CIRCULAR DICHROISM OF C5a ANAPHYLATOXIN OF PORCINE COMPLEMENT

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SUMMARY

The far-ultraviolet circular dichroism spectrum of C5a anaphylatoxin of porcine complement implies that it has a substantial content of helical structure. The circular dichroism spectra of C5a in the 200-250 nm region at pH 7.2 and 3.7 are nearly identical and resemble those of C3a anaphylatoxin. Treatment of C5a with 2-mercaptoethanol progressively diminishes the ellipticity at 222 nm and its anaphylatoxic activity to limiting values. Removal of the reducing agent by dialysis completely restored both the ellipticity at 222 nm and the activity. This finding indicates that the integrity of the secondary conformation of the C5a molecule is largely dependent on disulfide bonds and is essential for its full activity.

The complement system has been the subject of extensive study in recent years (1-3). However, little is known of the molecular structure of the individual components which comprise this system and of the relationship of their structures to function. C5a is a fragment of component C5 produced by the action of C5 convertase of either the classic or alternate complement pathway (3-5). It is one of the two anaphylatoxins of the complement system, the other being C3a, which is derived from component C3. These anaphylatoxins may act as mediators of inflammation and smooth muscle contraction (6,7), and they are inactivated upon cleavage of the carboxyl-terminal arginine residue by a carboxypeptidase B-like enzyme of serum (8).

Highly active, purified material was subjected to a study of structure-function relationships of C5a. In this report we present the circular dichroism* (CD) spectra of

* Abbreviation used: CD, circular dichroism.

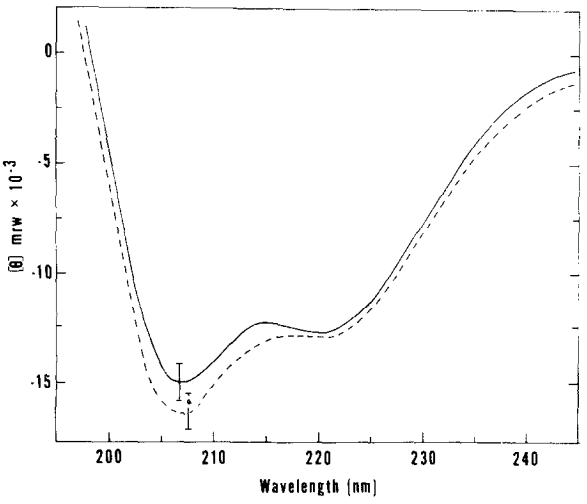


Figure 1 - CD spectra of C5a. Shown are: C5a in 0.02 M sodium phosphate, 0.85% NaCl, pH 7.2 (——) and 0.1 M sodium acetate, pH 3.7 (----) at 27°. The ellipticity is presented in terms of mean residue ellipticity, deg-cm^2/dmole of amino acid residue. The bars across the spectra represent the maximum deviations observed from the reported curves.

C5a in the ultraviolet region and the effect of 2-mercaptoethanol on the activity and conformation of C5a.

MATERIALS AND METHODS

Porcine C5a, molecular weight 14,000, was purified from serum (9,10) and its activity determined by the guinea pig ileum assay, as described previously (4). The protein exhibited a single band on acrylamide gel electrophoresis and contained only a slight trace of des-arg-C5a on cellulose acetate electrophoresis (5). Amino acid analysis showed the preparation to possess the expected amino acid composition which is characteristic for C5a (8,9). Maximal contraction of guinea pig ileum was obtained with 60 nanograms of the purified material in a 20 ml organ bath (5,11). Protein concentrations were determined by the method of Lowry et al. (12).

CD spectra were recorded in duplicate or triplicate in a Cary 61 spectropolarimeter calibrated with d-10-camphorsulfonic acid (13). A mean residue molecular weight of 115 calculated from the amino acid composition of C5a (9,10) was used. Samples were

examined in 1-mm pathlength quartz cuvettes at 27°, and the dynode voltage of the instrument did not exceed 600 volts.

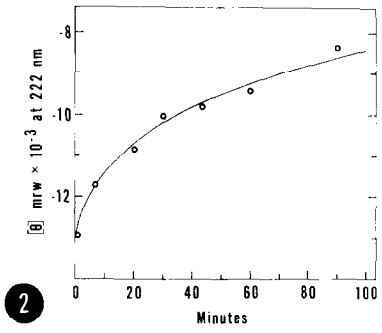
Solutions of C5a were dialyzed overnight against the desired solvent, and their pH and concentrations were measured. The effect of 2-mercaptoethanol on the activity of C5a was determined by adding the reagent to the protein in 0.02 M sodium phosphate, 0.85% sodium chloride, pH 7.2 (phosphate-buffered saline). The ellipticity at 222 nm of C5a was determined at various time intervals after addition of 2-mercaptoethanol. The activity of the protein was tested at comparable time intervals in the organ bath assay system (5). Control experiments showed this system to be unaffected by the concentrations of 2-mercaptoethanol employed.

RESULTS AND DISCUSSION

The CD spectra of C5a from 200-250 nm at pH 3.7 and pH 7.2 are shown in Figure 1. No significant differences are evident between these spectra, which suggests that the secondary structure of the molecule is stable over this pH range. Examination of the near-ultraviolet CD spectrum of C5a was precluded by lack of material and by the weak signals in the 350-240 nm region. The spectra qualitatively imply that the C5a molecule has a considerable content of α-helical structure. Indeed, calculations by three methods (14-16) based on empirical relations of CD spectra to secondary structure indicate that the molecule contains approximately 40% α-helix. Notably, the C3a molecule (molecular weight 8900) also contains 40-45% α-helical structure (11).

