CONFORMATION OF NATIVE *PSEUDOMONAS TESTOSTERONI* $\Delta_{5\rightarrow4}$ 3-OXOSTEROID ISOMERASE

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1. Introduction

The $\Delta_{5\rightarrow4}$ 3-oxosteroid isomerase (isomerase) was discovered by Wang and Talalay [1]. Inducible by testosterone and progesterone, this enzyme promotes the isomerization of $\Delta^{5(6)}$ and $\Delta^{5(10)}$ -3-oxosteroids to the corresponding $\Delta^{4(5)}$ -3-oxosteroids. Its molecular weight is 40,800 ± 5%. All evidence points to the existence of three identical subunits of about 13,600 molecular weight. The sequence of the isomerase subunit was recently determined [2].

However, nothing is known about its secondary and tertiary structure. The present results show that isomerase studied by the circular dichroism technique is well ordered, and has a very high percentage of β-structure (pleated sheets).

2. Experimental

Isomerase was purified and crystallized by the usual procedure [1]. The pure protein yielded a single component in polyacrylamide gel electrophoresis, and had a constant specific activity of about 64,000 units/mg protein [1]. The concentration of isomerase in 0.03 M potassium phosphate buffer, pH 7.0, was determined by absorbance measurements at 280 nm, using an extinction coefficient: $E^{1\%}_{1\,cm} = 4.13$. Absorption spectra were recorded on a Cary 15 spectrophotometer.

Circular dichroism spectra were performed with a "Cary 61" dichrograph. Cylindrical quartz cells of 0.5 mm path length were used in the 184–250 nm spectral region, and of 20 mm in the 250–310 nm range. The sample temperature was controlled by a circulating bath (Haake). The ellipticity $(\Theta)_m$ of isomerase was expressed as deg·cm^2/dmole and was normalized to the mean residue weight of the protein i.e.: 109; it was calculated on the basis of 40,800 molecular weight and 375 amino acid residues. $(\Theta)_m$ values were not corrected for the refractive index of the medium.

3. Results

3.1. *Circular dichroism and ultra-violet spectra*

The circular dichroism spectrum at 184–250 nm of a 8.95 μM native isomerase solution in 0.03 M potassium phosphate buffer, pH 7, was recorded between 184 and 250 nm at several temperatures (4–27°) (fig. 1). It is characterized by 2 dichroic bands: a positive one at 193–194 nm of a high magnitude ($(\Theta)_m$ = + 21,000 deg·cm^2/dmole) and a negative broad band centered at 222 nm with a weaker ellipticity ($(\Theta)_m$ = – 9,000 deg·cm^2/dmole). A very small shoulder appears near 210 nm. The general shape of the dichroic curve suggests a considerable amount of β structure.

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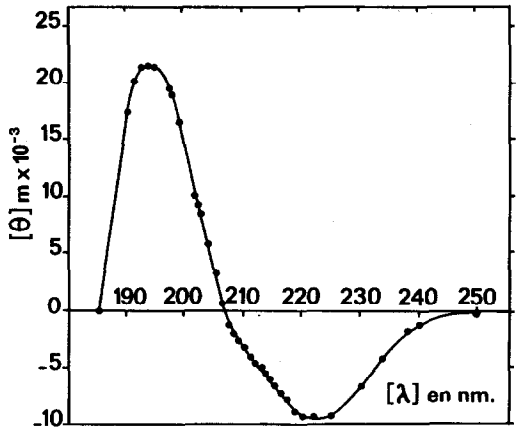

Fig. 1. Circular dichroism spectrum of isomerase in the far ultra-violet region. Experiments were performed with isomerase (8.95 μM) in 0.03 M phosphate buffer, pH 7.0. The temperature was 25° and cell path length 0.5 mm. The $(\Theta)_m$ values are in degrees · cm^2/dmole and are normalized to the mean residue weight of isomerase, i.e.: 109.

In table 1 are listed $(\Theta)_m$ values at different wavelengths for isomerase and for a model polypeptide (poly-L-lysine in different known conformations).

In the near ultra-violet 250–310 nm, the optical activity is due to electronic transitions of the aromatic amino acids present in isomerase i.e.: tyrosine and phenylalanine. Fig. 2 shows the absorption spectrum of a concentrated solution of isomerase (33 μM) on curve a. The dichroic spectrum is shown on curve b. Several dichroic bands are observed at 257 and 264 nm, which are attributed to the phenylalanine transitions; those observed from 270 to 284 nm are due to the tyrosine content. In this wavelength range, the highest $(\Theta)_m$ value is 73.5 deg · cm^2/dmole at 277.5 nm.

In table 2 are listed dichroic band positions (250–320 nm) and their corresponding $(\Theta)_m$ values, as well as absorbance peaks of isomerase and of a model mixture of 9 tyrosines and 24 phenylalanines, corresponding to the aromatic amino acid content of the enzyme, at the same molar concentration

3.2. *Determination of the α-helix and of the β-structure content of isomerase*

The calculation of the percentage in the α-helix content at 208 nm was attempted according to the Fasman's [3] equation based on poly-L-lysine $(\Theta)_m$ values in different conformations. This method gave a negative value for the α-helix content, due to a very weak $(\Theta)_m$ value of about – 2,000 deg · cm^2/dmole.

