SHORT COMMUNICATION

A POSSIBLE ROLE FOR PROTEIN DISSOCIATION IN THE FUNCTIONING OF EMBRYONIC HAEMOGLOBINS

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During early mammalian development the changing demands for oxygen in embryonic tissues are met, in part, by the synthesis of a series of embryonic haemoglobins. At the earliest stages of development three embryonic haemoglobins are synthesized in both humans and mice (Melderis, Steinheider & Ostertag, 1974; Shimizu & Watanabe, 1978; Brotherton, Chui, Gauldie & Patterson, 1979; Purdie, Wells & Brittain, 1983; Brittain & Wells, 1983). The proportions of each haemoglobin present in the mouse red blood cells vary significantly from the ninth day of gestation until parturition (Purdie et al. 1983). Although studies have been made on this developmental system of gene switching and haemoglobin composition (Brotherton et al. 1979) very few studies have been made on the functional characteristics of these embryonic haemoglobins, mainly because of the limited amount of material available (Bauer et al. 1975; Brittain & Wells, 1983; Purdie et al. 1983). The oxygen binding curves for whole blood from the earliest embryos show anomalous binding patterns (Wells & Brittain, 1981; Purdie et al. 1983; Brittain & Wells, 1983). The high oxygen affinity component present in the red blood cells of early embryonic mice has been correlated with the presence of embryonic haemoglobin EI, which has a subunit structure $\alpha_2\epsilon_2$ (Melderis et al. 1974). This haemoglobin species EI shows very low cooperativity in both its equilibrium and kinetic functions (Purdie et al. 1983; Brittain, Sutherland & Greenwood, 1986). Recent investigations have indicated that the functioning of this haemoglobin is not related to any unusual redox balance within the embryonic red blood cell (Brittain & Tottle, 1986) and thus the occurrence of an essentially non-cooperative haemoglobin with a tetrameric subunit structure has posed something of a paradox. This study presents data on the dimerization of mouse embryonic haemoglobin EI over a range of conditions and, together with numerical simulations of oxygen binding curves, illustrates how, within the early embryonic red blood cell, high oxygen affinity and sensitivity to pH may be obtained by protein dimerization.

Embryonic haemoglobin EI was isolated from embryo C57 BL6J mice of 13 days gestation and purified as previously described (Purdie et al. 1983). Equilibrium constants for the dissociation of haemoglobin tetramers to dimers were calculated...
from broad-band gel exclusion chromatography experiments using procedures described previously (Ackers & Thompson, 1965; Sasaki et al. 1978). The pH dependence of the dissociation constant for the oxy form of the protein was determined using air-equilibrated buffers in the range of pH 6–8. (A single measurement made on the deoxy form of the protein in the presence of sodium dithionite, using nitrogen-equilibrated buffers, yielded an apparent molecular mass indistinguishable, by this method, from that expected for the tetrameric form of the protein.)

Oxygen binding curves at various haemoglobin concentrations were simulated using the equations of Imai & Yonetani (1977) which relate binding affinity to the degree of dimerization:

\[ Y = f Y_2 + (1-f) Y_4, \]  
where

\[ f = \left( \frac{K_{4,2} Y_4}{2CY_2^4} \right) \left[ \sqrt{1 + \left( \frac{4CY_2^4}{K_{4,2} Y_4} \right)} - 1 \right] \]

and

\[ Y_4 = \left( \frac{K_{4p}^n}{1 + (K_{4p})^n} \right), \]
\[ Y_2 = \left( \frac{K_{2p}}{1 + (K_{2p})} \right), \]

where \( Y \) is the fractional saturation of the system at oxygen tension \( p \); \( f \) is the fraction of haems present as dimers at a total haemoglobin concentration \( C \); \( K_{4,2} \) is the tetramer–dimer equilibrium constant; \( Y_4 \) and \( Y_2 \) are the fractional saturations of tetramers and dimers, respectively; \( K_4 \) and \( K_2 \) are the affinity constants of tetramers and dimers, respectively; and \( n \) is the Hill coefficient associated with the cooperative tetramers. \( K_{4,2} \) was assigned a value determined from the dissociation experiments and, in the absence of accurate experimentally determined values, the constants \( K_4 \) and \( K_2 \) were given values reported in the literature for human haemoglobin (Imai & Yonetani, 1977). In order to obtain curves which could be expected to approach those due to EI in embryonic red blood cells the concentration of EI at 13 days gestation was employed.

