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BBA 3979

SOME ASPECTS OF THE COMBINATION OF ASCARIS HAEMOGLOBINS WITH OXYGEN AND CARBON MONOXIDE

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(Received August 22nd, 1962)

SUMMARY

1. Previous studies on the kinetics of the deoxygenation of the two Ascaris haemoglobins have been confirmed and extended. It has been shown that biochemical reducing systems produce the same results as sodium dithionite, so that no artifacts are introduced by using the latter under the conditions specified.

2. The deoxygenation velocity of the body-wall haemoglobin has a Q_{10} of about 3 and changes markedly with pH, whereas that of the perienteric-fluid haemoglobin has a Q_{10} of 5 and is little affected by change in pH. The reaction of the body-wall haemoglobin is about 17 times as fast as that of the perienteric fluid at 38°.

3. Further data are given to support the previous observation that oxyhaemoglobins in which the β band is more intense than the α band always seem to have a high affinity for O_2. Both the Ascaris haemoglobins are of this type.

4. Data obtained in this study have made it possible to estimate the value of the partition coefficient for both haemoglobins. It is shown that in both cases this value is not more than 0.1, and is probably nearer to 0.01. These values are much lower than any previously reported for haemoglobins.

INTRODUCTION

The properties and function of the body-wall and perienteric-fluid haemoglobins of pig *Ascaris lumbricoides* have recently been extensively investigated[1,2]. This paper

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reports further work connected with the reactions of these haemoglobins with O_2 and CO. Although the general properties are similar to those of other haemoglobins, particular details show considerable differences. Materials and methods were as stated in the accompanying paper[1] except where noted below. The work confirms and extends the original work of DAVENPORT[3] on these reactions.

RESULTS

Deoxygenation experiments

Perienteric-fluid haemoglobin: In his studies of this haemoglobin, DAVENPORT[3] reported kinetic data on addition of dithionite. The disadvantages in using dithionite are now better understood[4,5] and since DAVENPORT used a 30-fold excess of dithionite at his lowest concentration it was thought desirable to confirm his results with a biochemical reducing system.

Two systems were used in deoxygenating solutions of Ascaris haemoglobin contained under N_2, and the progress of the reaction was followed at 580 mμ using a Thunberg tube with an optical cuvette attached. The systems employed were a mixture of ascorbic acid and horse-heart cytochrome *c*, added to the haemoglobin plus a soluble pig-heart cytochrome *c* oxidase; and commercial glucose oxidase (containing a little catalase), added to the haemoglobin plus a little glucose.

The haemoglobin could be completely deoxygenated in either system, but the cytochrome system gave a biphasic responce (both phases appeared to be unimolecular), whereas with glucose oxidase there was a single unimolecular reaction with a half time of 80 sec at pH 6.8 and 25.5°. A similar reaction was obtained with dithionite under the same conditions, the half time being 85 sec. These results are in reasonable agreement with a value of 70 sec at pH 6.5 and 25° calculated from DAVENPORT's data. It was concluded that the use of dithionite was quite satisfactory for this particular purpose.

In studies on the metabolism of haematin compounds in Ascaris, SMITH AND LEE[2] have demonstrated that when horse haemoglobin is provided in the surrounding medium there is a large increase in the amount of haemoglobin in the perienteric fluid of the worms. Perienteric fluid taken from worms kept in horse haemoglobin solution was tested for evidence of a horse haemoglobin component by deoxygenation with dithionite and high vacuum. In the first instance visual observation showed that the haemoglobin was slowly deoxygenated without any sign of an initial rapid reaction. In the second case no sign of deoxygenation could be observed even when the sample was taken down to 0.2 mm of mercury. In comparison experiments with horse oxyhaemoglobin of similar concentration, conversion to the deoxygenated form was easily achieved by evacuation with a water pump accompanied by flushing with N_2. In a mixture of equal amounts of horse and Ascaris oxyhaemoglobins, the deoxygenation of the horse haemoglobin in this way was readily apparent.

