BIOCHEMISTRY

Haemoglobin Association and the Sigmoid Oxygen Equilibrium Curve

REDUCED haemoglobin is generally considered to have the same molecular weight as oxyhaemoglobin. The osmotic pressure measurements of Adair[1] and the light scattering experiments of Rossi-Fanelli, Antonini and Caputo[2] agree with this concept, but our light scattering measurements have shown that reduced horse haemoglobin is in a state of close association when in 0·1 molar phosphate (*p*H 7·4).

The following experiments were carried out. Thoroughly purified carbonylhaemoglobin was cleared of carbon monoxide by means of a vacuum and by rinsing with nitrogen. The solution of reduced haemoglobin thus obtained was transferred under nitrogen to a tube containing a thick layer of paraffin oil. Light scattering measurements were made at 700 mμ, and oxygen was admitted and the measurements repeated on the same solution under identical conditions.

These measurements can be expressed in the following way for oxyhaemoglobin and reduced haemoglobin respectively:

$$\frac{1}{M'_{ox}} = 1{\cdot}556 \times 10^{-5}\ (1 + 19{\cdot}5\ g - 58{\cdot}0\ g^2)$$

$$\frac{1}{M'_{red}} = 0{\cdot}689 \times 10^{-5}\ (1 + 42{\cdot}7\ g - 305{\cdot}4\ g^2)$$

M' is the apparent molecular weight at any concentration g.

These formulae give molecular weights at infinite dilution of 64,300 for oxyhaemoglobin and of 145,000 for reduced haemoglobin.

The extrapolation curve for reduced haemoglobin would be expected to slope upward at very low concentrations and to reach the same value as oxyhaemoglobin. At the lowest concentration used (4 mg/ml.) there is no such upward slope, which suggests strong binding of the molecules. The association increases with concentration, indicated by the high value of the coefficient of g^2 in the extrapolation formula.

Our results provide an experimental basis for the interpretation of the oxygen equilibrium of haemoglobin. (We prefer the term "association" for the reversible joining of molecules rather than "aggregation", which is irreversible.) Oxyhaemoglobin does not associate. This may result from some change at the oxygen binding groups occurring during association of reduced haemoglobin, which prevents combination with oxygen. This suggestion for the origin of the sigmoid oxygen equilibrium curve was put forward by Hill[3] and by Douglas, Haldane and Haldane[4]. More recently, association has been shown to occur in the haemoglobin of the lamprey by Briehl[5], and Roughton[6] considered some other cases. Our experiments show that it is the whole molecules (so-called "tetramers") which become associated.

The following equation has been derived:

$$R = \frac{Kp}{1+Kp} \times \frac{\frac{q}{4n} \times \frac{1}{Z} + \left(1 - \frac{q}{4n}\right)(1+Kp)^4}{\frac{1}{Z} \times \left(1 - \frac{q}{4n}\right)(1+Kp)^4}$$

R is the oxyhaemoglobin fraction and K is the intrinsic constant of the oxydissociation of a single haem group, which should be the same for all haemoglobins so long as binding to the globin is the same. We take K as 1·48 as with the myoglobins, and measure the oxygen tension, p, in millimetres of mercury. $q/4n$ is the fraction of free haemgroups in a reduced haemoglobin association of n molecules and Z is the dissociation constant of the associated molecules. If $q/4n$ is taken as 0·0252 and $1/Z$ as 2,111, a sigmoid curve is obtained which is in good agreement with the data of Otis and Roughton[7].

The relation between oxygen pressure and degree of association is now being studied.

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Citrate Condensing Enzyme in Citrus Fruit

CITRATE condensing enzyme has been demonstrated in a wide variety of living tissues: animal tissues[1–3], molds[4], bacteria[1,5,6], and higher plants[7,8]. It is somewhat surprising that there has been no direct demonstration of its occurrence in citrus fruits. There is no reason to doubt the existence of the enzyme because isolated mitochondria from many plant sources including citrus fruit[9] carried out the overall reactions of the Krebs cycle. None the less, we thought it worth while to demonstrate directly its presence in citrus fruit.

Fresh fruit and leaves were brought to the laboratory within an hour of picking. The fruit was peeled and the rind, fruit and leaves homogenized separately in a blender equipped with a jacket filled with ice. The temperature was kept below 10° C during homogenization. Homogenization proceeded for three 30 sec periods with 30 sec intervals between each homogenization period. The homogenates were centrifuged at 8,500*g* at 5° C for 15 min and the supernatant fractions were assayed for activity.

The assay system which measures the release of coenzyme *A* (Co*A*) has been described previously[10]. For routine assays oxaloacetate was the last addition to the cuvette, but all components (enzyme, acetyl Co*A* and oxaloacetate) are necessary for the reaction. Some extracts showed the presence of an acetyl-Co*A* deacylase. We have also shown the presence of citrate condensing enzyme using the coupled spectrophotometric assay[11], which measures oxaloacetate utilization.

In a recent review of acid metabolism in plants, Lioret and Moyse[12] state that the origin of citric and other fruit acids is not known and that it is assumed that they are principally synthesized in the leaves and transported to the fruit. They further conclude that a large number of fruits lack condensing enzyme activity although they cite avocado fruit as a possible exception. Our present results (Table 1) not only demonstrate that the leaves of citrus

Table 1. DISTRIBUTION OF ACTIVITY OF CITRATE CONDENSING ENZYME (TOTAL UNITS/G WET WEIGHT*) IN CITRUS FRUITS

		Expt. 1	Expt. 2
Lemon	Leaves	0·23	0·05
	Rind	0·15	0·10
	Pulp	0·10	0·04
	Bud	0·72	
Orange	Leaves	0·33	
	Ripe rind	0·073	
	Green rind	0·23	
	Ripe pulp	0·066	
	Green pulp	0·079	

* One unit of activity is 1 μmole of reaction product per min at 25° C. In Expt. 1 the tissues were obtained shortly after the tree started to bear fruit. In Expt. 2 the fruit was obtained later in the season when the tree had no green fruit or buds. About 10 g of tissue were homogenized in 30–60 ml. of 1 M *tris*-hydrochloric acid buffer (*p*H 8·2).