the partial relief of strain in the liganded structure. Haemoglobin in solution with strong allosteric effectors bound has a T-state affinity comparable with that of haemoglobin in the crystal^{27,28} suggesting that it also has a liganded conformation similar to that of the crystal and will exhibit a much reduced or absent tertiary Bohr effect²⁶

Some tertiary conformational changes on ligand binding do, however, occur in the crystal¹⁹. As for crystals of haemoglobin in the R quaternary structure^{9,29,30}, the decrease in polarization ratio on oxygenation (Fig. 1b) indicates a change in β haem orientation (the X-ray structure shows that there is very little change in the α haem orientation). The calculated β haem tilt (A. M. et al., unpublished observation) is very close to that found for carbon monoxide binding in the T quaternary structure of the metal hybrid, $\alpha(Ni)_2\beta(FeCO)_2^{31}$. This finding suggests that the tertiary conformational change in β subunits is very similar in the crystal for oxygen and carbon monoxide binding. These conformational changes do not, however, generate any cooperativity or Bohr effect.

The final point to discuss is the difference between the affinities of the α and β subunits. In the electron density map calculated from the X-ray data, the binding site for the α haems was fully occupied by an oxygen molecule, whereas no oxygen molecule was bound to the β haems¹⁷⁻¹⁹. This result predicts a distinctly biphasic binding curve (provided that binding is noncooperative). By contrast, the observed binding curve is monophasic. Furthermore, the linearity of the Hill plots (Fig. 2) indicates that the α and β binding constants differ by less than a factor of 3, compared with a factor of at least 15 predicted by the X-ray result (assuming an uncertainty of 20% in the occupancies of the oxygen binding sites³²). Also, the α and β haems have different orientations with respect to the a and c crystal axes, and therefore contribute differently to the absorption. The p50 calculated from the a-axis data, where the α and β haems contribute equally to the absorption, is about 5% higher than for the c-axis data, where the α haems contribute 55-60% of the absorption. We can thus estimate that the α haems have about double the affinity of the β haems, which is similar to what is observed in solution for the T state both in the presence and in the absence of inositol hexaphosphate^{33,34}. Could the discrepancy between the X-ray and optical studies in the fractional oxygenation of the β haems be due to the fact that the oxygen molecules are 'invisible' in the electron density map because of disordering of the distal oxygen atom? To answer this question will require the determination of the X-ray structure of crystals for which the fractional saturation with oxygen and fractional oxidation are known from their optical absorption spectra. \Box

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CORRECTIONS

Negative regulation of human c-fos expression by the retinoblastoma gene product

Paul D. Robbins, Jonathan M. Horowitz & Richard C. Mulligan

Nature 346, 668-671 (1990)

THIS letter in the 16 August 1990 issue of Nature reported that the protein product of the retinoblastoma susceptibility gene (RB-1) can repress c-fos expression and AP-1 transcriptional activity in both serum-induced and cycling 3T3 cells. We also identified a cis-acting element in the human c-fos promoter that can confer repression by Rb to a heterologous promoter. We now report that the intact c-fos promoter and the deletion mutants of the c-fos promoter used in the study were incorrectly described as being of human origin. They are, in fact, of mouse origin. The oligonucleotide RCE element, however, is indeed of human origin as stated. Aside from the erroneous description of the promoter sequences, the data and conclusions of the study remain as reported. Our recent unpublished studies confirm that, as expected, an intact human c-fos promoter (which contains the RCE sequences identified in our report) is negatively regulated by Rb, and that an oligonucleotide representing mouse sequences analogous to the human RCE (-100 to -62) also confers negative regulation by Rb upon the TK promoter. We regret the confusion that the error has caused.

Action potentials must admit calcium to evoke transmitter release

Rosel M. Mulkey & Robert S. Zucker

Nature 350, 153-155 (1991)

IN the above letter in the 14 March issue, the number given for the increase in frequency of miniature e.p.s.ps due to DMnitrophen photolysis in normal Ringer's (1,700-22,500%) is incorrect and should read 1,700-12,500 Hz (middle of the left column, page 155).

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