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A NEW METHOD FOR DETERMINING PROTEIN SECONDARY STRUCTURE BY LASER RAMAN SPECTROSCOPY APPLIED TO fd PHAGE

R. W. Williams and A. K. Dunker, *Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164*
W. L. Peticolas, *Chemistry Department, University of Oregon, Eugene, Oregon 97403 U.S.A.*

INTRODUCTION

A new method is presented for estimating the secondary structure of proteins from the intensity distribution of the laser Raman amide I spectrum. We have applied this method to the coat protein of the fd filamentous phage.

At a fixed frequency, the observed Raman scattering intensity for a single protein may be expressed as $f_{ho}I_{ho} + f_{hd}I_{hd} + f_{ba}I_{ba} + f_{\beta p}I_{\beta p} + f_tI_t + f_uI_u = Ie$, where Ie is the normalized experimental Raman intensity, each f is the fraction of a type of secondary structure and each I is the intensity that would be observed for a polypeptide with 100% of the indicated structure type. The structure types are: *ho*, ordered helix; *hd*, disordered helix; βa, antiparallel β-sheet; βp, parallel β-sheet; *t*, turn; and *u*, undefined. The experimental Raman intensity is normalized by dividing the observed intensity at each wavenumber by the sum over all wavenumbers of the observed intensities.

METHODS

Laser Raman spectra of the proteins listed in Table I were collected and solvent spectra were substracted generally as described elsewhere (Dunker et al., 1979).

Six reference intensities, I, representing the spectra of polypeptides with a single structure type, were computed from eleven equations, each equation representing a different protein of known structure. This set of solutions provides the reference spectra.

Linear combinations of the reference spectra were then fitted to the amide I spectrum of each protein for which an estimate of structure, f, was desired.

In our calculations of reference spectra, we have relied on the x-ray diffraction derived estimates of secondary structure obtained by Levitt and Greer (1977) or on the references therein. We have made a distinction between central helical residues that are likely to be hydrogen bonded at both the amide hydrogen and at the carbonyl oxygen (ordered helix) and end residues that have only one hydrogen bond (disordered helix).

RESULTS

Results are shown in Table I.

TABLE I

FRACTIONS OF SECONDARY STRUCTURE AS DETERMINED BY LASER RAMAN SPECTROSCOPY FOR SEVERAL PROTEINS COMPARED WITH FRACTIONS OF STRUCTURE DERIVED FROM X-RAY DIFFRACTION DATA

	Structure type					
Protein	*ho*	*hd*	*βa*	*βp*	*t*	*u*
Myokinase	0.266*	0.436	−0.015	0.177	0.143	−0.007
Suspension	0.211‡	0.407	0.0	0.186	0.149	0.047
Insulin	0.065	0.512	0.201	−0.049	0.141	0.131
Crystals	0.088	0.520	0.127	0.0	0.117	0.148
Lactate dehydrogenase	0.176	0.250	0.102	0.014	0.202	0.257
pH 7	0.179	0.237	0.119	0.052	0.195	0.218
Triose phosphate	0.136	0.323	0.004	0.267	0.105	0.165
Isomerase crystals	0.174	0.344	0.0	0.243	0.119	0.120
Thermolysin	0.134	0.214	0.262	−0.012	0.259	0.144
Crystal	0.218	0.177	0.237	0.047	0.190	0.131
Lysozyme	0.120	0.304	0.140	0.064	0.193	0.176
pH 7	0.109	0.349	0.171	0.0	0.225	0.146
Alcohol dehydrogenase	0.096	0.154	0.261	0.082	0.204	0.202
Crystals	0.061	0.184	0.243	0.078	0.188	0.246
Ribonuclease S	0.062	0.251	0.317	0.069	0.122	0.177
pH 5	0.040	0.194	0.460	0.0	0.154	0.152
Concanavalin A	−0.014	−0.048	0.703	−0.069	0.250	0.181
pH 6	0.0	0.025	0.586	0.0	0.220	0.169
Chymotrypsin	0.037	0.120	0.401	0.049	0.197	0.195
0.001 M HCl	0.013	0.068	0.436	0.0	0.246	0.237
fd phage pH 8	0.416*	0.341	0.062	-0.005	0.276	-0.088
fd coat:SDS 1:1.4 wt/wt	0.095*	0.456	-0.020	0.051	0.250	0.173

*Upper values were determined by the Raman method described here.

‡Lower values were obtained from Levitt and Greer (1977), and as described in Methods.

DISCUSSION

Filamentous phages have been studied extensively by x-ray diffraction by Marvin and coworkers (1975), who have proposed a detailed stereochemical model for these viruses in which the coat protein is 100% helical. The results shown here may be useful in further attempts to interpret the x-ray diffraction patterns of these phages.

Finally, Nozaki et al. (1978) have proposed that during phage assembly, in which the membrane bound coat protein forms a sheath around the DNA, the coat protein undergoes a major β-sheet to helix conformational change. This model is based on circular dichroism studies of the coat protein mixed with detergents and lipids as compared to the CD spectrum

of the phage. The results shown in the table for coat protein mixed with sodium dodecyl sulfate (SDS) suggest that the structural change upon DNA binding may not be great, and that β-sheet is not involved.

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INVESTIGATION OF SECONDARY STRUCTURES AND MACROMOLECULAR INTERACTIONS IN BACTERIOPHAGE P22 BY LASER RAMAN SPECTROSCOPY*

S. R. Fish and K. A. Hartman, *Department of Biochemistry and Biophysics, University of Rhode Island, Kingston, Rhode Island 02881*

M. T. Fuller and Jonathan King, *Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

G. J. Thomas, Jr., *Department of Chemistry, Southeastern Massachusetts University, North Dartmouth, Massachusetts 02747 U.S.A.*

ABSTRACT Laser Raman spectra of the DNA bacteriophage P22 and of its precursor particles and related structures have been obtained using 514.5-nm excitation. The spectra show that P22 DNA exists in the B form both inside of the phage head and after extraction from the phage. The major coat protein (gp5) contains a secondary structure composed of 18% α-helix, 20% β-sheet and 62% irregular conformations. The scaffolding protein (gp8) in the phage prohead is substantially richer than gp5 in α-helical content. Among the amino acid residues which give prominent Raman lines, the spectra show that tryptophans are exposed to solvent and most tyrosines are hydrogen bonded to positive donor groups. The above features of phage DNA and protein structures are nearly invariant to changes in temperature up to 80°C, indicating a remarkable thermal stability of the phage head and its encapsulated DNA.

INTRODUCTION

The laser-Raman spectrum of bacteriophage P22 consists of numerous lines due to radiation scattered by the normal modes of vibration of protein and DNA molecules of the virion. Each line is identified by its frequency shift (in cm^{-1} units) from the laser excitation frequency, and its intensity relative to an arbitrary standard. Among the many factors which determine the frequency and intensity of a given Raman line are the intramolecular and intermolecular

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