Vol. 105, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1982 Pages 398-403

β SHEET TO α HELIX TRANSITION IN THE BINDING SUBUNIT OF CHOLERA TOXIN

Randall M. Robinson, Maher M. Hamed, and Wayne L. Mattice

Department of Chemistry
Louisiana State University
Baton Rouge, Louisiana 70803

Received January 28, 1982

SUMMARY: Circular dichroism spectra have been measured for the binding subunit of cholera toxin in water and in the presence of dodecyl sulfate. In water the protein has an appreciable amount of β structure and almost no α helix. In the presence of dodecyl sulfate the spectrum undergoes drastic change over a time period of approximately four hours, and at equilibrium resembles that expected for a chain with an appreciable amount of α helix but no β structure. The change in helicity is in good agreement with that expected from our formulation of the configuration partition function for the binding subunit. The conformational change may have an important relationship to the means by which the binding subunit permits penetration of the active subunit into the cell.

The enterotoxin of Vibrio cholerae has the subunit structure AB_5 (1,2). Subunit B, molecular weight 11,604 (3), is responsible for binding of the toxin to the cell membrane via specific interaction with ganglioside G_{M1} (4-8). The specificity of this interaction has been shown to reside in the oligosaccharide portion of the ganglioside (9). Arginyl 35 of subunit B has been implicated in the binding process (10). There is a lag of about 2-4 hours between binding of the toxin and fluid loss (11). This lag may arise from the necessity for a conformational change in the toxin (6).

Experimental circular dichroism studies and theoretical statistical mechanical methods have been combined to successfully rationalize conformational changes produced in endogenous opioid peptides and hormonal peptides upon their interaction with anionic lipids (12-15). When applied to subunit B of cholera toxin, these theoretical methods (16) predict a conformation in the presence of anionic lipid which is quite different from the structure thought to exist in water (9). If the ganglioside G_{M1} - cell membrane environment bears a similarity to that provided by anionic lipid,

0006-291X/82/060398-06$01.00/0

the conformation of subunit B might indeed change upon binding. Circular dichroism studies reported here provide experimental confirmation for the major conclusions expected from theory. The conformational change observed experimentally is found to take place on a time scale comparable to the lag period between toxin binding and fluid loss.

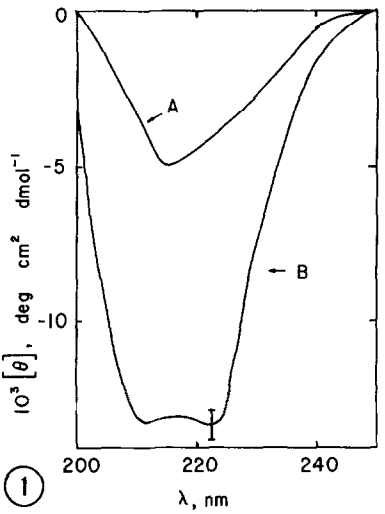
METHODS

Subunit B of cholera toxin from Vibrio cholerae and dithiothreitol were obtained from Sigma Chemical Co. Sodium dodecyl sulfate was from Matheson, Coleman, and Bell. Protein solutions investigated had an approximate concentration of 0.0187 mg/ml. Concentration was determined from the absorbance and extinction coefficient at 280 nm (17). Circular dichroism spectra were measured using a Durrum-Jasco recording spectropolarimeter calibrated with d-10-camphorsulfonic acid (18). The theoretical and experimental basis for the statistical mechanical calculations has been described earlier (16,19). The specific equations used are those numbered 1, 2, 4, and 6 in Robinson et al. (15)

RESULTS

Circular dichroism of subunit B in water (Figure 1) is negative throughout the spectral range studied, with a single minimum near 215 nm having $[\theta] = -5000$ deg cm^2 dmol^{-1}. Shape and intensity are in reasonable agreement with spectra reported earlier (9,10). The spectrum in water indicates the virtual absence of α helix and the presence of appreciable β structure.