Body-wall haemoglobin: DAVENPORT[3] recorded only one value for the deoxygenation rate of the body-wall haemoglobin, namely a half time of 80 sec at pH 6.3 and 3°. This result has now been confirmed and extended. Oxyhaemoglobin solutions were examined in Thunberg tubes, as in the experiments with perienteric-fluid haemoglobin, and dithionite was used as the deoxygenating agent. Weighed amounts of about 2 mg of dithionite were placed in the bulb of the Thunberg tube and dissolved in about 0.5 ml of the haemoglobin solution just prior to equilibrating with N_2.

The experiments were carried out over the range 0° to 20° at pH 6.5, 7.3 and 8.5. The course of the reaction was unimolecular. The results are quoted in Table I, being taken at each pH from the estimated best straight line through at least four concordant results at three or more temperatures in a plot of log k against the reciprocal of the

TABLE I

VALUES OF DEOXYGENATION VELOCITY CONSTANT

The values were obtained from the estimated best straight line through the experimental points in a plot of k against T_{abs}^{-1}.

pH	$k(sec^{-1})$ at			Q_{10}
	38°	1.°	3°	
8.5	0.79	0.068	0.025	2.72
7.3	1.05	0.114	0.040	2.85
6.5	1.55	0.100	0.031	3.22
6.3[3]			0.009	

absolute temperature. They are believed to be reasonably accurate but are particularly subject to experimental error at the highest temperatures since the half times were then only about 5 sec, making measurements difficult. The values for Q_{10} and k at 38° in the table are therefore given only to indicate the order of magnitude of these values and the substantial agreement between the Q values. The average Q_{10} is 2.93,

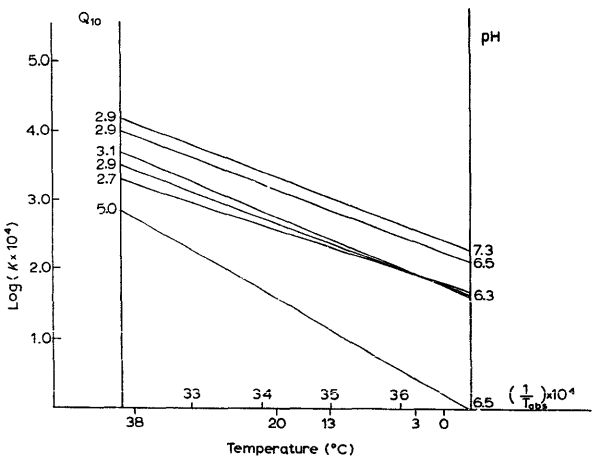

Fig. 1. Influence of temperature on the deoxygenation velocity constant of Ascaris haemoglobins. Lowest line shows the change in reaction rate for the perienteric-fluid haemoglobin at pH 6.5 (from DAVENPORT[3]). Upper lines show the relationship for the body-wall haemoglobin at the various pH, based on the velocity constant at 3° and assuming a Q_{10} of 2.9. Values are also given for Q_{10} of 2.7 and 3.1 at pH 6.3 in order to show the probable limits of error in this assumption.

and it is quite possible from the present experimental results that the Q_{10} is in fact the same at each pH. The relationship between the deoxygenation velocity and the pH is shown in Fig. 1, which shows the effect of temperature at several pH's, taking a Q_{10} of 5 (3°–13°) for the perienteric-fluid haemoglobin[3], and of 2.9 for the body-wall haemoglobin at all pH's. It is clear that the curves can only intersect at low pH's and high temperatures, and there is little likelihood of any reversal of affinity taking place *in vivo*. The activation energies calculated from these curves are 26 200 and 17 400 cal, respectively.

