A Study on the Molecular Weight of Hemoglobin from *Myxine glutinosa* L.

R. QUAST,* S. PALÉUS, G. BLOOM* and E. ÖSTLUND

Biochemical Department, Nobel Medical Institute, Wallenberg Foundation Laboratory 1960, and Department of Cell Research, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

The molecular weight of hemoglobin from *Myxine glutinosa* L. was determined by Svedberg and coworkers [1–3] to be 23 100 and the $s_{20,w}$ ranged from 2.0 to 2.33. These values correspond to a much smaller molecular weight than that of human hemoglobin. They also differ from the values found for the hemoglobins of other cyclostomes, *e.g.* *Lampetra fluviatilis*,[1,2,4,5] *Petromyzon marinus*,[6,7] and *Polistotrema stouti*.[8] Since the above determinations on *Myxine* hemoglobin were performed on whole hemolysates or erythrocytes, a redetermination of the sedimentation coefficient and of the molecular weight of the separated hemoglobins seemed desirable.

Experimental. Pooled hemolysates of the Atlantic hagfish were prepared and the hemoglobins were separated by isoelectric fractionation as has been recently described.[9] The hemoglobin solutions containing ampholytes and sucrose were brought to pH 8.0 with phosphate buffer in order to prevent the autoxidation of hemoglobins 1 and 2 and were dialyzed twice against large volumes of CO-saturated phosphate buffer (0.1 M; pH 8.0) at 4°C for about 24 h.[10] A third dialysis was done against a CO-saturated solution of M/15 potassium phosphate, pH 7.6, for 16 h at 4°C. The hemoglobin solutions were kept in the dialysis bags, immersed in the last buffer solution, until further treatment. Prior to ultracentrifugal analysis the COHb-solutions were concentrated under CO with dried rods of 20 % w/v polyacrylamide according to the procedure of Curtain.[11]

* Present addresses: (R.Q.) Hygiene Institut der Universität, 355 Marburg/Lahn, Bundesrepublik Deutschland; (G.B.) Department of Histology, University Umeå, S-901 85 Umeå, Sweden.

Sedimentation and diffusion experiments were carried out in a Spinco E analytical ultracentrifuge equipped with a temperature control and with a phaseplate instead of a diaphragm in the schlieren optics. A synthetic boundary cell (12 mm) with rubber valve and the conventional 12 mm cell were used. Photographic records were made on Kodak spectroscopic plates, type 103a-E, using a red filter in the light path.

The diffusion coefficient of Hb 2 was determined using Hb concentrations of 324 μmole heme/l and 150 μmole/l. In both cases a 12 mm synthetic boundary cell was used in the AN-D rotor, and the rotor speed was 11 150 rpm. The temperature during the experiment was 24.00 ± 0.05 and 25.43 ± 0.04°C, respectively. Exposures of 30 sec each were made at intervals of 8 or 16 min. The negatives were enlarged 10 times and projected on mm grid paper, on which the curves were redrawn. From these curves the values for the calculation of the diffusion coefficient, D, were taken according to the "maximum ordinate" method.[12] A plot of d_e^2 *versus* t gave a straight line cutting the abscissa at a negative t value.

The sedimentation constants of Hb 1 (two subgroups of Hb 1, with pI's of 4.7 and 5.0, respectively, were investigated), Hb 2 and Hb 3 were determined in a 12 mm single cell at four different Hb concentrations. The rotor speed was 59 780 rpm in all runs. Five or six exposures of 30 sec each were made in each run at intervals of 8 or 16 min. The experiments were run at room temperature.

At the end of each run, after replacing the CO of the sample with oxygen, the hemoglobin concentration of the solution was measured as the pyridine-hemochromogen according to the method of Paul *et al.*[13] The spectra of the pyridine-hemochromogen were recorded with a Cary Model 14R spectrophotometer. Values of the millimolar absorptivity as compiled by Falk [14] were used for the calculation of the heme content.

Results. The diffusion coefficient of Hb 2 was found to be 9.2 ± 0.4 F-units (10^{-7} cm^2 sec^{-1}) at 20°C.

