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Circular dichroism of isolated ricin A- and B-chains

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An analysis of the circular dichroism (CD) spectra of isolated ricin A- and B-chains revealed several bands not apparent in the spectrum of intact ricin. Arithmetic combination of the A- and B-chain spectra gave a composite spectrum resembling that of native ricin, indicating that the two chains did not undergo any major conformational change upon dissociation. The addition of lactose to the B-chain at pH 7.2 caused a slight perturbation of a tryptophan-derived negative CD band centred at 283 nm without change to the overall structure of the polypeptide.

1. Introduction

The plant toxin ricin, from the castor bean *Ricinus communis,* consists of two polypeptide subunits, the A- and B-chains, which have different functions. The A-chain catalytically inactivates eukaryotic ribosomes. The B-chain is a lectin that binds to galactose-terminating oligosaccharides present on the surface of many cell types. The two subunits are linked covalently by a single disulphide bond. Toxin attached to the cell surface via the B-chain is internalised by endocytosis and the A-chain is then translocated, probably across the membrane of the endocytic vesicle, into the cytosol where it inhibits protein synthesis [1,2].

Ricin has two independent saccharide-binding sites that bind lactose with different affinities [3] and are located on separate domains of the B-chain polypeptide [4]. Chemical modification studies have provided evidence for the presence of Tyr-248 [5,6] and Trp-37 [7] in or near these lactose-binding sites. These assignments have been subsequently confirmed by three-dimensional structure analysis using X-ray crystallography [8].

We previously reported that lactose protected one of the two oligomannose chains of ricin B-chain against enzymic removal with peptide: *N*-glycosidase F, suggesting that the binding of lactose may have induced a conformational change in the B-chain [9]. This finding prompted us to undertake a CD analysis of the effect of lactose binding on B-chain structure. Lactose slightly perturbed a negative CD band attributable to tryptophan but had no detectable effect on the overall structure of the polypeptide. These results are discussed in relation to previous CD studies on intact ricin [10,11].

2. Materials and methods

Ricin was purified from African castor beans (Croda Premier Oils, Hull, U.K.). The B-chain was separated from A-chain in the presence of 5% (v/v) 2-mercaptoethanol by the procedure of Fulton et al. [12] and dialysed to remove the reducing

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agent without further purification. The A-chain was purified as described previously [12]. The concentration of protein solutions was calculated assuming values of $E^{1\%}_{280\ nm}$(1 cm) of 7.65 for A-chain and 14.9 for B-chain [13].

CD spectra were measured at ambient temperature with a Jasco J40CS instrument and the spectra were processed on a BBC Acorn computer attached to the CD spectrometer. Differential absorption ($\Delta\epsilon$) values were calculated on the basis of the mean residue molecular weight deduced from the known amino acid sequences of the A- and B-chains [14] (110 for A-chain and 109 for B-chain).

3. Results

The near-ultraviolet CD spectrum of ricin B-chain at pH 7.2 (fig. 1) is dominated by a band of negative differential absorption with a maximum at 283 nm and shoulders at approx. 275 and 291 nm. This band resembles the ultraviolet spectrum of the B-chain and its structure suggests that it arises from tryptophan residues although the possibility of contributions from other aromatic amino acid residues and disulphide bonds in this region cannot be ruled out. A small negative band at 300–325 nm is attributable to disulphide bond transitions. Below 270 nm, there is a large positive band peaking at 232 nm. This band is probably derived from transitions of one or more disulphide bonds present in a fixed asymmetric environment [15,16]. In the far-ultraviolet region, there is a single negative band at 205–215 nm which resembles the far-ultraviolet CD spectra of the Elapid snake toxins (unrelated to ricin) which possess a backbone conformation comprising a mixture of β-sheet, polypeptide loops and reverse β-turns [17]. This structural assignment is consistent with the recently determined polypeptide fold of the B-chain in intact ricin [8].

In the presence of lactose, the negative band in the region 270–300 nm is increased slightly. However, the CD spectra of the B-chain in the presence and absence of lactose are superimposible between 300–340 and 200–270 nm, demonstrating that these features and the band at 270–300 nm probably arise from unrelated chromophores. The invariance of the CD spectrum below 230 nm is certainly consistent with the view that lactose causes only a local perturbation of a tryptophan residue or residues (see below) present in or near the lactose-binding sites, leaving the overall protein structure unchanged.

