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THE HAEM-ACCESSIBILITY TO SOLVENT AND ALLOSTERIC EFFECTS IN DIFFERENT PROTOMERS OF HUMAN HAEMOGLOBINS AS OBSERVED BY PROTON MAGNETIC RELAXATION

S. VUK-PAVLOVIĆ,[1] B. BENKO,[1] S. MARIČIĆ,[1] B. MARKOVSKA[2] AND G. D. EFREMOV[2]

[1]Macromolecular Biophysics Laboratory, Institute of Immunology, Rockefellerova 2, 41000 Zagreb, Croatia;
and [2]Division of Biological Sciences and Division of Biochemistry, Faculty of Agriculture, University of Skopje, Skopje, Macedonia, Yugoslavia

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Abstract—1. The temperature dependence of the paramagnetically induced molar solvent-proton longitudinal magnetic relaxation (PMR) rates in the phosphate-free solutions of aquomet derivatives of human haemoglobins A_0 and A_2 are almost identical.

2. The addition of inositol hexaphosphate to either of the solutions increases the PMR rates, but this effect is significantly smaller for HbA_2.

3. Taking into account the non-equivalent influence of α and β chains on PMR rates, it is concluded that the mechanism of transfer of the allosteric information upon binding of inositol hexaphosphate is less effective in HbA_2 than in HbA_0. Some implications of this finding are discussed here.

INTRODUCTION

Human blood contains several haemoglobin oligomers (see Lehmann & Huntsman, 1974), most of which have the common pair of α-protomers, but differ in the second protomer pair. The $(\alpha\epsilon)_2$ combination is present during the early stage of human foetal development and hence not easily available for structural studies. The so-called foetal haemoglobin, $(\alpha\gamma)_2$ or HbF, is the major component in the foetal blood and is retained in appreciable amounts in the first months after birth. While the best characterized form is that of the major component in adult blood, HbA_0, i.e. $(\alpha\beta)_2$, there is also the minor form HbA_2—$(\alpha\delta)_2$.

The basis of our understanding of the haemoglobin function is the knowledge of the three-dimensional structure of HbA_0 (Fermi, 1975 and references therein) and its area of interaction with the allosteric effectors 2,3-diphosphoglycerate (Arnone, 1972) and inositol hexaphosphate (IHP; Arnone & Perutz, 1974), all gained by X-ray structure analysis, i.e. in the crystalline state. Structural information of great importance has been obtained by Chien Ho and his coworkers (see a review by Maričić, 1975) for haemoglobins in solution by proton magnetic resonance spectroscopy of high resolution in a comparative study of mutant human Hb's. Among the Hb's studied in that way was also the foetal Hb, but not HbA_2. This mode of nuclear magnetic resonance yields most specific structural information. The relaxation mode (PMR) is less specific but complementary and lends itself in particular for comparative studies of the solvent-and-protein dynamics of the active domain in haemoproteins, that of the 'haem-pocket' (Vuk-Pavlović *et al.*, 1974; Markovska *et al.*, 1975). It was shown, for instance, that the previously known non-equivalence of α and β chains in human HbA_0 (Lindstrom & Ho, 1972) is due to a much more accessible α-haem compared to its β-partner (Markovska *et al.*, 1975). On the other hand, HbF was found to be non-responsive to IHP-binding regarding haem-accessibility to solvent as distinct from both α and β chains (Pifat *et al.*, 1974). Therefore, it seemed of certain interest to complete a comparative study by PMR of the haem-pocket in haemoglobins of human blood present after birth. The measurements and analysis presented in this report indicate a gradual diminution of the haem-iron-accessibility and responsiveness to IHP in the row $\alpha > \delta > \beta > \gamma$. It was found that α, β and δ haem-pockets open when IHP binds to Hb's.

EXPERIMENTAL

Two independent preparations of human HbA_0 and HbA_2 were performed on DEAE Sephadex (Pharmacia) columns (55 × 2.5 cm) using 0.05 M Tris–HCl buffer, pH 8.0 (Huisman & Dozy, 1965; Dozy *et al.*, 1968). Isolated Hb fractions were concentrated on columns of CM Sephadex, and further concentrated by ultrafiltration at 4°C under reduced pressure with concurrent dialysis against distilled water. The purity of the isolated haemoglobins was checked by starch gel electrophoresis using Tris–EDTA–boric acid buffer, pH 9.0 (Efremov, 1974). After oxidation with potassium ferricyanide the samples were extensively dialysed against 0.1 M NaCl containing 0.05 M Bis-Tris–propane and 5×10^{-4} M EDTA, pH 6.0.