Fig. 2 gives the value of k at 3° plotted against pH. Data for the perienteric-fluid haemoglobin are shown for comparison. In the first attempt to measure the pH-6.5 values, the haemoglobin was equilibrated against the buffer overnight, and was found to have gone almost completely to methaemoglobin by the morning. A fresh preparation was therefore made and only equilibrated against the buffer for 1.5 h in narrow dialysis tubing immediately prior to carrying out the experiments. All the experiments at any one pH were carried out consecutively on a single sample of haemoglobin extracted from stored body walls 2 days previously. I did not encounter changes in the preparation such as DAVENPORT found on keeping solutions of the haemoglobin for longer periods. The striking change in the deoxygenation velocity of Ascaris body-wall haemoglobin with pH does not have any previous parallel so far as I am aware. In mammalian haemoglobins there is an increase in the rate of deoxygenation with decreasing pH, but this follows an S-shaped curve which reaches a maximum at about pH 5. This behaviour is due to the occurrence of two ionising groups which are

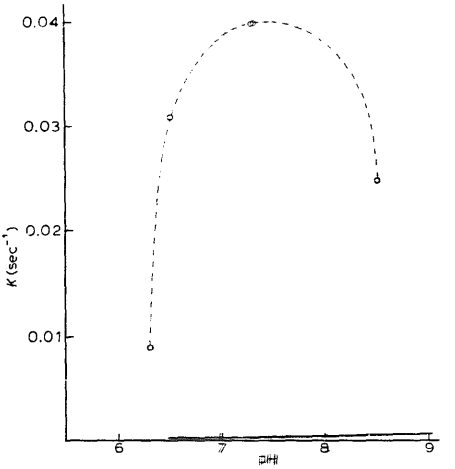

Fig. 2. Relationship between deoxygenation velocity constant and pH for Ascaris haemoglobins. Plot of k at 3° against pH. The dashed line is the presumed curve for the body-wall haemoglobin, joining up the experimental values (lowest value at pH 6.3 is from DAVENPORT[3]). Solid line is for the perienteric-fluid haemoglobin, calculated from DAVENPORT[3].

haem-linked and cause the O_2 affinity to change with pH. If a mechanism of this type is responsible for the changes in Ascaris, then there must be at least two such groups, with pK's of about 6.5 and 8.5. It is possible that the positive Bohr effect below pH 7 has a physiological importance. The tissues of Ascaris are known to be acid—HOBSON[6] reported that the pH of freshly extracted perienteric fluid was between 6 and 7. This is exactly the range in which there is maximum change in the deoxygenation velocity, increase in alkalinity increasing the rate at which O_2 is released in the tissues. The perienteric-fluid haemoglobin on the other hand is very little affected by change in pH, which is in accord with the views expressed by SMITH AND LEE[2] that this haemoglobin does not function to provide O_2 for the general metabolism of the worm.

O_2 affinity and spectra

The oxygenated form of both haemoglobins has the curious feature, pointed out by DAVENPORT[3,7] of a reversal of the usual intensity of the α and β bands. This presence of a more intense α band appears to be accompanied in all cases by a very high affinity for O_2, though haemoglobins with a high O_2 affinity do not always have this spectral feature (Table II). The haemoglobins listed in Table II range from protozoan to fish haemoglobins; include those found intracellularly, free in solution, and in corpuscles; and have molecular weights ranging from 13400 (Paramecium) to $3 \cdot 10^6$ (Tubifex). The only common denominator seems to be the high O_2 affinity, which is usually associated with some degree of O_2 deficiency in the environment. I do not know of any theoretical explanation for this phenomenon.

TABLE II

HAEMOGLOBINS KNOWN TO BE HALF SATURATED WITH O_2 AT 1 mm PARTIAL PRESSURE OR LESS

A, β band less intense than α (normal); B, β band more intense than α; C, relative intensities not known.

	Haemoglobin	*Half saturation (mm)*	*Reference*
A	Nippostrongylus (body wall)	0.1	7, 8
	Gasterophilus (tracheal cells)	< 0.02	9
B	Tubifex	0.6	10, 11
		2.0	
	Paramecium	< 0.6	12
	Ascaris (body wall)	< 0.1	2
	Strongylus (perienteric fluid)	< 0.1	7
	Legume haemoglobin (3 fractions)	0.040 0.068 0.063	13
	Ascaris (perienteric fluid)	0.1*	14
C	Mormyrus	1.0	15
	Bagrus	1.0	
	Ceriodaphnia	0.8	10
	Chironomus	0.6	
	Haemonchus	0.05	8
	Nematodirus	0.05	

* Fully saturated.

