TRYPTOPHANYL CIRCULAR-DICHROIC BAND IN SOYBEAN LEGHEMOGLOBIN *a*

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Received 23 October 1975

1. Introduction

The covalent structure of soybean *(Glycine max)* leghemoglobin has recently been elucidated [1]. The circular-dichroic (CD) spectra of soybean leghemoglobin and its liganded derivatives have been reported on [2]. The ultraviolet region of the dichroic spectrum showed a negative extremum at 296 nm, and positive extrema at 290 and 283 nm. The positive dichroic bands have been assigned to the ^1L_b transition of tryptophan from the close resemblance to the spectrum of *N*-acetyl-L-tryptophanamide [13]. The negative extremum has been assigned to the ^1L_a transition of tryptophan. Two tryptophans are found in leghemoglobin *a*, i.e. at positions 120 and 128 [1].

In this communication the reactivity of the tryptophan residues to *N*-bromsuccinimide (NBS) has been studied in order to elucidate which of these two residues gives rise to the dichroic spectra observed, and in addition how exposed to the environment the two residues are in the leghemoglobin molecule.

2. Materials and methods

Leghemoglobin *a* from soybean root nodules was prepared as previously described [4]. The protein concentration of the solution was calculated using millimolar absorption coefficients at 240, 260 and 280 nm [2]. Tryptophan was reacted with NBS (Serva, Heidelberg, GFR) at room temperature in 0.1 M sodium acetate buffer, pH 4, for 2 min [5]. After reaction the pH was immediately raised to 6 by adding 0.5 M sodium hydroxide dropwise, because leghemoglobin *a* denatures at a lower pH [6]. CD spectra were recorded with a Cary model 61 spectropolarimeter calibrated with d-camphosulphonic acid. The protein was digested with trypsin (Serva, Heidelberg, GFR) as described previously [7], with the exception that the heme group was not removed before digestion. High voltage electrophoresis at pH 6.5 was carried out and peptides were detected on the electropherogram as described earlier [7].

3. Results and discussion

The near-ultraviolet dichroic spectra of leghemoglobin *a* and leghemoglobin *a* reacted with one and two equivalents respectively of NBS per mole protein are shown in fig.1. The addition of one equivalent of NBS per mole leghemoglobin *a* causes only a small change in the negative band at 296 nm but almost totally cancels the positive dichroic band at 290 nm. NBS also causes a change at the broad negative CD band at 358 nm (fig.1). This band has been shown to be a heme chromophore band in a cytochrome *c* undecapeptide lacking aromatic side chains [8], and it represents the N-band, which is considered to arise from porphyrin $\pi-\pi^*$ transitions [9].

The amino acid sequence of soybean leghemoglobin *a* around Trp-120 and Trp-128 is shown in fig.2. To find out which of the tryptophan residues gives the CD maximum at 290 nm, the NBS-modified protein was digested with trypsin. The peptides *a*T16 and *a*T17 were isolated and identified of high-voltage paper electrophoresis. With one equivalent of NBS per mole protein the Ehrlich test gives only a faint colour reaction with peptide *a* T17 (Trp-128). After adding a further equivalent of NBS to the protein, tryptophan in *a* T17 is totally oxidised whereas tryptophan in *a* T16 is still detectable by the Ehrlich

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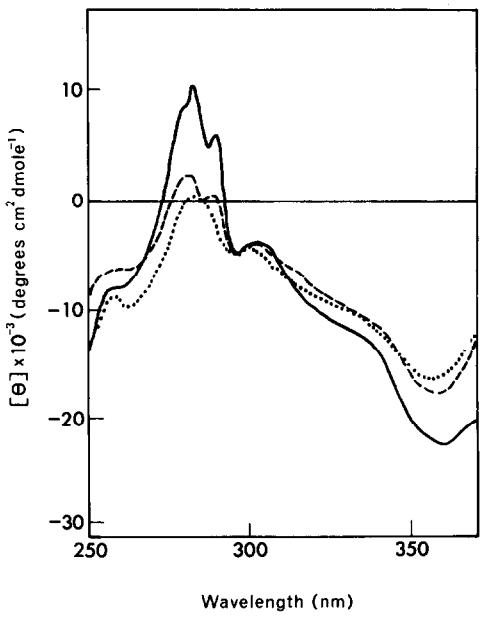

Fig.1. The near-ultraviolet circular-dichroic spectra of leghemoglobin *a* (———) and leghemoglobin *a* with one (– – – –) and two (. . . . .) equivalents of NBS per mole protein in 0.1 M sodium acetate buffer, pH 6.

test. This would indicate that Trp-128, which first reacts with NBS, is the origin of the positive CD maximum at 290 nm.

These results indicate that Trp-128 is more exposed to the external environment than Trp-120, which is buried in the molecule but not totally inaccessible to oxidation by NBS. Trp-128 is considered to be located at an equivalent position in the leghemoglobin chain as Tyr-130 in the human β-chain [10], which contributes to the heme chromophore bands [11]. In the reaction of Trp-128 of the leghemoglobin chain with NBS, the modification of tryptophan or a concomitant structural change in the tertiary structure of the protein molecule also alters the

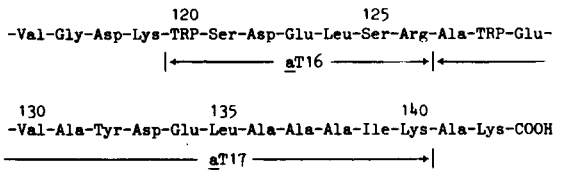

Fig.2. The amino acid sequence of the C-terminal portion of the leghemoglobin *a* chain including the tryptic peptides *a*T16 and *a*T17.

heme chromophore N-band at 358 nm, suggesting that the spatial relationship of this tryptophan to the heme group resembles that of Tyr-130 in the human β-chain.

Acknowledgement

This study was in part supported by Magnus Ehrnroots stiftelse.

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