Inositol hexaphosphate (Sigma) was added from a stock solution buffered at pH 6.0 up to a concentration equal to that of the haem. Concentrations of Hb's were determined spectrophotometrically as the cyanmet complexes (Zijlstra & Van Kampen, 1960). The concentrations of the samples used in PMR measurements were 3 and 4 mM (per haem).

The longitudinal PMR times, T_1, were measured at 24 MHz by the $\pi - t - \pi/2$ pulse sequence with the same equipment and measuring procedure as before (Vuk-Pavlović *et al.*, 1974).

RESULTS AND DISCUSSION

The logarithms of the molar (per haem) paramagnetically induced longitudinal (T_1) solvent-proton magnetic relaxation rates vs reciprocal absolute temperature are plotted in Fig. 1. These rates are obtained by subtracting those measured for the solutions of diamagnetic carbonmonoxy derivative of HbA_0 from the total relaxation rates obtained with both methaemoglobin solutions.

The PMR rates in the solution of phosphate-free aquometHbA_0 are presented as open triangles in Fig. 1, while the full triangles stand for these rates when IHP is added. The corresponding molar PMR rates measured for the aquometHbA_2 are denoted as open and full circles.

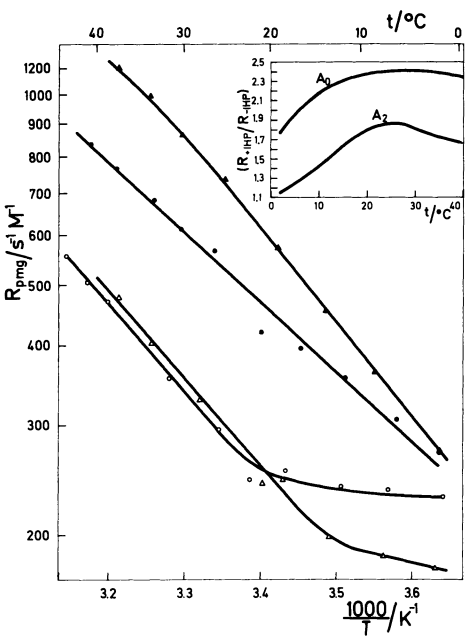

Fig. 1. The reciprocal temperature dependence of the paramagnetically induced molar longitudinal magnetic relaxation rates (R_{pmg}) in the acid solutions (pH 6.0) of human metHbA_0 (triangles) and metHbA_2 (circles). Open symbols: phosphate-free solutions; filled symbols: solutions containing an equimolar (per haem) concentration of inositol hexaphosphate. Protein concentrations were 3–4 mM per haem. Insert: The temperature dependence of the ratio of the paramagnetically induced PMR rates in the presence and absence of inositol hexaphosphate (R_{+IHP}/R_{-IHP}) for solutions of HbA_0 and HbA_2.

The mechanisms of the temperature dependence of the PMR rates in methaemoprotein solutions have been elaborated elsewhere in detail (Vuk-Pavlović *et al.*, 1974). We point out here only that the PMR rates in solutions of mammalian aquomethaemoglobins are dominated by the thermally activated rate of interchange of protons between the haem-pocket and bulk solvent. The difference in the kinetics of this interchange, due to the stereochemical specificities of a particular haem-pocket, thus enable a relative comparison between various haem-environments (Vuk-Pavlović, 1975).

The comparison of the temperature dependences of the paramagnetically induced PMR rates in solutions of phosphate-free aquomet forms of HbA_0 and HbA_2 (Fig. 1, open triangles and open circles, respectively) shows that the solvent dynamics in the immediate environment of the iron-ion is almost the same in the temperature range above 20°C. Below this temperature slight distinction between these two Hb's can be observed. This effect is due to higher outer-sphere relaxation rates (Vuk-Pavlović *et al.*, 1974) in HbA_2: here the haem-iron enhances more efficiently the relaxation rates of the solvent-protons which are located outside the nearest possible approach to the paramagnetic centre. In other words, these data would suggest a closer approach of protons in the bulk of the solvent towards the haem-pocket, i.e. a more open haem-crevice at its mouthpiece in metHbA_2. In view of the fact that α chains are identical in HbA_0 and HbA_2 and that the contribution of the β chain in HbA_0 to the PMR rates is significant only at lower temperatures (Markovska *et al.*, 1975), it is highly probable that the difference between HbA_0 and HbA_2 discussed here is, in fact, caused by a more open δ chain haem-pocket to the β.