The values of $s_{20,w}$ found for Hb 2 at concentrations between 150 μmole heme/l and 324 μmole heme/l were 1.98 and 2.16, respectively. No concentration dependence of $s_{20,w}$ could be observed in these experiments. The sedimentation constant of Hb 3 was found to be 1.95 at a concentration of 196 μmole heme/l. One subgroup of Hb 1 (with a pI of 4.7) had a sedimentation coefficient (2.72 – 2.73) higher than that of the other fractions. The other

subgroup of Hb 1 (with a pI of 5.0) had $s_{20,w}$-values of 1.64 and 1.72.

The molecular weight was calculated from the measured sedimentation constant and diffusion coefficient. Tentatively using the diffusion coefficient obtained for Hb 2 in the calculation of the molecular weights of all three hemoglobins, the molecular weight determined for Hb 1 will be 28 000 $\pm$ 5 000, for Hb 2 21 000 $\pm$ 2 000 and for Hb 3 20 500 $\pm$ 2 000.

Discussion. The values of the sedimentation coefficients presented in this study are in agreement with those reported by Svedberg and Hedenius [2] for *Myxine* hemoglobin, 2.33 and 2.20. The differences between our findings and the older values are probably due to difficulties in temperature control in the oil-turbine ultracentrifuge, leading to 10 % higher values.[15,16] The temperature error has no influence on the molecular weight determined by equilibrium runs.[15] It is suggested that the molecular weight of 23 100 found by Svedberg and Eriksson-Quensel [1] for hemoglobin in unpurified hemolysates represents an average value for all hemoglobins present, and it agrees with the values presented for the three isolated hemoglobins within the limits of error of the methods.

The conclusion is drawn that a difference seems to exist between the molecular weights and the sedimentation coefficients of the hemoglobins of *Myxine glutinosa* L. and those of other cyclostomes, and that the molecular weights of the *Myxine glutinosa* hemoglobins are higher than those of the single chains of other vertebral hemoglobins. The finding that a difference seems to exist between the molecular weight of Hb 1 and that of the other hemoglobins of the hagfish is tentative and awaits confirmation by extended experimentation.

Acknowledgement. The authors gratefully acknowledge the aid of Professor A. Ehrenberg and Miss G. Heimbürger with the ultracentrifuge experiments. One of us (R.Q.) is especially indebted to Professor Hugo Theorell for laboratory facilities during the work.

This work was supported by a grant from *Statens naturvetenskapliga forskningsråd*. Dr. Quast is grateful to the *Deutsche Forschungsgemeinschaft* for personal support.

1. Svedberg, T. and Eriksson-Quensel, I. B. *J. Am. Chem. Soc.* **56** (1934) 1700.
2. Svedberg, T. and Hedenius, A. *Biol. Bull.* **66** (1934) 191.
3. Svedberg, T. *J. Biol. Chem.* **103** (1933) 311.
4. Svedberg, T. *Kolloid-Z.* **85** (1938) 119.
5. Allison, A. C., Cecil. R., Charlwood, P. A., Gratzer, W. B., Jacobs, S. and Snow, N. S. *Biochim. Biophys. Acta* **42** (1960) 43.
6. Lenhert, P. G., Love, W. E. and Carlson, F. D. *Biol. Bull.* **111** (1956) 293.
7. Rumen, N. M. and Love W. E. *Arch. Biochem. Biophys.* **103** (1963) 24.
8. Zuckerkandl, E., Jones, R. T. and Pauling, L. *Proc. Natl. Acad. Sci. U.S.* **46** (1960) 1349.
9. Quast, R. and Vesterberg, O. *Acta Chem. Scand.* **22** (1968) 1499.
10. Quast, R., Paléus, S., Östlund, E. and Bloom, G. *Acta Chem. Scand.* **21** (1967) 2849.
11. Curtain, C. *Nature* **203** (1964) 1380.
12. Ehrenberg, A. *Acta Chem. Scand.* **11** (1957) 1257.
13. Paul, K. G., Theorell, H. and Åkeson, Å. *Acta Chem. Scand.* **7** (1953) 1284.
14. Falk, J. E. *Porphyrins and Metalloporphyrins, Their General Physical and Coordination Chemistry, and Laboratory Methods.* BBA Library Vol. 2, Elsevier, Amsterdam-London-New York 1964, pp. 240–241.
15. Pedersen, K. O. *The molecular weights of the proteins.* In *Les Protéines*, Neuvième Conseil de Chimie, Solvay, Bruxelles 1953, p. 19.
16. Ehrenberg, A. and Dalziel, K. *Acta Chem. Scand.* **12** (1958) 465.

Received November 13, 1969.