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Vacuum-ultraviolet circular dichroism of *Escherichia coli* dihydrofolate reductase: Insight into the contribution of tryptophan residues

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ABSTRACT

To elucidate the contribution of tryptophan side chains to the vacuum-ultraviolet (VUV) circular dichroism (CD) of *Escherichia coli* dihydrofolate reductase, we measured the VUVCD spectra of eight tryptophan mutants down to 175 nm. The difference spectra between the wild-type and the mutants clearly demonstrated that the contribution of tryptophan side chains extends to the high-energy peptide CD in the VUV region. These results should be useful for a theoretical study on improving protein secondary-structure analysis by VUVCD spectroscopy.

1. Introduction

Circular dichroism (CD) spectroscopy in the far-UV region has been widely used for secondary-structure analysis of proteins because it is sensitive to local peptide structure and can be applied to any protein [1,2]. However, aromatic side chains make a significant contribution to peptide CD in some proteins, although these contributions have not been explicitly considered in secondary-structure analysis [3]. Sreerama and Woody showed that the difference between experimental and theoretical CD spectra is decreased when incorporating the contribution of aromatic side chains into the theory [4]. However, such theoretical and experimental studies on the contribution of aromatic side chains have not been performed for CD spectra in the vacuum-ultraviolet (VUV) region, although VUVCD spectroscopy using synchrotron radiation can predict secondary structures more accurately than conventional CD spectroscopy and hence is becoming more important in protein structural biology [5–8]. Experimentally, the contribution of an aromatic residue can be inferred by examining the difference in CD spectra between wild-type and mutant proteins in which an aromatic amino acid residue is replaced with a non-aromatic or weaker aromatic one. Among the aromatic amino acids, tryptophan exerts the most significant contribution to peptide CD. We previously found that the far-UV CD spectrum of *Escherichia coli* dihydrofolate reductase (DHFR), which consists of 159 amino acid residues including 5 tryptophan, 4 tyrosine, and 6 phenylalanine residues, is largely affected by the mutation of tryptophan residues, Trp22, Trp30, Trp47, Trp74, and Trp133 (Figure 1), to leucine, phenylalanine, or valine [9]. In the present Letter, we measured the VUVCD spectra of wild-type and these mutant DHFRs without any ligands down to 175 nm to elucidate how the contribution of tryptophan side chains extends to high-energy transitions of peptide CD.

2. Materials and methods

The wild-type and mutant DHFRs were sufficiently purified as described previously [9] to a single band on SDS–PAGE gel (Figure S1). The VUVCD spectra of these proteins were measured from 260 to 175 nm using the VUVCD spectrophotometer constructed at the Hiroshima Synchrotron Radiation Center (HiSOR) and an assembled-type optical cell at 25 °C under high vacuum (10^{-4} Pa) [10,11]. The light-path length of the cell was adjusted to 10 μm using a Teflon spacer. The solvent used was 10 mM potassium phosphate (pH 7.0) containing 0.1 mM EDTA and 0.1 mM dithiothreitol. The protein concentration was 350 μM.

3. Results and discussion

3.1. VUVCD spectra of DHFRs

Figure 2 shows the VUVCD spectra of the wild-type and eight mutant DHFRs at pH 7.0 and 25 °C. The spectra of all DHFRs from 260 to 190 nm were consistent with our previous results measured with a conventional CD spectrophotometer at 15 °C [9]. Interestingly, a substitution of only a single tryptophan residue markedly

Abbreviations: CD, circular dichroism; DHFR, dihydrofolate reductase; VUV, vacuum ultraviolet.

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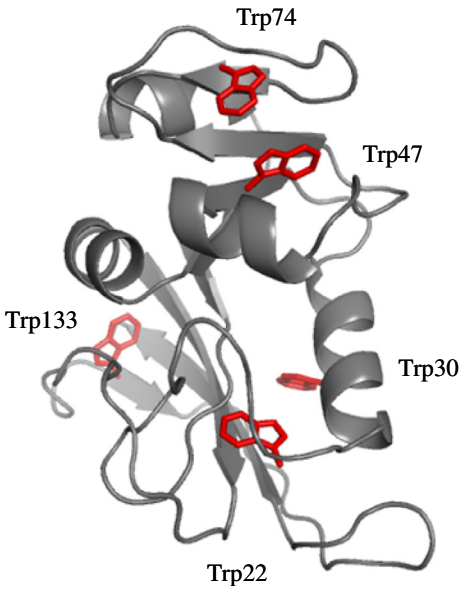

Figure 1. Crystal structure of wild-type DHFR (PDB code 1rx2). Folate and NADPH have been removed from the figure because apo-form DHFR was used in the VUVCD measurements. Five tryptophan side chains are indicated by the red stick model. The figure was created in PyMol [http://www.pymol.org/].