At pH 7, adult mouse oxyhaemoglobin showed a pattern of dissociation characterized by a \( K_{4,2} \) value of 0.7 \( \mu \)mol l\(^{-1} \) (±0.3 \( \mu \)mol l\(^{-1} \) s.e.) (Fig. 1) which is very similar to the value obtained for adult human oxyhaemoglobin in phosphate-containing media (Chiancone, Gilbert, Gilbert & Kellett, 1968; Barksdale & Rosenberg, 1978). Mouse embryonic oxyhaemoglobin EI showed a more marked tendency to dissociate into dimers (Fig. 1) characterized by a \( K_{4,2} \) constant at pH 7 of 200 \( \mu \)mol l\(^{-1} \) (±30 \( \mu \)mol l\(^{-1} \)), a value similar to that for human oxyhaemoglobin Kansas (Atha & Riggs, 1976). At very low concentrations oxyhaemoglobin EI also showed some evidence of further dissociation to monomers. When the pH at which the measurements were made was altered, EI showed significant changes in its tetramer–dimer dissociation constant (Fig. 2). Although the magnitude of the equilibrium constant associated with EI is larger, at all pH values, than that associated with the adult protein, the pattern of its pH dependence closely matches that previously reported for the adult human protein (Barksdale & Rosenberg, 1978).

The combination of a high dissociation constant and the marked pH sensitivity seen for EI leads to the possibility that dissociation might play a role in the control of
Fig. 1. Dissociation as a function of concentration for adult mouse (○) and embryonic
El (●) haemoglobins. The samples were run in 0·1 mol l\(^{-1}\) phosphate buffer at pH 7·0.
\(N = 3\). Oxygen tension = 155 mmHg (1 mmHg = 133·3 Pa).

Oxygen delivery in the embryo, a situation not possible in the case of the adult
protein. Even though the adult protein dissociates, the equilibrium constant is so low
that under normal conditions dimerization of adult haemoglobin is minimal. The
intracellular concentration of El is only 600 \(\mu\)mol l\(^{-1}\). Thus a dissociation constant of
the order of 200 \(\mu\)mol l\(^{-1}\) implies that changes in intracellular solution conditions,
such as pH, organic phosphate concentration, ionic strength or oxygen concen-
tration, all of which are known to affect dissociation (Antonini & Brunori, 1971),
would produce very significant alterations to the proportions of dimers present
(Fig. 3). It is well known that dimers of haemoglobin are non-cooperative and have a
very high oxygen affinity (Hewitt, Kilmartin, Ten Eyck & Perutz, 1972; Ackers,
Johnson, Mills & Ip, 1976). Thus any change in the proportions of dimers present

Fig. 2. The pH dependence of the tetramer–dimer equilibrium constant (\(K_{4,2}\)) obtained
for embryonic haemoglobin El. \(N = 3\).
Fig. 3. Simulated oxygen binding curves for haemoglobin E1, which include the effect of dimerization. The curves shown are those for the pure tetramer (——) and haemoglobin at the concentration of 40 µmol L⁻¹ employed in previous kinetic experiments (— — — ). A curve for E1 at its concentration within the embryonic red blood cell is also shown, calculated on the assumption that the dimerization constant is the same in the red blood cell as in solution (— — — —). 1 mmHg = 133.3 Pa.

can readily change both the cooperativity and oxygen affinity of the system. At present the intracellular composition of embryonic red blood cells has not been determined. However, the correlation of an essentially hyperbolic component in the oxygen binding curves of whole embryonic blood with the amount of E1 present (Purdie et al. 1983) suggests that oxyhaemoglobin E1 is appreciably dissociated into dimers within the embryonic red blood cell.

The fact that the deoxy protein appears to be essentially tetrameric in form suggests that, in the case of E1, a lowering of oxygen concentration might lead to a shift towards a low-affinity protein and hence an enhanced oxygen delivery in embryonic red blood cells. The dissociation mechanism outlined above thus explains the former apparent paradox, seen in kinetic experiments performed at haemoglobin concentrations of 50 µmol L⁻¹ (Brittain et al. 1986), of a tetrameric haemoglobin with essentially no cooperativity, and may represent a mechanism for the control of haemoglobin oxygen delivery within the embryonic red blood cell modulated by intracellular composition. Some preliminary evidence is available that this mechanism may be common to a number of mammalian embryonic systems (Wells & Brittain, 1981, 1983; Purdie et al. 1983).

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REFERENCES

Functioning of embryonic haemoglobins


