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

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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 indi-
cated that the antigen possesses a high \( \alpha \)-helical content similar to human high-
density lipoproteins.

In our previous communication, loss of struc-
ture and antigenicity of Australia antigen particle by reduction with dithiothreitol (DTT), and re-
constitution by reoxidation, was reported, emphasi-
sing 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). Immuno-electrophoresis 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 spec-
trophotometer, model-124. Measurements of
ORD and CD in the ultraviolet region were car-
rried out with a JASCO model J-20 spectropola-
rimeter at 25°C with the use of cells of 0.1-mm
path length. All spectra were measured in trip-
licate.

An ultraviolet spectrum of the purified Aus-
tralia 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 re-
gion for nucleic acid absorption. In addition, all
highly purified preparations showed a character-
istic shoulder at 285 nm, which disappeared at
alkaline pH (Fig. 1). This shoulder, due to tryp-
tophan residues, seems to be significant in dis-
criminating the Australia antigen from other pro-
teins.

The ORD and CD spectra of intact Australia
antigen are shown in Fig. 2 and 3. The ORD spec-
trum (Fig. 2) is typical of an \( \alpha \)-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
\( \alpha \)-helical protein (4), with minima at 207 and 222
nm and a crossover at 200 nm. The calculation of
\( \alpha \)-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
\( \alpha \)-helix content of 70 to 80%.

The same conformational analysis was con-
ducted with four different chemically modified
Australia antigens. These were 8 M urea-treated
antigen (unaltered antigenicity), 1% sodium dode-
cyl sulfate-treated antigen (nonantigenic),
DTT-reduced antigen (nonantigenic), and carba-
midomethylated antigen (nonantigenic). All gave
ORD and CD spectra similar to native Australia
antigen. These results indicate that the gross sec-
dary 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 \( \alpha \)-helix
content of 10 to 25%. Among the viruses, only
filamentous phage fd (2) has been reported to
have an \( \alpha \)-helix content of 90%. In contrast,
the human high-density lipoproteins are known to
have about 70% \( \alpha \)-helical structure (7, 8). Thus,
the present results indicate that Australia antigen
**NOTES**

**J. VIROL.**

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**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 [θ] against λ (nm).

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

**LITERATURE CITED**