Relative affinities for O_2 and CO

Sufficient kinetic data are now available to make it possible to attempt to calculate the partition coefficient between oxygen and carbon monoxide for the body-wall haemoglobin at 20° and pH 6.5. This constant depends on the magnitude of the individual combination and dissociation constants and varies from 530 in vertebrate haemoglobins to 0.67 in Gasterophilus[9]. GIBSON[16] has reported that at room temperature and unspecified pH the CO dissociation constant is 10 sec^{-1}, and the combination constant about the same for that of mammalian myoglobin, say $5 \cdot 10^5$ $M^{-1}sec^{-1}$. From the work reported in this paper, the deoxygenation velocity constant may be taken as 0.2 sec^{-1}. The combination constant has not been directly measured. However, in the equilibrium

$$Hb + O_2 \underset{k}{\overset{k'}{\rightleftharpoons}} Hb\text{–}O_2$$

the ratio of k' over k gives the concentration of O_2 when the haemoglobin is half saturated. Hence the knowledge of the half-saturation value would enable us to calculate k'. SMITH AND LEE[2] have attempted to estimate this value in assessing the physiological behaviour of the haemoglobin. They concluded that it was probably 0.02 mm at 38°. If the combination reaction has a similar temperature coefficient to the dissociation reaction, then the half-saturation value will be the same at 20°, as at 38°, and $k' = 0.20/(0.02 \times 1.8 \cdot 10^{-6}) = 5.6 \cdot 10^6$ $M^{-1}sec^{-1}$. This compares with, values of $0.4\text{–}19 \cdot 10^6$ $M^{-1}sec^{-1}$ for other myoglobins[17–19]. The probable limits of error of the calculated value are a factor of ± 5 times for variation in the temperature coefficient (between 1.4 for mammalian myoglobin[16] and 5 for Ascaris perienteric haemoglobin[3]); and a factor of $+2$ times for the half-saturation value. The partition coefficient is then calculated to be

$$\frac{5 \cdot 10^5}{10} \times \frac{0.20}{5.6 \cdot 10^6} = 1.8 \cdot 10^{-3}.$$

The probable limits are now in the opposite sense, so that the true value is unlikely to be more than $0.9 \cdot 10^{-2}$. The lowest coefficient previously known for any haemoglobin was 0.67 for that of Gasterophilus, and for any other material was 0.1 for cytochrome oxidase[9]. Confirmation that the coefficient really is lower than these comes from an experiment by DAVENPORT[3] in which "Equilibration against pure CO for 1 h at 20° resulted in a shift of ..... 80 Å for the [α band of the] body-wall [oxy-]haemoglobin". He gives the span of this haemoglobin as 90–95 Å, so that a shift of 80 Å represents at most 90% conversion. The partial pressure of O_2 in the system cannot be determined, but experience suggests that it would not be more than 1 mm at the very most. Therefore the partition coefficient

$$\left(= \frac{P_{O_2}}{P_{CO}} \cdot \frac{[COHb]}{[O_2Hb]} \right)$$

would not be more than

$$\frac{1}{760} \cdot \frac{90}{10} = 1.2 \cdot 10^{-1}$$

The coefficient for the perienteric-fluid haemoglobin cannot yet be estimated from kinetic data but must be even lower than that for the body-wall haemoglobin since

in a similar direct equilibration experiment there was a shift of only 60 Å, the span being the same. These astonishingly low partition coefficients are due chiefly to the extremely small dissociation constants, whereas the combination velocity constants are not substantially different from those of other known haemoglobins.

COMMENT

The data reported here extend our knowledge of the properties of Ascaris haemoglobins without providing any obvious explanation of how they can be so different from mammalian haemoglobins in some respects, yet still retain the characteristics of haemoglobins in general. Possibly they have some common structural feature which results in the altered properties of the haem group. The observation that they and several other haemoglobins with high O_2 affinity share the same unusual type of oxyhaemoglobin spectrum suggests that the two anomalies are connected, and that it may be possible to obtain further data from spectrophotometric experiments.

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