acid appears to be more available to the organism as a precursor than the hydroxy derivative.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN RECEIVED JUNE 13, 1946

The Molecular Size and Shape of Botulinus Toxin[1]

BY GERSON KEGELES[2]

Type A Botulinus toxin has been prepared in crystalline form by two independent groups of workers.[3,4] Concurrent with fractionation studies by these groups, the physicochemical properties of the toxin were investigated.[5] Electrophoretic investigations are reported elsewhere.[4] This communication reports conclusions as to the size and shape of the toxin molecule, based on studies of diffusion, apparent specific volume, and viscosity performed on crystalline materials.

The diffusion measurements were carried out in 0.06 molar sodium acetate buffer at *p*H 4.48 in the Tiselius electrophoresis apparatus[6] as described by Longsworth.[7] The results appear in Table I, where the diffusion constants have been averaged from both channels.

TABLE I

Da is calculated from height and area; *Dm* calculated by method of moments; $D_{20,w}$ calculated from average of *Da* and *Dm*.

Source	C-50 (Lamanna)[3]	XII (Abrams)[4]	XII (Abrams)[4]
Protein concn., %	0.47	0.98	0.50
Temp., °C.	1.0	1.0	20.0
$Da(10)^7$ cm.²/sec.	0.93	1.04	1.99
$Dm(10)^7$ cm.²/sec.	0.95	1.22	2.00
$D_{20,w}(10)^7$ cm.²/sec.	1.79	2.16	2.10

The lower value observed for the electrophoretically homogeneous fraction C-50 may be due, possibly, to partial denaturation resulting from the use of chloroform in its purification.

Density measurements on fractions prepared by both methods were made in an uncapped pycnometer at 30°. Since the protein becomes insoluble when dried, solutions for density determinations were prepared by dialysis against the buffers used as reference solvents. Protein concentrations in these solutions were determined by Kjeldahl nitrogen, using 14.2% as the best available figure for nitrogen content.[3,4] Apparent specific volumes in 0.06 molar sodium acetate buffer at *p*H 4.48 varied somewhat with concentration. On the assumption that the variation was a charge effect, the density measurement was repeated nearer to the isoelectric point[4] in the presence of a large excess of salt. The apparent specific volume obtained for a 0.59% solution of the salt-fractionated protein[4] in 0.2 molar sodium chloride–0.02 molar sodium acetate buffer at *p*H 5.38 was 0.76. The value of the partial specific volume corrected[8] to 20° is taken as $V_{20} = 0.75_5$.

Viscosity measurements at seven protein concentrations from 0.1 to 0.8 per cent. by weight in 0.06 molar sodium acetate buffer at *p*H 4.48 were made with an Ostwald viscometer, using fractions prepared by both methods. Although subsequent examination revealed extensive electrophoretic inhomogeneity in the crystalline chloroform-treated protein fraction studied,[3] satisfactory agreement in the viscosity data was obtained, giving an intrinsic viscosity of 10.6. This corresponds to an axial ratio of 8.3 according to the Simha theory[9] for elongated ellipsoids and a frictional ratio f/f_0 of 1.45 from the Perrin theory.[10] The isoelectric point[4] is 5.60 and further addition of neutral salt would suppress charge effects, giving a lower value for the frictional ratio.

The molecular weight *M* is 1,130,000 as calculated from the diffusion constant $D_{20,w} = 2.10(10)^{-7}$ cm²/sec., the partial specific volume $V_{20} = 0.75_5$ and the frictional ratio $f/f_0 = 1.45$ with the equation[11]

$$MV_{20} = [2.89(10)^{-5}/D_{20,w}(f/f_0)]^3$$

This must be regarded as a lower limit, because the error in the frictional ratio is tripled by this method of calculation. It is hoped that future ultracentrifuge studies which were not possible at the time of this investigation will improve the accuracy of the data. The large size of the molecule is particularly surprising in view of previous studies on bacterial toxins.[12]

(1) Investigations conducted at Camp Detrick, Frederick, Maryland, from July through October, 1945, by Gerson Kegeles, 1st Lt., CWS.

(2) Present address: Chemistry Department, University of Wisconsin, Madison, Wisconsin.

(3) Lamanna, McElroy and Ecklund, *Science*, **103**, 613 (1946).

(4) Abrams, Kegeles and Hottle, *J. Biol. Chem.*, **164**, 63 (1946).

(5) The author is indebted to the senior author of each group for the materials for these studies.

(6) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(7) Longsworth, *Ann. N. Y. Acad. Sci.*, **41**, 267 (1941).

(8) Svedberg and Pedersen, "The Ultracentrifuge," Oxford Press, 1940, Appendix II.

(9) Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(10) Perrin, *J. phys. rad.* **VII**, **7**, 1 (1936).

(11) Reference 8, equation (70a).

(12) Krejci, Stock, Sanigar and Kraemer, *J. Biol. Chem.*, **142**, 737 (1942).

LABORATORIES, TECHNICAL DEPARTMENT
CAMP DETRICK
FREDERICK, MARYLAND RECEIVED JUNE 8, 1946

Preparation of *p*-Alkylbenzyl Chlorides

BY G. M. KOSOLAPOFF

The preparation of *p*-alkylbenzyl chlorides has been effected usually by the method of Blanc[1] or by minor variations thereof. Such procedures utilize the catalytic effect of zinc chloride, which necessitates rather strict temperature control to avoid resinification and, generally, polysubstitu-

(1) Blanc, *Bull. soc. chim.*, (4) **33**, 313 (1923).