Upon addition of dodecyl sulfate, the circular dichroism changes over a period of several hours. There is an increase in intensity and development of the double minimum characteristic of the α helix. The equilibrium spectrum is depicted in Figure 1, and the time dependence of $[\theta]_{222}$ is depicted in Figure 2. Half of the change has been completed in about 20 minutes, but roughly ten times as long is required to attain equilibrium. The profile suggests multiple steps are involved in the rearrangement. The equilibrium spectrum in dodecyl sulfate is less intense, by about 15%, in the presence of excess dithiothreitol. Development of helicity is therefore favored by the presence of an intact disulfide bond between cysteinyl residues 9 and 86. The temperature dependence of the equilibrium spectra is also smaller when the disulfide bond is intact ($d[\theta]_{222}/dT$ are 54 and 84 deg cm^2 dmol^{-1} K^{-1}). The



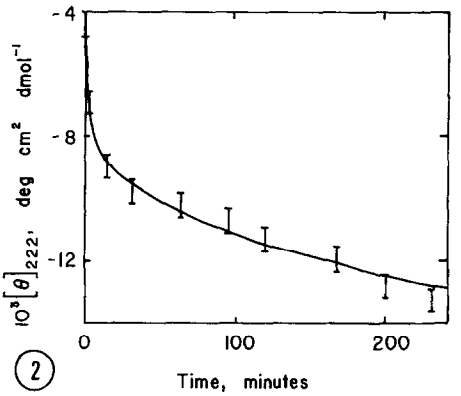


Fig. 1. Circular dichroism of subunit B in (A) water, and (B) 0.003 M dodecyl sulfate. Conditions were pH 7.0 at 30° C and pH 7.04 at 25° C, respectively.

Fig. 2. Time dependence of $[\theta]_{222}$ for subunit B in 0.003 M dodecyl sulfate, pH 7.04, 25° C. The line through the points was calculated for two consecutive first-order reactions. Rate constants were k_1 = 0.21 min^{-1} and k_2 = 0.0069 min^{-1}, and ellipticities of the three species, in order of their appearance, were -4400, -8700, and -13900 deg cm^2 dmol^{-1}.

value of $[\theta]_{222}$ obtained at equilibrium in dodecyl sulfate suggests a helical content of about 33%. A helical content near zero is deduced from the circular dichroism in water. The matrix calculation (15,16) predicts helical contents of 8% in water and 38% in the presence of dodecyl sulfate. Experimental circular dichroism spectra change in the manner predicted by the theory.

DISCUSSION

The theoretical prediction (8% helix in absence of dodecyl sulfate, 38% in its presence) is in reasonable agreement with the circular dichroism reported in Figure 1. Agreement between observed and calculated helicities for the native state may be, in part, fortuitous. The calculation does not take account of long range interactions which are undoubtedly important in determination of the conformation of the native state. Greater significance should be attached to the agreement between experiment and theory in the presence of dodecyl sulfate. Since the configuration partition function

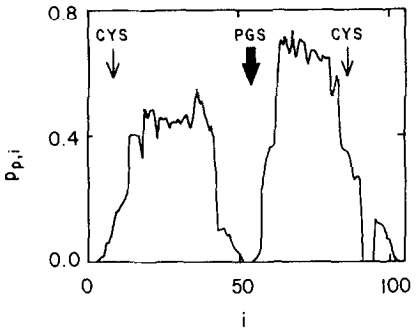


Fig. 3. Computed helix propagation probability profile for subunit B in the presence of dodecyl sulfate. Locations of the cysteinyl residues involved in disulfide bond formation, as well as the Pro-Gly-Ser (PGS) sequence, are noted.

successfully reproduces the fraction helix under these conditions, it is reasonable to extract from this configuration partition function the helix propagation probability profile (Figure 3). Formation of two helical segments containing as many as 30 residues each is clearly predicted. Experiment shows slightly more helical structure (33% vs. 28%) is induced when the disulfide bond is intact than in the presence of excess dithiothreitol. Residues 53-55, Pro-Gly-Ser, have a high probability for reverse turn formation (20,21). This tripeptide forms a reverse turn in adenylate kinase (residues 17-19) (20). A simple model for subunit B in dodecyl sulfate might be a helical hairpin, with the Pro-Gly-Ser sequence forming a reverse turn at the head of the hairpin and the disulfide bond crosslinking the ends. Helix-helix interaction (22,23) could then occur along much of the length of the hairpin. Rupture of the disulfide bond would make accessible conformations in which the helices are sufficiently remote from one another that stabilizing helix-helix interactions are negated. The helical content would then decrease upon reduction of the disulfide bond, in harmony with the response of the circular dichroism spectra to excess dithiothreitol.