The CD spectra of ricin B-chain at pH 7.2 and 10.2 (fig. 2) show significant differences. The band at 270–300 nm is reduced but there is no evidence

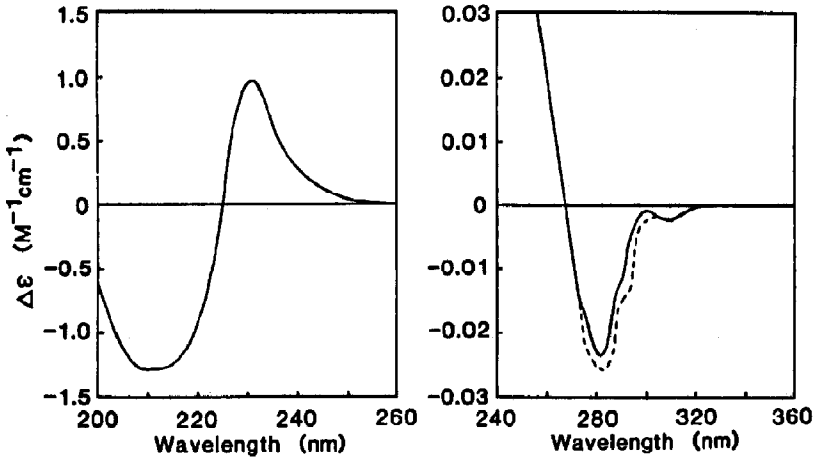

Fig. 1. CD spectrum of ricin B-chain at pH 7.2. B-chain (1.01 mg/ml) in 10 mM sodium phosphate (pH 7.2) in either the absence (———) or presence of a saturating concentration of lactose (50 mM) (— — —).

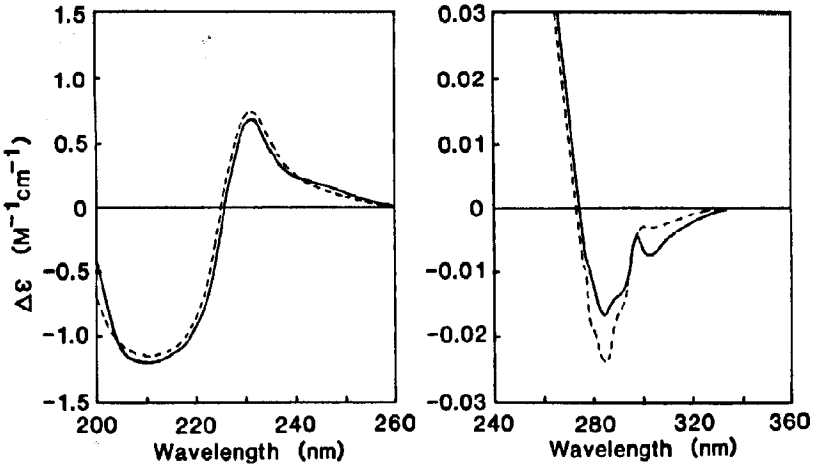

Fig. 2. CD spectrum of ricin B-chain at pH 10.2. B-chain (1.01 mg/ml) in 10 mM sodium phosphate titrated to pH 10.2 with NaOH in either the absence (———) or presence of 50 mM lactose (— — —).

of a red-shift which might have been expected upon ionization of tyrosine residues. We conclude that the major contribution to this band is from tryptophan residues. Concomitant differences are observed in the bands attributable to disulphide bonds, namely, an increase in the band at 300–325 nm, a decrease in the 232 nm band and an increased positive band at 240–275 nm which accounts, at least in part, for the decrease in size of the negative band at 270–300 nm. The backbone configuration of ricin B-chain at pH 10.2 is unaltered, since there is no significant change in the negative band at 200–225 nm. These results suggest that the ionization of tyrosine residues alters the conformation of ricin B-chain only slightly if at all. Indeed, ricin B-chain at pH 10.2 retains its affinity for the galactose-based adsorbent Sepharose 4B and there is clear evidence

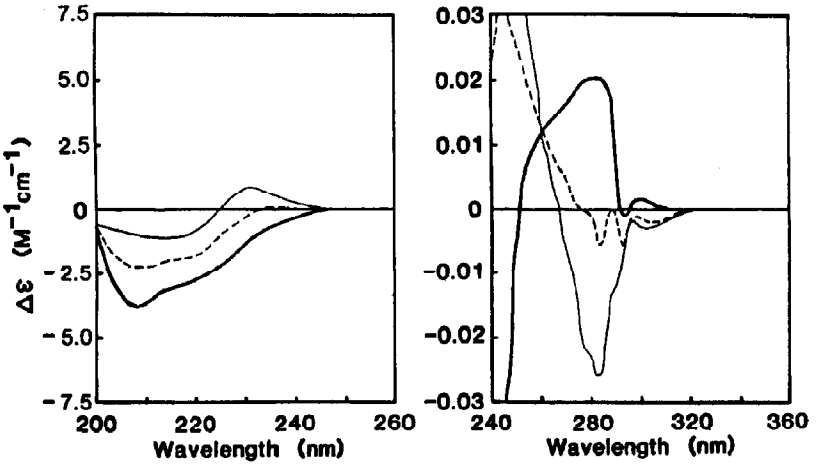

Fig. 3. Comparison of CD spectra of ricin A-chain and B-chain. A-chain (1.03 mg/ml) (———) and B-chain (1.03 mg/ml) (———) in 50 mM sodium phosphate, pH 7.2. Calculated composite spectrum (— — —).

of B-chain-lactose interaction from CD at pH 10.2 (fig. 2) as at pH 7.2.

