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SHORT COMMUNICATION

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THE EFFECT OF SICKLE CELL HAEMOGLOBIN POLYMERIZATION ON HYDROGEN ION DISSOCIATION

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Summary

An increase in hydrogen ion dissociation was found on sickling due to sickle cell haemoglobin polymerization. Since a decrease in pH favours sickling, this might enhance the sickling process in a vicious cycle.

Introduction

A number of reports in the recent literature have featured the intracellular concentration of haemoglobin as a potentially important biological factor in oxygen transport [1,2]. In a preliminary report Brewer et al. [3] have shown that the higher the concentration of haemoglobin, the greater the dissociation of hydrogen ion (H^+). This raises the possibility that in sickle cell disease H^+ dissociation due to sickle cell haemoglobin (Hb—S) polymerization (the ultimate in decreased molecular spacing) could occur during sickling. Such an effect might autocatalyze sickling, since a decrease in pH favours Hb-S polymerization. In the present study we report the results of Hb-S polymerization on H^+ dissociation.

Material and methods

The study was done in two groups. In group I, blood from 6 cases of homozygous sickle cell anaemia were compared individually with normal blood. The red cells were washed, suspended in 0.146 M NaCl—0.005 M Tris buffer at pH 7.2 and the haemoglobin concentration adjusted to 5 g/dl. In group II, two

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experiments were performed with pooled haemoglobin-S and results were compared with pooled haemoglobin-A. For this, partially-purified haemoglobin solutions were prepared from red cells obtained from several haemoglobin-S and haemoglobin-A cases. These cells were washed and lysed with toluene and stripped of organic phosphates by elution with 0.1 M NaCl through a Sephadex G-25 (coarse) column at pH 7.2 [4]. The eluted haemoglobin was dialysed against distilled water for 48 h and concentrated by either oxygen (for haemoglobin-S) or nitrogen (for haemoglobin-A) gas at 2 atmospheres pressure while the haemoglobin solution was contained in dialysis tubing. The haemoglobin concentrate contained less than 3% methaemoglobin. The haemoglobin solution thus obtained was approximately 20 g/dl.

All studies were carried out at 4°C and the pH and gas tensions were measured at 37°C in a radiometer BMS-3 blood gas system. Nitrogen was used to de-oxygenate the samples. Measurement of haemoglobin concentration, oxygen saturation, oxygen pressure (PO_2), carbon dioxide pressure (PCO_2), pH and lactate were done in the oxygenated and deoxygenated states, as well as after reoxygenation. The contribution to change in pH due to lactate production during the experiments was calculated. The degree of haemolysis present after each step was assessed by spectrophotometry.

Results

Desaturation of haemoglobin solutions could be achieved to about 80% with nitrogen treatment and this gave a fair degree of gelling of Hb-S samples to work with. The observations on change in pH are given in Table I and II.

For every 10% increase in desaturation an increase in pH of about 0.018 units occurred in Hb-A samples, whereas the increase was only 0.008 units in Hb-S samples. The results were statistically significant in both groups of experiments. This change of pH could be reversed on reoxygenation of the samples. When increase in pH due to desaturation was calculated there was found to be an actual decrease in pH in Hb-S samples. On deoxygenation a small increase in haemoglobin concentration was noted for which correction was made in the net pH change. Influence of lactate and haemolysis produced during the experiments on the net pH change was insignificant.

TABLE I

CHANGE IN pH ON DEOXYGENATION OF HAEMOGLOBIN SAMPLES

Expt.	Sample	Concentration of Hb in oxygenated stage (g/dl)	Concentration of Hb in deoxygenated stage (g/dl)	Percentage oxygen desaturation	Mean change in pH	*P*
Group I (mean of 6 experiments)	Hb-A	4.9 ± 0.3	5.2 ± 0.4	82.2 ± 6.7	0.142	<0.001
	Hb-S	4.9 ± 0.3	5.2 ± 0.4	82.2 ± 7.2	0.064	
Group II	Hb-A	18.7 ± 0.8	21.7 ± 3.3	80.7 ± 6.2	0.194	<0.001
	Hb-S	17.8 ± 2.3	20.2 ± 1.2	80.7 ± 5.6	0.075	

TABLE II

CALCULATED CHANGE IN pH IN Hb-S SAMPLES

	Hb-A	Hb-S	Calculated decrease in Hb-S samples
Change in pH/ % oxygen desaturation	0.0018 ± 0.0008	0.0008 ± 0.0005	0.0010 ± 0.0005

Discussion

In the present experiment the pH changes in Hb-A samples were subjected to desaturation effect and in Hb-S samples to both oxygen desaturation and haemoglobin concentration (represented by gelling) effect. In the previous report Brewer et al. [3] have shown that there occurs an increase of 0.017 pH units for every 10% increase in desaturation. This is based on the fact that due to molecular organization at the deoxygenated state there is an increase in affinity of basic groups in the molecule for H^+ and decrease in affinity for oxygen. Thus the discharge of oxygen by haemoglobin is accompanied by an uptake of H^+ which results in an increase of the pH of the solution. Our normal control samples show consistency with this observation. Assuming that the same amount of increase in pH has also occurred in the Hb-S samples due to desaturation one finds an actual mean decrease of 0.010 pH units in these samples. This finding of decrease in pH in Hb-S samples can be attributed to the effect of gelling which has also occurred in these samples. This increase in H^+ dissociation can be explained on the basis of a very close alignment of molecules during gelling so that the H^+ ions which are normally present between helix are squeezed out, resulting in a decreased pH of the solution. This decrease in pH during sickling may enhance the sickling process in a vicious cycle.

Acknowledgement

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