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Amino Acid Composition of *Clostridium botulinum* Type A Toxin

DIANE VAN ALSTYNE, JULIA GERWING, AND JACK H. TREMAINE

Department of Microbiology, University of British Columbia, and Canada Department of Agriculture Research Station, Vancouver, British Columbia, Canada

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In a recent publication, it was shown that a toxic protein could be isolated from *Clostridium botulinum* type A; the molecular weight of this protein was reported to be between 12,000 and 13,000 when calculated on the basis of sedimentation and diffusion properties (J. Gerwing, C. E. Dolman, and H. S. Bains, J. Bacteriol. **89**:1383, 1965). Previous data on type A toxin indicated that its molecular weight was in the region of 1,000,000 (Abrams, Kegeles, and Hottle, J. Biol. Chem. **164**:63, 1946; Kegeles, J. Am. Chem. Soc. **68**:1670, 1946; Lamanna, McElroy, and Eklund, Science **103**:613, 1946; Putnam, Lamanna, and Sharp, J. Biol. Chem. **176**:401, 1948; J. T. Duff et al., J. Bacteriol. **73**:42, 1957). The assumption was made that earlier investigators had been working with an aggregated form of the toxin.

The amino acid composition of the high molecular weight material has been calculated (Buehler, Schantz, and Lamanna, J. Biol. Chem. **169**:295, 1947). Analyses of the low molecular weight toxic protein have also been carried out, and indicate a minimal molecular weight of about 15,000. The toxin was prepared by the method described for the purification of type B toxin (J. Gerwing et al., J. Bacteriol. **91**:484, 1966) from *C. botulinum* type A (strain corn T). Methods for protein hydrolysis and performic acid oxidation have been described previously (J. Gerwing, C. E. Dolman, and A. Ko, J. Bacteriol. **89**:1176, 1965).

TABLE 1. *Comparison of amino acid residues found in crystalline type A toxin (calculated by Buehler et al., J. Biol. Chem. **169**:295, 1947) and the low molecular weight toxic moiety isolated from type A cultures by Gerwing et al. (J. Bacteriol. **89**:1383, 1965)*

Amino acid	Crystalline type A toxin			Low molecular weight type A toxin		
	Per cent of constituent	Molar ratio (cysteine = 1.0), minimal mol wt = 45,000	Molar ratio (cysteine + ½ cystine = 1.0), minimal mol wt = 15,000	Per cent of constituent	Molar ratio (cysteic acid = 1.0), minimal mol wt = 15,000	Probable no. of residues
Lysine	7.74	29.0	9.7	7.74	10.95	11
Histidine	1.03	3.9	1.3	1.41	2.00	2
Arginine	4.62	17.2	5.8	2.80	3.95	4
Aspartic acid	20.26	76.0	25.3	13.93	19.7	20
Threonine	8.49	31.7	10.6	5.54	7.86	8
Serine	4.36	16.3	5.4	5.51	7.79	8
Glutamic acid	15.57	58.1	19.4	12.18	17.2	17
Proline	2.60	9.7	3.2	3.28	4.64	5
Glycine	1.38	5.1	1.7	10.08	14.25	14
Alanine	3.92	15.0	4.9	9.87	13.95	14
Valine	5.29	19.7	6.6	7.23	10.23	10
Methionine	1.06	4.0	1.3	1.36	1.93	2
Isoleucine	10.30	38.5	12.8	6.12	8.64	9
Leucine	11.94	44.5	14.9	7.57	10.70	11
Tyrosine	13.50	50.4	16.8	1.41	2.00	2
Phenylalanine	1.17	4.4	1.5	3.89	5.50	6
Tryptophan	1.86	6.9	2.3			
½ Cystine	0.534	2.0	1.0			
Cysteine	0.268	1.0				
Cysteic acid				0.81	1.17	1

Amino acid analyses were carried out on a Beckman-Spinco automatic amino acid analyzer. Tryptophan estimations were done by method K of Spies and Chambers (Anal. Chem. **21**:1649, 1949), by which we were unable to demonstrate the presence of this amino acid in our toxic preparations.

The results of repeated amino acid analyses of this material are summarized in Table 1. The first three columns of the table show the results obtained previously by Buehler et al. (J. Biol. Chem. **169**:295, 1947) on the crystalline toxin with a calculated molecular weight of 900,000. These authors showed that, on the basis of the cysteine content, a minimal molecular weight of about 45,000 could be calculated. Analysis of the low molecular weight toxic material shows the presence of a single residue of cysteine. In the light of this finding, we have suggested that previous calculations for cysteine and ½ cystine should be grouped together; by so doing, a minimal molecular weight of 15,000 may also be calculated for the crystalline toxin (see Table 1). It is possible that the cysteine residue underwent oxidation and disulfide bridge formation in the process of the crystallization of the toxin. This could account in part for the high molecular weight estimation as well as for the estimation of cysteine and ½ cystine.

Although discrepancies exist between the two analyses, there is a remarkable similarity in the overall ratios between individual amino acids.

It should be mentioned at this time that in a previous publication (J. Gerwing et al., J. Bacteriol. **89**:1176, 1965) we stated that the probable number of cysteine residues in trypsin-activated type E toxin was two. Repeated analyses of type E toxin have shown our previous calculation to be incorrect and that, like types A and B toxins, type E also has a single cysteine residue.