Fig. 3 compares the CD spectra of the isolated A- and B-chains. The A-chain displays a negative band that peaks at 205 nm and resembles the far-ultraviolet spectrum of intact ricin [10,11]. The mean residue differential absorption of this A-chain CD band is 3–4-fold greater than that of the far-ultraviolet negative CD band of the B-chain, showing that for the intact toxin, the major contribution in this region is from the A-chain. In the near-ultraviolet CD spectrum of the A-chain, the main feature is a positive band at 250–290 cm. A CD spectrum for ricin was calculated by adding together the CD spectra measured for the A- and B-chains separately assuming an equivalent weight ratio of the two chains. In the near-ultraviolet, the composite CD spectrum (fig. 3) shows two sharp bands centred at 285 and 293 nm and in this and other respects, namely, a negative band at 300–320 nm and a positive band peaking at 245 nm, closely resembles the CD spectrum reported for intact ricin [10,11]. The same spectrum was observed when the isolated A- and B-chains were reassociated by mixing in stoichiometric amounts, indicating that the non-covalent interaction between the two chains in the holotoxin does not involve significant changes in the environments of the chromophores giving rise to the CD bands observed for the isolated subunits.

4. Discussion

The CD spectrum of B-chain shows some interesting features compared with that of ricin. Firstly, in the near-ultraviolet, the B-chain CD spectrum gave a single negative band centred at 283 nm with a small shoulder at 291 nm. Ricin A-chain gave no negative bands in this region of the spectrum but a composite spectrum calculated for a mixture of A- and B-chains produced two negative bands at 285 and 293 nm (fig. 3). This strongly suggests that the two negative bands reported for ricin at 285 and 294 nm [10,11] do not represent individual chromophores but the combination of bands from several chromophores in both A- and B-chains. A second feature of the B-chain spectrum is the large positive band at 232 nm arising from disulphide bond transitions. This CD band is virtually absent from the ricin CD spectrum because it is almost completely cancelled out by a negative band of similar magnitude due to the A-chain (see fig. 3). The resemblance of the CD spectrum calculated for ricin by simple arithmetic combination of the isolated A- and B-chain spectra and the spectrum determined after mixing of the isolated subunits to the measured CD spectrum of ricin [10,11] indicates that the two chains do not undergo any major conformational changes upon dissociation. This is consistent with the analysis of the three-dimensional structure of ricin deduced from X-ray crystallographic data [8] showing that relatively few amino acid residues of the A- and B-chain interact and suggests that the B-chain may inhibit the catalytic ribosome-inactivating activity of the A-chain by directly binding close to a site on the A-chain that is essential for catalysis or binding to the ribosome.

The 285 nm band of ricin has been tentatively assigned to a tyrosine chromophore on the basis of two kinds of evidence. Firstly, the 285 nm band was diminished by increasing the pH of the ricin solution to induce ionization of tyrosine residues [11]. When the pH of the B-chain solution was raised from pH 7.2 to 10.2, concerted changes were observed in several bands attributable to disulphide bond transitions, notably an increase in the magnitude of the positive band at 240–275 nm. This increase was associated with a decrease in the negative band centred at 283 nm. Therefore, the effect of elevated pH on the 285 nm band of ricin could have been due to an increase in the positive 240–275 nm band of the B-chain subunit. Moreover, as discussed above, the 285 nm band in ricin may also be sensitive to changes in bands arising from the A-chain. Secondly, the 285 nm band of ricin was diminished by partial acetylation of tyrosine residues in the intact toxin [10]. However, changes were also clearly evident in other regions of the CD spectrum, suggesting that tyrosine modification per se may not have been responsible for this effect. We were unable to analyse the CD spectrum of acetylated B-chain because B-chain extensively modified with *N*-acetylimidazole in the absence of lactose tended

to precipitate and non-tyrosine residues were also modified [6].

In experiments with ricin, the presence of lactose led to only a slight perturbation of the negative bands in the near-ultraviolet CD spectrum [10]. In the case of isolated B-chain, we have likewise observed that lactose slightly increased the size of the negative band centred at 283 nm. This indicates that the binding of lactose to the isolated B-chain caused no detectable change to the overall structure of the lectin in common with the intact toxin. It is unlikely, therefore, that the protection of one of the oligomannose chains of the B-chain against removal by protein:*N*-glycosidase F [9] was due to a large conformational change induced by lactose. In principle, lactose could bring about more subtle conformational adjustments of the B-chain that might not be detectable by CD analysis. However, we have recently obtained evidence [18] that lactose serves to protect the B-chain against denaturation which occurs readily in the absence of lactose under the conditions used for enzymic deglycosylation and which exposes both oligomannose chains to enzymic attack.

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