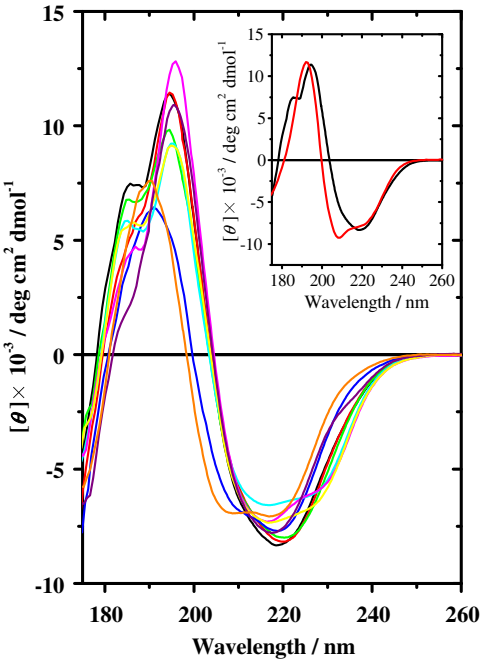

Figure 2. VUVCD spectra of the wild-type and tryptophan-mutant DHFRs at pH 7.0 and 25 °C. The solvent used was 10 mM potassium phosphate containing 0.1 mM EDTA and 0.1 mM dithiothreitol. The protein concentration was 350 μM. Wild-type (—), W22F (——), W22L (——), W30L (——), W47L (——), W74F (——), W74L (——), W133F (——), and W133V (——). (Inset) Experimental (—) and calculated (——) VUVCD spectra of wild-type DHFR.

affected the VUVCD spectrum of DHFR. Since all mutants retained substantial enzyme activity (>50% of the wild-type activity except for W22F, which had 13%) [9], significant unfolding of the secondary structures is unlikely, although some structural perturbation may have occurred in W30L and W133V mutants, as suggested by their relatively low stability for urea unfolding [9]. The large difference in the spectra below 200 nm clearly indicates that the tryptophan side chains contributed to the chiroptical property of DHFR down to the VUV region.

Before considering the difference spectra between wild-type and mutants, we compared the wild type spectrum with the calculated one based on the crystal structure of apo DHFR (PDB code, 5dfr) using the component spectra of the α-helix (21.4%), β-strand (30.8%), turn (6.9%), and unordered structure (40.9%), which were extracted by deconvolution analysis from the VUVCD spectra of 31 reference proteins down to 160 nm [12,13]. In this calculation, undetectable regions in 5dfr were assigned to unordered structures referring to the crystal structure of the ternary complex of DHFR (Figure 1). As shown in the inset of Figure 2, the experimental and calculated spectra present different features: the calculated spectrum generates a negative peak around 208 nm and no shoulder around 185 nm. Since the negative peak around 208 nm corresponds to the π–π^* transition of the backbone peptides along the helix axis [4,14,15], the CD of α-helices in DHFR may be susceptible to their environment.

The shoulder around 185 nm does not exist in any component spectra of above four secondary structures and cannot be explicitly explained, although such a shoulder was also found for cytochrome *c*, ribonuclease A, and thioredoxin [12,13]. A possible explanation for this shoulder is that the positive peak around 195 nm is split into two by the contribution of the aromatic side chains because the positive peak around 192 nm involves two components polarized perpendicular to the helix axis [4]. Among the π–π^* transitions of aromatic side chains, *L* bands are observed in near-UV region except for L_a bands of phenylalanine and tyrosine at about 210 and 230 nm, respectively, whereas *B* bands are observed at 180–200 nm except for a B_b band of tryptophan around 230 nm [4]. Actually, N-acetyl-L-tyrosine amide and N-acetyl-L-tryptophan amide have large negative CD peaks of less than -10^4 deg cm^2 dmol^{-1} at 185 and 195 nm, respectively, with a similar negative peak being expected for N-acetyl-L-phenylalanine amide at 180–190 nm [1]. Therefore, the π–π^* transitions (*B* bands) of aromatic side chains could couple with the π–π^* transition of backbone peptides of helices to split the CD peak around 192 nm although the relative conformations between backbone peptides and aromatic side chains in the protein are different from those in the model compounds [16,17]. Such coupling effects of π–π^* transitions between backbone peptides and aromatic side chains might be significant for DHFR as judged from a large difference in VUVCD spectra below 200 nm (Figure 2). However, the theoretical CD spectra calculated by Sreerama and Woody for eight proteins including cytochrome *c* did not show such a shoulder, regardless of excluding or including the contribution of aromatic side chains in the calculation [4]. On the other hand, theoretical calculations by Bulheller et al. revealed that the charge transfer between neighboring main chain peptides significantly contributes to CD spectra in the VUV region [18]. Therefore, it is unlikely that this shoulder originates from only the direct contribution of the aromatic side chains, although these aromatic contributions would be fairly large for DHFR since this shoulder was affected by the mutation of tryptophan residues.

3.2. VUVCD difference spectra between wild-type and mutant DHFRs

Figure 3 shows the difference in VUVCD spectra (wild-type VUVCD – mutant VUVCD) for the eight tryptophan mutants. In wild-type DHFR, two tryptophan side chains, W74 and W47, are in close proximity and give rise to strong coupling. Such exciton coupling is absent in the W74L and W47L mutants, so the difference spectra for W74L and W47L are close to each other and distinctly show a couplet with negative and positive peaks centered around 220 and 230 nm, respectively. This finding is consistent