Conformational Studies of Australia Antigen by Optical Rotatory Dispersion and Circular Dichroism

NORIYOSHI SUKENO, RYOICHI SHIRACHI, HIROYUKI SHIRAISHI, AND
NAKAO ISHIDA

*Department of Bacteriology, Tohoku University School of Medicine and Miyagi
Prefectural Institute of Public Health, Sendai, Japan*

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The optical rotatory dispersion and circular dichroism of intact, 8 M urea- or sodium dodecyl sulfate-treated, and carbamidomethylated Australia antigen indicated that the antigen possesses a high α -helical content similar to human high-density lipoproteins.

In our previous communication, loss of structure and antigenicity of Australia antigen particle by reduction with dithiothreitol (DTT), and reconstitution by reoxidation, was reported, emphasizing the role of disulfide bonds in the structural integrity of Australia antigen (10). In this paper, we report conformational studies of the same antigen by optical rotatory dispersion (ORD) and circular dichroism (CD), to compare the antigen with viruses and human serum lipoproteins.

The Australia antigen was purified as described previously (10). Immunoelectrophoresis indicated the absence of any human serum components. For optical measurements, the purified antigen was dissolved in 0.01 M sodium phosphate buffer (pH 7.2) containing 0.15 M NaCl at a concentration of 1 to 2 mg of protein/ml as determined by the Lowry method (6). Ultraviolet absorption spectra of the antigen were measured on a Hitachi spectrophotometer, model-124. Measurements of ORD and CD in the ultraviolet region were carried out with a JASCO model J-20 spectropolarimeter at 25°C with the use of cells of 0.1-mm path length. All spectra were measured in triplicate.

An ultraviolet spectrum of the purified Australia antigen measured at acidic or neutral pH was characteristic for protein, with a maximum at 280 nm and minimum at 250 nm. There was no peak at 259 nm, which is the characteristic region for nucleic acid absorption. In addition, all highly purified preparations showed a characteristic shoulder at 285 nm, which disappeared at alkaline pH (Fig. 1). This shoulder, due to tryptophan residues, seems to be significant in dis-

criminating the Australia antigen from other proteins.

The ORD and CD spectra of intact Australia antigen are shown in Fig. 2 and 3. The ORD spectrum (Fig. 2) is typical of an α -helical protein, having a trough at 233 nm, a crossover at 223 nm, a shoulder between 215 and 220 nm, and a peak at about 198 nm (11). The CD spectrum of Australia antigen (Fig. 3) is again typical of an α -helical protein (4), with minima at 207 and 222 nm and a crossover at 200 nm. The calculation of α -helix content in the Australia antigen made by the usual method (1, 3), based upon the values shown in Fig. 2 and 3 (mean residual rotation in ORD and ellipticity in CD), gives a value for α -helix content of 70 to 80%.

The same conformational analysis was conducted with four different chemically modified Australia antigens. These were 8 M urea-treated antigen (unaltered antigenicity), 1% sodium dodecyl sulfate-treated antigen (nonantigenic), DTT-reduced antigen (nonantigenic), and carbamidomethylated antigen (nonantigenic). All gave ORD and CD spectra similar to native Australia antigen. These results indicate that the gross secondary structure of the antigen is not influenced by these chemical modifications.

Most common viruses such as mengovirus (5) and tobacco mosaic virus (9) have an α -helix content of 10 to 25%. Among the viruses, only filamentous phage fd (2) has been reported to have an α -helix content of 90%. In contrast, the human high-density lipoproteins are known to have about 70% α -helical structure (7, 8). Thus, the present results indicate that Australia antigen

FIG. 1. Ultraviolet absorption spectrum of purified Australia antigen.

FIG. 2. Optical rotatory dispersion of Australia antigen plotted as mean residue rotation $[m']$ against λ (nm).

FIG. 3. Circular dichroism of Australia antigen plotted as mean residue ellipticity $[\theta]$ against λ (nm).

is much more similar to human lipoproteins than it is to normal viruses.

LITERATURE CITED

1. Blout, E. R., I. Schmier, and N. S. Simmons. 1962. New cotton effects in polypeptides and proteins. *J. Amer. Chem. Soc.* **84**:3193-3194.
2. Day, L. A. 1966. Protein conformation in fd bacteriophage as investigated by optical rotatory dispersion. *J. Mol. Biol.* **15**:395-398.
3. Greenfield, N., and G. D. Fasman. 1969. Computed circular dichroism spectra for the evaluation of protein conformations. *Biochemistry* **8**:4108-4116.
4. Holzwarth, G., and P. Doty. 1965. The ultraviolet circular dichroism of polypeptides. *J. Amer. Chem. Soc.* **87**:218-228.
5. Kay, C. M., J. S. Cothier, and K. Oikawa. 1970. Circular dichroism studies on mengo virus variants and their constituent ribonucleates. *Can. J. Biochem.* **48**:940-943.
6. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
7. Scanu, A. 1965. Studies on the conformation of human serum high-density lipoprotein HDL₂ and HDL₃. *Proc. Nat. Acad. Sci. U.S.A.* **54**:1699-1705.
8. Scanu, A., and R. Hirz. 1968. Human serum low-density lipoprotein protein: its conformation studied by circular dichroism. *Nature (London)* **218**:200-201.
9. Simmons, N. S., and E. R. Blout. 1960. The structure of tobacco mosaic virus and its components: ultraviolet and optical rotatory dispersion. *Biophys. J.* **1**:55-62.
10. Sukeno, N., R. Shirachi, J. Yamaguchi, and N. Ishida. 1972. Reduction and reoxidation of Australia antigen: loss and reconstitution of particle structure and antigenicity. *J. Virol.* **9**:182-183.
11. Yang, J. T. 1967. Optical activity of the α , β and coiled conformations in polypeptides and proteins, p. 157-172. In G. N. Ramachandran (ed.), *Conformations of biopolymers*, vol. 1. Academic Press Inc., New York.