INFLUENCE OF DPG ON THE BOHR EFFECT OF HUMAN HEMOGLOBIN

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1. Introduction

The Bohr effect of human hemoglobin is release of protons on binding of heme ligands above pH 6.0 (the alkaline Bohr effect) or on uptake of protons below pH 6.0 (the acid Bohr effect). 2,3-Diphosphoglycerate (DPG) increases both the acid and alkaline Bohr effects [1–4] because on binding it raises the pK's of the positively charged groups on hemoglobin while the pK of its own phosphate groups are lowered. On binding of DPG at any pH, protons will be either taken up or released depending on these pK values and the observed Bohr effect will be changed.

This paper describes the independent measurements of the release and uptake of protons on binding of DPG to both oxy- and deoxyhemoglobin. On mixing a solution of DPG and hemoglobin of identical pH, the uptake or release of protons on binding will change the pH, and back titration to the original pH with acid or alkali will give the number of protons taken up released on binding. The results show that in explaining the changes in the Bohr effect caused by DPG the two quite independent effects of DPG on the charge of oxy- and deoxyhemoglobin must be taken into account.

2. Materials and methods

DPG was obtained as the pentacyclohexyl-ammonium salt from Calbiochem and converted to a neutral solution of the sodium salt [5]. The preparation of stripped human hemoglobin and the mixing of solutions of DPG and hemoglobin of identical pH was carried out as described for inositol hexaphosphate [6]. The DPG was in a 1.5 molar excess per mole excess per mole of hemoglobin tetramer. The methemoglobin content at the most acid points was less than 5%.

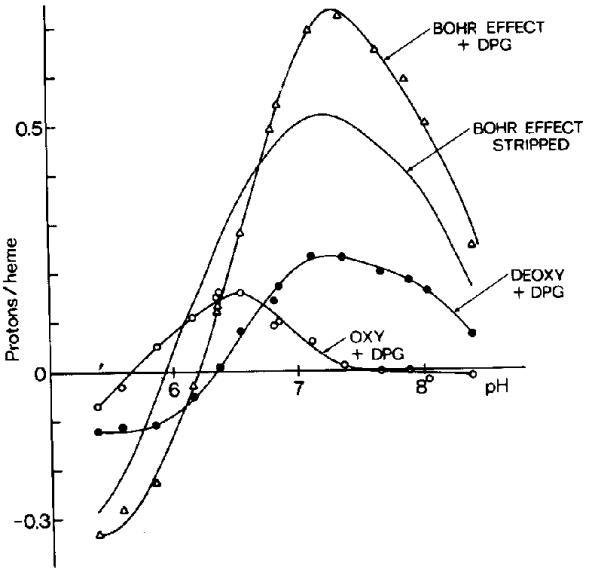

Fig. 1. The top two continuous lines are the measured Bohr effect for stripped hemoglobin and for hemoglobin with DPG expressed as protons/heme released on oxygenation. The filled and unfilled circles are the proton uptakes on mixing DPG with deoxy- and oxyhemoglobin respectively. The triangles are the calculated Bohr effect with DPG which is the difference between the proton uptake on adding DPG to oxy- and deoxyhemoglobin added to the Bohr effect for stripped hemoglobin. Hemoglobin concentration 10 mg/ml (0.15 mM tetramer) in 0.1 M KCl at 25°C, DPG concentration 0.23 mM.

3. Results and discussion

Fig. 1 shows the proton uptake on addition of DPG to either oxy- or deoxyhemoglobin. Oxyhemoglobin above pH 7.0 shows little proton uptake on

addition of DPG, in agreement with the low binding observed in this pH range [5]; however, below pH 7.0 protons are taken up, indicating that DPG is bound as previously reported by others [7–9]. The binding site for DPG to deoxyhemoglobin lies in the central cavity between the two β-chains [10–13] but this site no longer exists in oxyhemoglobin because due to the change in quaternary structure the two sides of the cavity close up and expel the DPG molecule. When DPG binds to oxyhemoglobin below pH 7.0 it must therefore bind to a site different from that in deoxyhemoglobin, leading to a different pattern of proton release as indicated by fig. 1. Below pH 6.7 the uptake of protons associated with the binding of DPG to oxyhemoglobin is greater than to deoxyhemoglobin but this does not mean that DPG has a higher affinity for oxyhemoglobin; clearly this cannot be so because the lowering of the oxygen affinity by DPG in this pH range [4] means that it has a higher affinity for deoxyhemoglobin. Instead, the oxy-binding site takes up more protons on binding less DPG than the deoxy-binding site does at the same pH.

If the values given here for the proton uptake on addition of DPG to oxy- and deoxyhemoglobin are correct, then the measured Bohr effect with DPG effect with DPG should correspond to the difference between the proton uptake on adding DPG to oxy- and deoxyhemoglobin, added to the normal Bohr effect for stripped hemoglobin. This sum agrees exactly with the measured Bohr effect in the presence of DPG (fig. 1). This shows that both DPG and inositol hexaphosphate [6] influence the Bohr effect by binding to both oxy- and deoxyhemoglobin.

The difference between the proton uptake on binding of DPG to oxy- and deoxyhemoglobin invalidates previous analyses of the influence of DPG on the Bohr effect which assumed that the binding of the same amount of DPG to oxy- and deoxyhemoglobin causes an identical uptake of protons [14, 15].

The lowering of the pK's of the phosphate groups of DPG on binding to hemoglobin will cause a release of protons [15]. In deoxyhemoglobin this takes place below pH 6.3 and rises to a maximum at pH 5.5 (fig. 1). This is probably due not only to the phosphates because these have an initial pK of 7.0 [15], which would have to be lowered by more than one pK unit to give the observed proton release at pH 5.5, but also at this pH, DPG might bind to more than one site of deoxyhemoglobin thereby causing the charged groups on the globin to release protons.

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