ON THE RATE OF REACTION OF AN ORGANIC PHOSPHATE (ATP) WITH DEOXY HEMOGLOBIN

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Organic phosphates (such as diphosphoglycerate or ATP) interact reversibly with hemoglobin and affect the ligand binding behaviour of the protein [1–3]. The reversible binding of organic phosphates is therefore oxygen linked, the affinity of deoxy hemoglobin being much greater than that of ligand bound hemoglobin [3]. Other ions, notably from inorganic salts, produce the same effect on the O_2 equilibrium as do organic phosphates but are effective at much lower concentrations [3, 4].

It appeared interesting to obtain information on the velocity of binding of hemoglobin to organic phosphates, particularly in view of the possibility that the combination or dissociation of organic phosphates may show up in the kinetics of the reaction of hemoglobin with ligands. This may occur especially when the reaction is initiated by sudden removal of the bound ligand from hemoglobin, as for instance in flash photolysis experiments.

The experiment reported in the present note clearly shows that binding of ATP to deoxy hemoglobin is a rapid process which is completed, even at low reagent concentrations, within the dead time of the rapid mixing apparatus, i.e. 3 to 4 msec. The experiment, shown in fig. 1, was performed as follows: deoxy hemoglobin in salt-free solution, freed previously as HbO_2 from organic and inorganic ions by passage through an ion exchange column, was allowed to react with CO in water. The rate of combination with CO (curve 1) was higher than that measurable in phosphate buffer, reflecting the change in ligand affinity in the absence of salts. The same deoxy hemoglobin solution was then mixed with the same CO solution but containing 10^{-3} M (before mixing) ATP; the rate of reaction was now similar to that in the presence of phosphate buffer (curve 2). In particular there was no fast component as would be expected if some or all of the reacting material had kinetic properties similar to hemoglobin in salt-free solution.

As a control ATP (5×10^{-4} M) was added to deoxy hemoglobin and the solution reacted again with CO solution containing ATP (10^{-3} M before mixing): the kinetics of combination (curve 3) was again similar to that observed in the presence of phosphate buffer.

In summary this experiment shows that:

(a) Removal of ions increases the rate of reaction of hemoglobin with CO, the initial second order rate constant being $\sim 5 \times 10^5$ M^{-1} sec^{-1} instead of $\sim 1.5 \times 10^5$ M^{-1} sec^{-1}, as observed in the presence of ATP or of 0.1 – 0.2 M phosphate buffer at the same temperature and pH (curve 3).

(b) When deoxy hemoglobin is allowed to react simultaneously with CO and ATP the rate of reaction with CO is already, at the time of the first observation, that characteristic of hemoglobin bound to ATP. Thus complete combination of deoxy hemoglobin with ATP has taken place within the dead time of the apparatus i.e. about 4 msec.

The rate constant for the combination of ATP with deoxy hemoglobin, assuming a second order behaviour, can be then estimated to be higher than 10^6 M^{-1} sec^{-1} at 30°. If the reaction of hemoglobin with ATP is a simple bimolecular reversible process with an equilibrium constant, at pH 6.5 and 30°, of

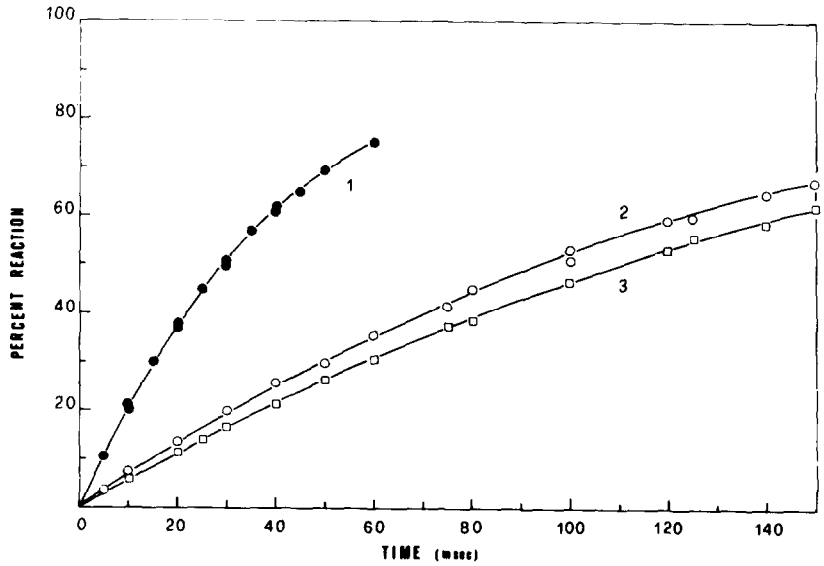

Fig. 1. Combination of human deoxy hemoglobin with carbon monoxide in the presence and absence of ATP. Curve 1: Hb 5×10^{-5} M in heme, CO 2.5×10^{-5} M; ATP absent. Curve 2: Hb 5×10^{-5} M, CO 2.25×10^{-5} M; ATP present. Curve 3: Hb 4.5×10^{-5} M, CO 2.25×10^{-5} M, ATP present. Conditions pH 6.5, 30°; observations at $\lambda = 538$ nm. For details of ATP additions see text.

the order of 10^5 M^{-1} [5, 6] the dissociation velocity constant should be higher than 10 sec^{-1}.

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