Crystalline Leghemoglobin

XIII. Sedimentation Studies

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Each of the two main components of crystalline leghemoglobin from soybean root nodules was previously shown to consist of a single polypeptide chain.[1] At pH 7.0, the molecular weight of the slow component (Lb*a*) was found to be 15 400 and that of the fast component (Lb*c*) 16 800.[1] Recent studies have shown that the sedimentation coefficients of some hemoglobins of low molecular weight depend on the pH [2,3] and on the oxidation state of the heme iron.[4] The binding of different ligands to the heme group also influences the sedimentation behaviour.[5] For example, the value of the sedimentation coefficient of lamprey (*Lampetra fluviatilis*) ferrohemoglobin, increased from 1.9 ± 0.1 S to 3.9 ± 0.2 S with decreasing pH values,[4] due to an association of the monomer molecule to give dimers and, partially, higher degrees of association. Though lamprey ferrihemoglobin in the pH range $4.5 - 10.0$ exists in a monomer form, complexes with azide, fluoride and cyanide, however, are dimers.[6] A corresponding influence on the sedimentation coefficients under similar conditions has not been observed in the case of myoglobin.[6,7] In order to elucidate whether any association of leghemoglobin molecules occurs under different conditions, the sedimentation coefficients of ferro-, ferri-, and azide leghemoglobin were determined in an analytical ultracentrifuge.

Materials and methods. The main components of leghemoglobin were isolated as described previously.[8] The leghemoglobin solutions were made in phosphate buffers. The azide complex was prepared by adding solid sodium azide (Arcochemie, Berlin) in excess to obtain completely liganded leghemoglobin. Reduced leghemoglobin was prepared by adding solid sodium dithionite (E. G. Merck, Darmstadt). Complex formation and reduction, respectively, were followed with a Cary 15 recording spectrophotometer. The concentrations of the leghemoglobin solutions were determined by means of pyridine hemochrome.[9]

Sedimentation coefficients were determined at 20.0 ± 0.1°C in a Beckman Spinco model E ultracentrifuge at 59 780 rpm, with schlieren optics. A single-sector cell and an An-D rotor

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Table 1. Sedimentation coefficients of leghemoglobin.

	pH	Lb mg/ml	Concentration of phosphate buffer	$s_{20,w}$
FerriLb*a*	4.92	4.1	0.10 M	1.82
FerriLb*a*-N_3	5.02	1.8	0.10 M	2.07
FerriLb*a*-N_3	5.70	2.3	0.05 M	1.96
FerroLb*a*	6.62	2.8	0.10 M	2.16
FerroLb*a*	6.10	2.0	0.10 M	2.00
FerriLb*a* + FerriLb*c* (1:1)	5.18	2.8	0.10 M	1.88
FerriLb*a*-N_3 + FerriLb*c*-N_3 (1:1)	5.52	2.8	0.10 M	2.24
FerroLb*a* + FerroLb*c* (1:1)	6.70	2.2	0.10 M	2.01
			Mean value	2.03 ± 0.21

were used in the experiments. The sedimentation coefficients were calculated from the sedimentation rate, and the $s_{20,w}$ values were corrected in the usual way for the temperature, viscosity and density of the medium. The absorption spectra of the leghemoglobin solutions were recorded before and after each run.

The pH measurements were performed with a Radiometer Titrator type TTT 1c. The pH of 0.05 M potassium hydrogen phthalate was taken to be 4.01 at 23°C.

Results and discussion. The sedimentation coefficients of Lb*a*, as complexes with different ligands, and of a mixture of equal concentrations of Lb*a* and Lb*c*, are presented in Table 1. The coefficients differ only slightly from one another, showing that leghemoglobin exists only in a monomeric form under the experimental conditions chosen for this study, and that there is no hybrid formation between the two main components. Thus leghemoglobin differs in this respect from lamprey (*Petromyzon marinus*) hemoglobin, the components of which have been found to form hybrids,[10] and shows some resemblance to the myoglobins in forming no associated forms.[6,7]

The function of leghemoglobin in root nodules is still obscure. However, the similarity between leghemoglobin and myoglobin shown by this study justifies the theory that leghemoglobin facilitates the diffusion of oxygen into the root nodule tissue [11] in a similar way to that in which myoglobin is assumed to function in animal tissue (*cf.* Wittenberg).[12] This is further supported by the fact that leghemoglobin *in situ* has been found to exist in the form of an oxygen complex.[13]

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On the Hydrolysis of Plutonyl Ion in Sodium Perchlorate Medium

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Very few data [1-4] are available on the hydrolysis of the plutonyl ions so it may be of some interest to publish preliminary data on this system, especially as the main complex found here (2,2) is not mentioned in any of the above papers.

Method. The investigations were performed as EMF-titrations at 25°C. The following cell was used:

glass electrode | plutonyl solution | RE

where RE = Ag,AgCl | 2.99 M Na^+, 0.01 M Ag^+, 3 M ClO_4^-.

A solution of the general composition

H M H^+, $(3-H)$ M Na^+, 3 M ClO_4^-

was added to a solution with the composition

B M PuO_2^{2+}, H M H^+, C M Ag^+, $(3-2B-H-C)$ M Na^+, 3 M ClO_4^-.

Thus the value of B was decreasing during the titration.

Preparation of plutonyl solutions. The plutonyl solutions were prepared from plutonium metal, which was dissolved in a known