Response to ''Comment on 'Improving protein circular dichroism calculations in the far-ultraviolet through reparameterizing the amide chromophore' '' [J. Chem. Phys. 111, 2844 (1999)]

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(Received 25 March 1999; accepted 13 May 1999)

[S0021-9606(99)00530-9]

In their Comment,[1] Woody and Sreerama report an improvement in the calculation of protein circular dichroism (CD) using the matrix method.[2] Their approach consists of a combination of experimental data, semiempirical calculations using the spectroscopic parametrization of intermediate neglect of differential overlap method (INDO/S), and better locations of monopoles used to describe charge distributions. The protein CD spectra thus calculated are a significant improvement over our previous calculations[3] based on multireference configuration interaction (MRCI) parameters[4] and over our previous assessment[3] of parameters derived from semiempirical calculations using the spectroscopic parametrization of complete neglect of differential overlap method (CNDO/S). Recently, we have independently realized a similar improvement,[5] also using the matrix method, but with a different approach to parameter development.

Our most recent approach has used complete active space self-consistent field/self consistent reaction field (CASSCF/SCRF) calculations on N-methylacetamide (NMA).[6] These *ab initio* calculations involve a self-consistent reaction field, treating solvent as a continuum dielectric. The CASSCF/SCRF calculations reproduce all the experimental solution phase data well, including the transition energies, the orientation, and magnitude of the $\pi\pi^*$ (NV_1) transition dipole moment. In our recent CD calculations, which do not employ any experimental data, transition and static charge densities are represented by charges fitted to reproduce the *ab initio* electrostatic potential, which they do with an error of about 5%.

Our approach gives a Spearman rank correlation coefficient of 0.89 between the calculated and experimental intensity at 220 nm for the 23 proteins studied previously,[3] and a correlation of 0.81 at 190 nm. Thus, our most recent work and that of Woody and Sreerama both achieve a near-quantitative accuracy at 220 nm. Both studies show a more modest, but nevertheless significant, correlation at 190 nm, and both also qualitatively reproduce the peak at 208 nm. These improvements appear to stem from similar sources. The orientation of the $\pi\pi^*$ (NV_1) transition dipole moment and its intensity are important. Furthermore, as noted by Woody and Sreerama, our previous representation of charge distributions introduced avoidable imprecision.

In Fig. 1, we compare our calculated CD spectra with the experimental spectra for four proteins. For the predominantly α-helical proteins, hemoglobin and triosephosphate isomerase, the intensity of the 190 nm band is reproduced well, but the intensity at 220 nm is a little underestimated. For the β-sheet proteins, the intensity at 190 nm is overestimated, more significantly for elastase. However, for both elastase and concanavalin A, the region close to 220 nm is well reproduced.

In their Comment, Woody and Sreerama make several valid points. However, their conclusion that semiempirical wave functions can be a basis for protein CD calculations of the quality reported, perhaps warrants some qualification. Their CD calculations rely on experimental data. The orientation of the $\pi\pi^*$ (NV_1) transition dipole moment is taken from experimental measurements reported by Clark on N-acetylglycine in a condensed phase.[7] INDO/S calculations reported by Clark on the same system show a significant 20° difference. These INDO/S calculations also overestimate the intensity of this transition. The calculated oscillator strength is 25% larger than that determined experimentally. Manning and Woody reported a similar problem with CNDO/S calculations on NMA.[8] Although it is unclear, based on their CD calculations, it appears that Woody and Sreerama have rescaled the calculated $\pi\pi^*$ (NV_1) transition dipole moment to agree with experiment. It also appears that Woody and

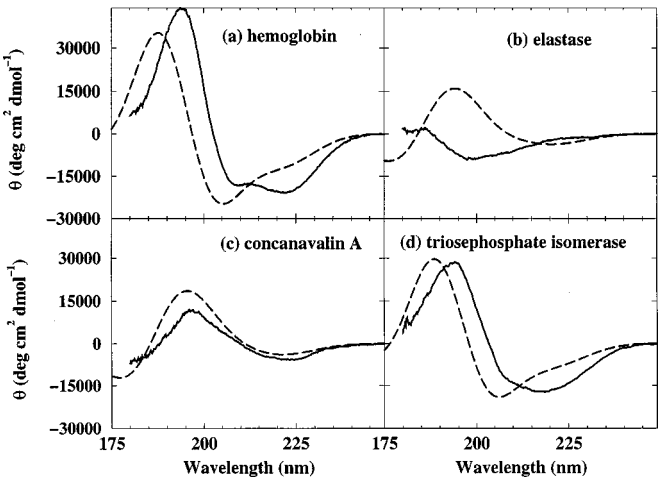

FIG. 1. Calculated (dashed lines) and experimental (solid lines) CD spectra for four proteins. Intensity is given as the mean residue ellipticity. The calculated spectra were generated using a Gaussian bandwidth of 12.5 nm for all transitions.

Sreerama use the experimental transition energies, as the INDO/S calculated energies differ from experiment, particularly for the $n\pi^*$ transition energy, which is calculated to be greater than 300 nm. Beyond the mixing of INDO/S parameters and experimental data, the parameters of Woody and Sreerama have other inconsistencies whose origin is less clear. In particular, the ground-state monopoles were taken from a third source[9] and the NV_1 and NV_2 transitions were not allowed to mix. Clark concludes that there is a general agreement between experiment and INDO/S calculations.[7] However, it seems clear that INDO/S calculations by themselves do not provide an adequate basis for the calculation of protein CD.

Finally, we note that recent calculations using the dipole interaction model are in almost quantitative agreement with experiment at 190 nm.[10] This observation, combined with the improved results at 220 nm obtained by Woody and Sreerama and by ourselves using the matrix method, suggests that after more than 40 years since Moffitt's seminal work,[11] fully quantitative protein CD calculations are almost within grasp.

ACKNOWLEDGMENTS

Financial support from the National Science Foundation (Grant No. MCB-9632124) is gratefully acknowledged. Acknowledgment is made to the donors of The Petroleum Research Fund, administered by the ACS, for partial support of this work.

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