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Synchrotron radiation circular dichroism: a new tool for identification of point-mutation protein

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Abstract

Many diseases are associated with the mutation of wild-type proteins. Usually, a point mutation can lead to severe clinical outcomes. Few techniques have the ability to detect the minute differences between the wild-type and mutant proteins in solution under near physiological conditions. Circular dichroism (CD) is an established and valuable technique for examining protein structure. Because of its ability to sensitively detect conformational changes, it has important potential for identification of mutant protein. Synchrotron radiation CD (SRCD) offers significant enhancements with respect to conventional CD spectroscopy, which will enable its usage for high-resolution conformation detection and as a tool in the point-mutation protein identification. In this report, SRCD was used, as an example, to identify the point-mutations of human phosphoribosyl pyrophosphate synthetase 1 which were associated with an X chromosome-linked disease.

Keywords: Synchrotron radiation circular dichroism; Secondary structure; Point mutation; Phosphoribosyl pyrophosphate synthetase 1

1. Introduction

Synchrotron radiation circular dichroism (SRCD) spectroscopy is an emerging technique which offers significant improvements to the well-established method of conventional circular dichroism (cCD) spectroscopy. It takes advantage of the higher light flux available from synchrotron sources, which results in lower wavelength data, higher information content and improved signal-to-noise ratios [1]. It is noteworthy that SRCD has enabled us to measure a functionally-significant difference between the wild-type and disease-associated mutant protein in

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solution that was not feasible using either *c*CD or X-ray crystallography. The latter is also because that no crystals have yet been obtained for the mutant although a crystal structure has been obtained for the native protein [2].

Human phosphoribosylpyrophosphate synthetase 1 (hPRS1), catalyzing the reaction of ribose-5-phosphate with ATP to yield 5-phosphoribosyl-a-1-pyrophosphate and AMP. Superactivity of mutant hPRS1 is an X-linked disorder of purine metabolism characterized by gout with uric acid overproduction [3] and neurodevelopmental impairment in some affected families [4]. Compared to wild-type hPRS1, a residue is replaced in the amino acid sequence of hPRS1 of the patients. Six point-mutants of hPRS1 have been found [5]. Point mutations provide the genetic basis for regulatory defects, indicating the functional significance of these amino-acid residues in hPRS1. Li et al. have reported the structure of hPRS1 [6]. To date, however, more details about how point mutations affect the regulatory mechanism of hPRS1 are not available. This situation is partly due to the difficulty in point-mutation protein crystallization. In this report, we have used SRCD spectroscopy to study the secondary structures of wild-type hPRS1 and its two point-mutants: D52H and N114S in solution. We have detected slightly but significantly alterations in secondary structures associated with single point mutations. Two patterns were suggested about how the point mutation affects the allosteric regulatory mechanism.

2. Materials and Methods

2.1 Sample preparation

Three proteins: wild-type hPRS1, D52H, and N114S were obtained. The protein expression and purification were as described in [7]. The accurate determination of protein concentration was to measure the absorbance of the protein at 280 nm when it was denatured in 6 M guanidinium hydrochloride. For native and mutant hPRS1, the same calculated extinction coefficients of 0.530 were obtained [8]. 12% cross-linked polyacrylamide gels were prepared according to the method of Davis. SDS-PAGE gels were analyzed by staining with 0.1% Coomassiebrilliant blue.

All proteins after purification were dialyzed against the buffer system with 20 mM sodium phosphate and 100 mM sodium sulphate (pH 8.0) to remove the strongly absorption of chlorine anions in the lower wavelength region and to provide sufficient ionic strength to keep the proteins stable. After dialysis, samples were centrifuged at 14,000 rpm for 10 min at 4℃. The protein was omitted from the solution in the control samples. All the prepared samples were degassed under vacuum condition before measurements to avoid optical artifacts such as differential light scattering and absorption flattening [9,10].

2.2 SRCD experiments

SRCD spectra were recorded on Beamline 4B8 at the BSRF (Beijing, China), and at Beamline U25 at NSRL (Hefei, China) respectively, using a scan speed of 120 nm/min, a time constant of 2 s and a bandwidth of 1 nm. Spectra were collected at a protein concentration of 0.7 mg/ml, at a temperature of 25°C controlled by a BTEC-77b temperature controller. Three scans of each spectrum over a wavelength range from 175 nm to 270 nm were averaged, smoothed, and baselines subtracted. In all measurements, the same sample cuvette with pathlengths of 0.01 cm (Hellma Ltd) was used. SRCD instrument was calibrated using camphor sulphonic acid. All spectra were only recorded down to wavelengths where the instrument dynode voltage indicated that the detection was still within the linear range. Spectra were converted to delta epsilon units using a mean residue weight value of 109.6. Data processing was performed using the CDTool software suite. [11]

2.3 Secondary structure analyses

The secondary structure analyses of SRCD spectra were performed on the DICHROWEB [12], using the self-consistent method CONTINLL and the SELCON3 method based on the 29 reference proteins dataset from Johnson et al.[13] The SRCD performance, δ, was characterized by the root mean square deviations (RMSD) between the DSSP assignment and the SRCD estimate. The quality of the fit of the calculated data to the experimental data was characterized by the normalized RMSD, whose values of <0.1 mean that they are in close agreement. [14]