We have tried therefore to adjust our experimental dichroic curve with the theoretical curves calculated from a linear combination of α-helix, random coil and β-structure in the 215–235 nm range. For these calculations, we have used $(\Theta)_m$ values reported by Fasman [3], corresponding to 100% α-helix and 100% random coil. The $(\Theta)_m$ values for 100% β-structure were taken from Li and Spector data [4]. The latter are in agreement with those obtained with isomerase; whereas those from Fasman are much higher than the values reported here. In fact Li and Spector have shown that the Fasman's values were overestimated. The best fit between 215 and 235 nm corresponds to 75 ± 10% of β-structure, 10 ± 5% of α-helix and the remainder being random coil.

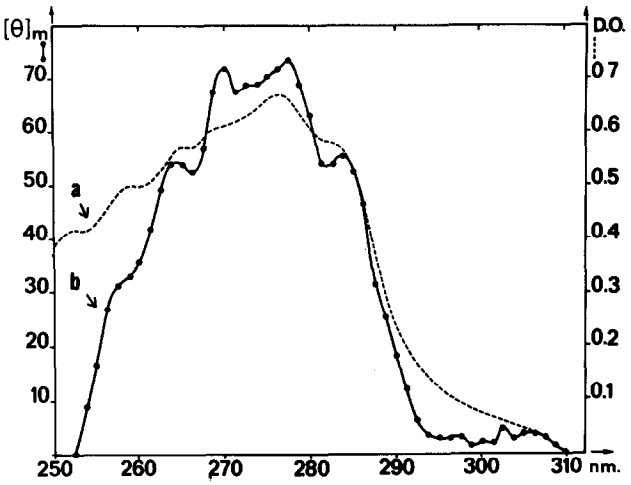

Fig. 2., Circular dichroism and absorption spectra of isomerase in the near ultra-violet region. a) Absorption spectrum of isomerase (33 μM). b) Circular dichroism spectrum of isomerase (8.95 μM). Experiments were performed in 0.03 M phosphate buffer, pH 7.0. The temperature was 25° and cell path length 20 mm. The $(\Theta)_m$ values are expressed in degrees · cm^2/dmole and normalized to the mean residue weight of isomerase, i.e.: 109.

Table 1
Comparison of the poly-L-lysine and isomerase dichroic parameters.

Peak position (nm)	$(\Theta)_m$ (degree · cm²/ dmole *)	Intersection with baseline
1) β structure (poly-L-lysine) [3,4]		
195	27,900±4,400[3]	207
217	−19,000±3,000[3] − 9,000±1,300[4]	
2) α helix structure (poly-L-lysine) [3]		
190.5	+71,500±6,300	200
207	−38,900±3,900	
221	−39,800±3,900	
222 (40)	−32,000	
3) Isomerase		
194	+ 21,600±1,650	206.5
208	−2,160	
220–225	−9,250	

* The $(\Theta)_m$ values are normalized to the mean residue weight of isomerase, i.e.: 109.

Table 2
Comparison of the dichroic parameters of isomerase with the absorption peaks of isomerase and its model mixture *.

Absorption spectra		D.C. spectra	
Mixture of phenylalanine and tyrosine	Native isomerase		
λ max (nm)	λ max (nm)	λ max (nm)	$(\Theta)_m$**
252.0	253.0		
258.0	259.0	258.0	31.5
264.0	265.5	264.0	54.5
268.0	269.0	269.0	72.0
275.5	277.0	277.5	73.5
281.5	283.0	284.0	55.5

* The model mixture was made up of 9 tyrosines and 24 phenylalanines at the same molar concentration as isomerase.

** The $(\Theta)_m$ values are expressed in deg · cm²/dmoles and normalized to the mean residue weight of isomerase, i.e.: 109.

4. Discussion

From the analysis of the dichroic characteristics in the 184–250 nm region, isomerase seems to contain a very high amount of β-structure. The positive circular dichroic band at 194 nm with a mean residue ellipticity of + 21,000 deg · cm²/dmole is a typical feature of this structure [3]. The $(\Theta)_m$ value at 208 nm is very weak, about 20% as intense as the 222–225 band. In α-helical polypeptides, the magnitude of both of these bands are approximately the same [3]. This constitutes further evidence for the presence of a very high content of β-structure.

However, some discrepancy exists between the dichroic characteristics of isomerase and those of the poly-L-lysine model compound studied in the β conformation. Particularly, isomerase presents a small red shift of its negative dichroic band. This could be due to the arbitrary choice of the poly-L-lysine as polypeptide model compound; for example poly-L-serine [5] has a negative dichroic band centering at 220–223 nm with a $(\Theta)_m$ value of $\simeq -9{,}000$ deg·cm²/dmole, very similar to isomerase. However all these models do not take into account the different micro-environments of the chromophore residues occurring in proteins. Therefore the reliability of using model polypeptides for calculation of percentage of various conformations in proteins is questionable.

In the near ultra-violet region, the dichroic bands occur approximately at the same wavelengths as the absorbance peaks. We have shown [7] by solvent perturbation difference spectra with 20% ethylene glycol, that isomerase has 4 tyrosines and 10 phenylalanines accessible to the solvent. The present dichroic data do not allow a decisive distinction between the contribution of exposed and buried chromophores to the circular dichroism spectrum.

References

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