Mercaptoethanol treatment of C5a produces a progressive decline in the magnitude of the ellipticity at 222 nm (Figure 2). This decline approaches a limiting value at which an appreciable fraction of the original ellipticity at 222 nm remains. A CD spectrum indistinguishable from the original spectrum was observed after removal of 2-mercaptoethanol by dialysis against phosphate-buffered saline.

The effect of 2-mercaptoethanol with time on the activity of C5a in the guinea



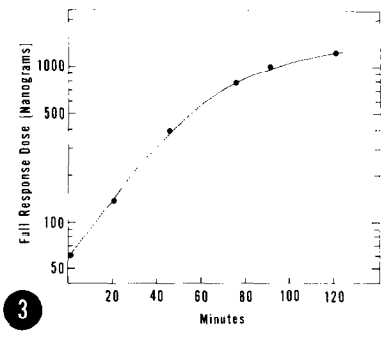


Figure 2 - Effect of 2-mercaptoethanol on the mean residue ellipticity at 222 nm of C5a. The reducing agent was added to C5a in phosphate-buffered saline at 27° to a final concentration of 0.07 M. The ellipticity at 222 nm was determined at the times indicated.

Figure 3 - Effect of 2-mercaptoethanol on the activity of C5a. The activity of C5a in the guinea pig ileum assay was determined at the indicated times after addition of 2-mercaptoethanol, final concentration of 0.25 M, to C5a in phosphate-buffered saline. At the indicated times, C5a activity was tested by determining the amount of C5a required to give a full response of the ileum in a 20 ml organ bath. The amount of C5a needed for full response is presented on a logarithmic scale against the time of incubation in the presence of reducing agents.

pig ileum assay is presented in Figure 3. The C5a activity, like the ellipticity at 222 nm, progressively declines with time of exposure to the reducing agent, as shown by the increasing amount of anaphylatoxin required to obtain full contraction of the ileum. The decline in activity also approached a limiting value.

In another experiment, C5a was inactivated by treatment with 0.05 M 2-mercaptoethanol. After dialysis of the inactivated C5a against phosphate-buffered saline for 48 hours in the cold, the preparation had recovered its original activity: a full contraction of the ileum was obtained with 60 nanograms. These observations indicate that the activity of the C5a molecule depends upon its secondary structure which in turn depends, to a large extent, on disulfide bonds.

As will be reported in detail elsewhere, similar results have been observed with human and porcine C3a anaphylatoxins (11). The resemblance between the CD spectra of C5a and C3a is striking and points to a similarity in their secondary structures. Both C3a

and C5a are activation peptides of their precursors, C3 and C5, respectively. Both peptides possess similar biological activities in that they cause histamine release from mast cells, smooth muscle contraction and directed migration of polymorphonuclear leukocytes (7,17,18). Apparently these peptides exert their biologic effects by interaction with cell membranes. In this connection, it is of interest that some proteins thought to interact with membranes (15) or lipids (19) have a considerable content of α-helix. This may point to a fundamental role of the α-helical structure in the interactions of proteins with membrane constituents. The results reported here and elsewhere (11) suggest that the extensive α-helical contents of C3a and C5a are structural features which might be intimately related to their physiological activities as anaphylatoxins.

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REFERENCES

1. Müller-Eberhard, H.J. (1971) in B. Amos, ed. Progress in Immunology, Vol. 1, pp. 553-565, Academic Press, New York.
2. Ruddy, S., Gigli, I., and Austen, K.F. (1972) New Engl. J. Med. 287, 489-495, 545-549, 592-596, 642-646.
3. Müller-Eberhard, H.J. (1972) in The Harvey Lectures, Series 66, pp. 75-104, Academic Press, New York.
4. Cochrane, C.G., and Müller-Eberhard, H.J. (1968) J. Exp. Med. 127, 371-386.
5. Vallota, E.H., and Müller-Eberhard, H.J. (1973) J. Exp. Med. 137, 1109-1123.
6. Lepow, I.H., Dias Da Silva, W., and Eisele, J.W. (1968) in K.F. Austen and E.L. Becker, eds. The Biochemistry of the Acute Allergic Reactions, pp. 265-282, Blackwell Scientific Publications, Oxford.
7. Müller-Eberhard, H.J., Vallota, E.H., Götze, O., and Zimmerman, T.S. (1972) in I.H. Lepow and P.A. Ward, eds. Inflammation, Mechanisms and Control, pp. 83-91, Academic Press, New York.

8. Bokisch, V.A., and Müller-Eberhard, H.J. (1970) J. Clin. Invest. 49, 2427-2436.
9. Liefländer, M., Dielenberg, D., Schmidt, G., and Vogt, W. (1972) Hoppe-Seyler's Z. f. Physiol. Chem. 353, 385-392.
10. Vallota, E.H., Hugli, T.E., and Müller-Eberhard, H.J. (1974) J. Immunol. (in press)
11. Hugli, T.E., Morgan, W.T., and Müller-Eberhard, H.J. (1974) J. Biol. Chem. (in press)
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
13. Cassim, J.Y., and Yang, J.T. (1969) Biochemistry 8, 1947-1951.
14. Greenfield, N., and Fasman, G.D. (1969) Biochemistry 8, 4108-4116.
15. Bewley, T.A., Brovetto-Cruz, J., and Li, C.H. (1969) Biochemistry 8, 4701-4708.
16. Chen, Y-H., Yang, J.T., and Martinez, H.M. (1972) Biochemistry 11, 4120-4131.
17. Ward, P.A. (1971) J. Exp. Med. 134, 109s-113s.
18. Johnson, A.R., and Müller-Eberhard, H.J. (1974) J. Immunol. (in press)
19. Scanu, A.M., Edelstein, C., Vitello, L., Jones, R., and Wissler, R. (1973) J. Biol. Chem. 248, 7648-7652.