3. Results and Discussion

The purified proteins showed a major band of 35 kDa on SDS–PAGE, which were in good agreement with the calculated value of 34.8 kDa (Fig. 1a). The SRCD spectrum of three proteins produced by two beamlines are same. As showed in Fig. 1b, the wild-type hPRS1 gives rise to a spectrum that is characteristic of a protein with α/β

tertiary structure [15], having peaks at ~222, 208, and 192 nm and a cross-over from positive to negative near 177nm. And the 222 nm band is of lager magnitude and more prominent than the 208 nm band. Compared to wild-type hPRS1, D52H has a significant increase in magnitude of the 208 nm peak and there is a significant increase of the β-strand and turn structures as showed in Table 2. N114S has an increase in magnitudes of the peaks 222, 192nm, and its peak of 208nm has the same magnitude with that of wild-type hPRS1. The calculated results show the slightly increase of α-helix content.

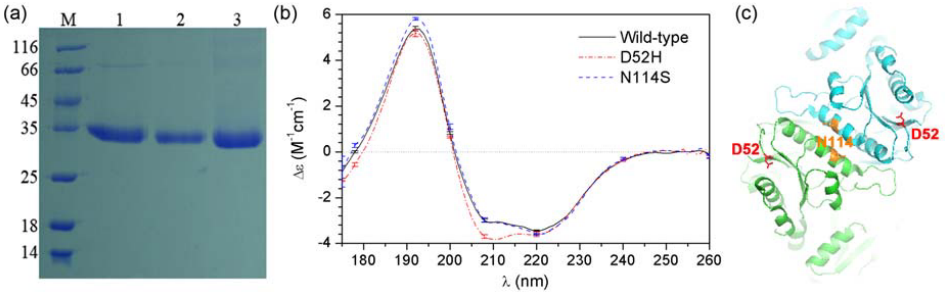

Fig. 1. (a) SDS–PAGE of hPRS1. Lane M, molecular-weight markers (kDa); Lane 1 to 2, purified fraction from Gel filtration (more than 95% purity) of wild-type hPRS1, N114S and D52H, respectively. (b) SRCD spectra of purified wild-type hPRS1, D52H, and N114S mutation. The error bars represent one standard deviation in the repeated measurements from three sequential scans of both spectra and baselines. (c) Crystal structure of wild-type hPRS1 two subunits highlighted in green and cyan, respectively. The D52 and N114 residues are in red and orange, respectively.

As showed in Table 1, the secondary structures generated from SRCD spectra using the CONTINLL analyses method are accordant with the secondary structures in crystal assigned by DSSP program. This increases our confidence level in the accuracy of SRCD technique. The data of CONTINLL analyse, which are similar with the SELCON3 analyse (data not shown), show that both the δ and NRMSD factors are small. As showed in Table 2, the point mutation causes slightly but significantly alterations in secondary structures. These alterations should be the structural basis of the regulatory defects of hPRS1 activity. It is possible to influence the activity of the enzyme from a mutational site distinct from where the reaction takes place on the enzyme. Therefore, the severe clinical diseases appear.

Table 1. Secondary-structure contents of wild-type hPRS1 determined by DSSP assignment and SRCD analysis.

Protein		α_R	α_D	β_R	β_D	Turn	Unordered	Total	NRMSD [a]	δ^b
Wild-type	DSSP	20.7	16.2	15.5	8.5	13.7	25.4	100	—	—
	SRCD	18.1	13.9	8.9	7.3	22.1	29.7	100.0	0.020	0.049

Table 2. Secondary-structure contents of wild-type hPRS1, D52H and N114S mutations determined by SRCD spectra in the wavelength region from 260nm to 175nm. The number of helix and strand segments is given per protein molecule corresponding to one subunit in the asymmetric unit of the X-ray structure.

Protein	Secondary Structure (%)				Number of helix and strand segments		NRMSD
	α-Helix	β-Strand	Turn	Unordered	α-Helix	β-Strand	
Wild-type	32.0	16.2	22.1	29.7	11	12	0.020
D52H	32.2	19.5	23.4	24.9	11	13	0.024
N114S	32.9	16.0	21.1	30.0	11	11	0.029

The X-Ray results show that D52 residue is located at the interface between three subunits of the hexamer where the ADP allosteric site is. D52H mutation might cause alteration of the space structure of the ADP binding site, by what the regulatory defect is caused. The N114S mutation induces the increase of the length of some helix. This residue is located at the α3 helix that is next to the flexible loop. If we considered that the length of α3 helix increased, the conformation of the flexible loop should be certainly changed. These results also agree well with the point that the allosteric regulation mainly occurs at the interfaces between subunits of the hexamer [6,16].

In conclusion, we have observed slightly but significantly conformational alterations in secondary structure of hPRS1 caused by D52H and N114S point-mutations. The SRCD spectra show enough accuracy to distinguish the point-mutation-induced alteration of the protein conformation. SRCD offers significant enhancements with respect to *c*CD spectroscopy, which will enable its usage for high-resolution conformation detect and as a tool in the point-mutation protein identification.

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