The hairpin conformation may be important to the biological activity of subunit B. The cellular receptor for subunit B in vivo is ganglioside G_{M1} (4-8), an anionic amphipathic molecule. In studies of cholera in canines,

Carpenter and coworkers (11) observed that the onset of fluid loss through the intestines always follows a lag period of several hours. Sterile filtrates of Vibrio cholerae were usually absorbed by jejeunal loops within an hour, and fluid secretion started after two hours, and reached a maximal rate at four hours, which lasted 4-6 hours. A lag period of 15-60 minutes has been observed in adenylate cyclase activation in isolated tissues and cells (24-29). The time scale of the conformational change observed in dodecyl sulfate is comparable to that of the lag period.

Further study of the conformational transition is warranted for several reasons: (i) It is an excellent model for a β structure to α helix transition in a protein. (ii) The molecule constitutes a large macrocycle, closed by a disulfide bond, which provides for helix-helix interaction. (iii) The conformational change may be related to the means by which subunit B facilitates penetration of subunit A into the cell.

ACKNOWLEDGEMENT

This work was supported by National Science Foundation research grant PCM 78-22916.

REFERENCES

1. Gill, D. M. (1976) Biochemistry 15, 1242-1248.
2. Lai, C. Y. (1980) CRC Crit. Rev. in Biochem. 171-206.
3. Kurosky, A., Markel, D. E., and Peterson, J. W. (1977) J. Biol. Chem. 252, 7257-7264.
4. Cuatrecasas, P. (1973) Biochemistry 12, 3547-3558.
5. Cuatrecasas, P. (1973) Biochemistry 12, 3558-3566.
6. Cuatrecasas, P. (1973) Biochemistry 12, 3566-3577.
7. King, C. A., and van Heyningen, W. E. (1973) J. Infect. Dis. 127, 639-647.
8. Holmgren, J., Lönnroth, I., and Svennerholm, L. (1973) Infect. Immun. 8, 208-214.
9. Fishman, P. H., Moss, J., and Osborne, J. C., Jr. (1978) Biochemistry 17, 711-716.
10. Duffy, L. K., and Lai, C. Y. (1979) Biochem. Biophys. Res. Commun. 91, 1005-1010.
11. Carpenter, C. C., Jr., Sack, R. B., Feeley, J. C., and Steenberg, R. W. (1968) J. Clin. Invest. 47, 1210-1220.
12. Mattice, W. L., and Robinson, R. M. (1981) Biopolymers 20, 1421-1434.
13. Mattice, W. L., and Robinson, R. M. (1981) Biochem. Biophys. Res. Commun. 101, 1311-1317.
14. Maroun, R. C., and Mattice, W. L. (1981) Biochem. Biophys. Res. Commun. 103, 442-446.
15. Robinson, R. M., Blakeney, E. W., and Mattice, W. L. (1982) Biopolymers 21, 000-000.
16. Mattice, W. L., Srinivasan, G., and Santiago, G. (1980) Macromolecules 13, 1254-1260.

17. Lospalluto, J. J., and Finkelstein, R. A. (1972) Biochim. Biophys. Acta 257, 158-166.
18. Cassim, J. Y., and Yang, J. T. (1970) Biochemistry 8, 1947-1951.
19. Mattice, W. L., Riser, J. M., and Clark, D. S. (1976) Biochemistry 15, 4264-4272.
20. Chou, P. Y., and Fasman, G. D. (1974) Biochemistry 13, 222-244.
21. Zimmerman, S. S., and Scheraga, H. A. (1977) Proc. Nat Acad. Sci. USA 74, 4126-4129.
22. Skolnick, J., and Holtzer, A. (1982) Macromolecules 15, 000-000.
23. Mattice, W. L., and Skolnick, J. (1982) Macromolecules 15, 000-000.
24. Field, M., Fromm, D., Wallace, L. K., and Greenough, W. B., III (1969) J. Clin. Invest. 48, 24a.
25. Vaughn, M., Pierce, N. F., and Greenough, W. B., III (1970) Nature (London) 226, 658-659.
26. Chen, L. C., Rohde, J. E., and Sharp, G. W. G. (1972) J. Clin. Invest. 51, 731-740.
27. Sharp, G. W. C., and Hynie, S. (1971) Nature (London) 229, 266-269.
28. Boyle, J. M., and Gardner, J. (1974) J. Clin. Invest. 53, 1149-1158.
29. Gill, D. M., and King, C. A. (1975) J. Biol. Chem. 250, 6424-6432.