant and feloid hemoglobins without DPG closely resemble the affinities of other mammalian hemoglobins in the presence of phosphate can be most reasonably understood as an evolutionary compensation for the loss or reduction of DPG binding by these hemoglobins. In each group the result was a red cell that provided oxygen delivery appropriate for animals of large size. Although we can be reasonably certain of the structural bases for DPG binding, the structural determinants contributing to the differences in oxygen affinity between mammalian hemoglobins is less clear. However, ruminant hemoglobin chains, for which sequence data are available, do not show a relatively large number of codon substitutions (Barnabas et al., 1971; Goodman et al., 1973), yet clearly structural changes have occurred which account for the altered oxygen affinity. On this basis we argue that positive Darwinian selection as reflected in function is not necessarily observed as significant perturbations in the rate of molecular evolution.

This does not eliminate the possibility that some, or indeed most, substiutions that occur in evolution are selectively neutral. For the mammals that regulate oxygen affinity with DPG there are few correlates of hemoglobin function with phylogeny. Indeed, the primates differ in their P_{50} values with and without DPG nearly as much as all other DPG synthesizing mammals (Fig.1A and B). Perhaps, by retaining hemoglobins which bind DPG and allow its allosteric regulation of whole blood oxygen affinity, drift can be permitted in other structural sites of the molecule even to the extent of changing certain intrinsic properties. This is because the different hemoglobins can be fine-tuned by appropriate DPG synthesis in order to optimize the oxygen carrying properties of the whole blood. The physiological flexibility afforded by such allosteric regulation may have allowed, in part, the rapid evolution of the mammals because the different oxygen demands required by animals of different size and metabolic rates did not require the necessity of specifically tailored hemoglobins.

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