When inositol hexaphosphate, a potent allosteric effector, is added to the acid solution of human metHbA_0, the paramagnetically induced PMR rates approximately double (Pifat *et al.*, 1974; Gupta & Mildvan, 1975). Recent results from this laboratory unequivocally prove that this effect is due to the 'loosening' of the haem-pockets (Benko *et al.*, 1976). Further, although IHP binds to β chains only, this effect is shared by both types of chains (Markovska *et al.*, 1975). Filled symbols in Fig. 1 represent the IHP-effect in HbA_0 (triangles) and HbA_2 (circles). It can clearly be seen that IHP affects the PMR rates in the two metHb solutions by increasing the rate of proton interchange from the place of closest interaction of haem-iron electron and water–proton spins. The increase is more pronounced for HbA_0 than HbA_2, although the amino acid sequences in the phosphate-binding regions are indentical in β and δ chains (Dayhoff, 1969; Arnone & Perutz, 1974), so that this difference cannot be ascribed to distinct dissociation constants of IHP for HbA_0 and HbA_2.

Delta chains in human haemoglobin A_2 differ from β chains of HbA_0 in 10 amino acids (Dayhoff, 1969). Most of them may be considered to occupy 'non-strategic' positions with respect to both the haem-globin and interchain contacts, and therefore no significant stereochemical distinction from the β chains should be expected. Nevertheless, some differences in the oxygenation properties (Huisman *et al.*, 1962) as well as in the thermal stability (Perutz & Raidt, 1975) between HbA_0 and HbA_2 have been demonstrated. The PMR data presented here confirm that in spite of no profound distinctions in the β and δ primary structures the α/β and α/δ route of allosteric transmission are not equivalent. The insert in Fig. 1 shows the temperature dependence of the ratios of PMR rates in the presence and absence of IHP for both types of haemoglobin. Throughout the temperature range measured the IHP-effect in HbA_0 is more than 30% stronger than it is for HbA_2; this implies that the overall haem-accessibility is more phosphate-dependent in the former than in the latter. It has been already shown that iron-ions in α chains dominantly influence the PMR rates and that the absolute magnitude of the IHP-effect is greater for α than β chains (Markovska *et al.*, 1975), although IHP binds directly to the latter ones. Since the PMR-dominant α chains are the same in HbA_0 and HbA_2, the smaller IHP-effect in HbA_2 may be rationalized in the following way(s): (1) IHP 'loosens' the haem-pocket in δ chains less than in β chains, and/or (2) the structural consequences of the IHP-binding for the accessibility of the haem in α chains are weaker in HbA_2 than in HbA_0 due to the less efficient mechanism of transfer of the allosteric information in the former. Bearing in mind the low contribution of β chains to the overall PMR rates in HbA_0 solutions, which is mainly due to the outer-sphere relaxation even in the presence of IHP (Markovska *et al.*, 1975), it is hard to believe that δ chains may significantly influence the measured PMR rates in the higher-temperature region. Therefore, conclusion (2) is much more probable. Further, it is consistent with the finding of Perutz and Raidt (1975) that in HbA_2, compared to HbA_0, two additional non-polar contacts exist within δ chains and an additional hydrogen bond at the $\alpha_1\delta_1$ contact in the T conformation of the tetramer. It is possible that these interactions contribute also to the observed 'resistance' of HbA_2 towards IHP.

We now extend the comparison of the PMR-behaviour of these two Hb's with that of human foetal haemoglobin (Pifat *et al.*, 1974). The interaction of IHP and β chains has been proved also by X-ray diffraction (Arnone & Perutz, 1974), while for γ chains in HbF this phenomenon was confirmed by difference spectrophotometry (Perutz *et al.*, 1974), and it is plausible to assume that here IHP binds to the analogous site as in HbA_0. The structural changes induced by the binding of this polyanion to non-α chains, as observed by PMR, are very different: The effect is most pronounced for HbA_0, less in HbA_2, and is undetectable in HbF (Pifat *et al.*, 1974). It has been reported that the oxygenation parameters of HbF are more 'resistant' to organic phosphates compared to HbA_0 (Tyuma & Shimizu, 1969). On the basis of the present study it may be predicted that the oxygenation properties of HbA_2 in the presence of phosphates may be an intermediate case. Such relative non-responsiveness of HbA_2 to phosphates in the ferrous state (as proved here for the ferric), compared with HbA_0, may be one of the reasons that HbA_2 remains a minor Hb component in humans during the entire life.

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