BINDING OF GANGLIOSIDE BY THE CHAINS OF TETANUS TOXIN

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1. Introduction

Tetanus toxin can be split by thiol reducing agents into two polypeptide chains: the heavy or β chain (molecular weight about 105 000) and the light or α chain (about 55 000). There is evidence that these two chains are the product of proteolytic cleavage of a single chain which is sometimes found intact [1–3].

The mode of action of the toxin is unknown in any detail; but it is known [4] that it binds strongly to certain sialic-acid-containing gangliosides, e.g. SGGnSSLC and GGnSSLC (for nomenclature of gangliosides, see [5]), and this binding is probably involved with its action. Ganglioside insolubilized by forming a complex with insoluble cerebrosides retains its ability to bind toxin [4].

This paper describes evidence that the ganglioside-binding site lies exclusively in the heavy chain: isolated heavy chain binds to ganglioside, but isolated light chain does not. This observation may be relevant to the mechanism of the toxin.

2. Experimental

2.1. *Preparation of the toxin*

Toxin (kindly given by Dr R. O. Thomson of the Wellcome Research Laboratories, batch numbers TD745D and TD754B) was purified by a modification of the method of Dawson and Mauritzen [6]. Crude material (containing about 5 mg toxin) was dialysed exhaustively against 0.01M sodium phosphate buffer, 0.1 mM triethylenetetramine, pH 7.2; diluted to 100 ml with this buffer, and applied to a column (2 cm diameter by 4.5 cm) of DEAE-cellulose (Whatman DE-52) that had been equilibrated with the buffer. Toxin was eluted from the column with a linear gradient of sodium phosphate concentration up to 0.04 M, pH 7.2. It could be further purified to homogeneity by gel-filtration on a column (2.6 × 85 cm) of Ultrogel AcA 34 (LKB) in 0.1 M Tris–HC1, 0.2M NaC1, mM EDTA, pH 7.5; but was usually used without further purification. There was no evidence (judging by dodecylsulphate gel electrophoresis) of any of the single-chain form of the toxin in this preparation.

2.2. *Separation of the chains*

The chains were separated and purified essentially as described by Matsuda and Yoneda [7], except that petreatment with 100 mM dithiothreitol in only 4 M urea as they suggest was not enough to split the chains completely: at least 6 M and preferably 8 M was required. However purification of the chains by gel-filtration on Ultrogel AcA 44 in 2 M urea [7] gave much better separation than gel-filtration in 8 M urea on Biogel A-5m [1] or on Sepharose 6B (S. van Heyningen, unpublished). After gel-filtration the chains were concentrated by ultrafiltration using Amicon PM 30 membranes. Care was taken to keep all concentrated solutions in the presence of phenylmethyl sulphonyl fluoride (about 10 mg/l, a protease inhibitor) since otherwise there was often some evidence of proteolysis, especially at higher urea concentrations.

2.3. *Gangliosides*

Insolubilized ganglioside was prepared by dissolving SGGnSSLC (1 mg) and brain cerebroside (9 mg) (prepared by Mr N. Gascoyne) in 1 ml chloroform and 1 ml methanol, and then evaporating a suitable volume of the solution to dryness in vacuo.

3. Results

3.1. *The chains*

Light chain could be separated from urea and salts by dialysis against neutral phosphate buffer, but heavy chain precipitated under these conditions. Except when very dilute (i.e. less than about 50 μg/ml) it would remain in solution only in the presence of at least M urea.

Surprisingly, and in distinction to other reports [7], heavy chain did not react with equine tetanus antitoxin on immunodiffusion even in the presence of M urea. Light chain did react, showing partial identity with whole toxin.

3.2. *Binding to ganglioside*

When a solution of whole toxin (1 mg/ml in 0.05 M Tris–HCl, pH 7.0) was incubated with 0.5 mg SGGnSSLC insolubilized with 4.5 mg cerebroside, it was adsorbed onto the insoluble complex (as judged by gel electrophoresis of the supernatant). It could be eluted from the complex by incubation with 8M urea or solutions of higher ionic strength (e.g. 2 M NaCl). These results are compatible with those of Habermann [8], who showed that tetanus toxin bound to synaptosomes on a Kieselgur column (presumably through ganglioside in the membrane) could be released from them by increasing the ionic strength.

When this experiment was repeated using purified heavy chain (in M urea), the result was very similar. Figure 1a is a trace of a dodecylsulphate gel of about 200 μl of a solution (E_{280} = 0.12) of heavy chain in 0.05 M sodium phosphate, M urea, mM EDTA pH 7.0. Figure 2b is the supernatant after incubating a similar solution for 2 h at 37°C with 0.05 mg SGGnSSLC and 0.45 mg cerebroside, and then centrifuging. Almost all the protein has been adsorbed. The pellet (an insoluble cerebroside-ganglioside-heavy-chain complex) was washed twice with the M urea buffer, suspended in 100 μl 8M urea, 0.05 M sodium phosphate, mM EDTA pH 7.0, incubated for 2 hr at 37°C, and then centrifuged again. Figure 1c is a gel of this supernatant, showing that the heavy chain has been eluted. Controls using cerebroside alone without SGGnSSLC did not adsorb the toxin. Experiments conducted without M urea gave similar results, but these are difficult to interpret unambiguously since the heavy chain may precipitate.

When these experiments were repeated using purified light chain, there was no evidence for any adsorption of the ganglioside under any conditions.

4. Discussion

These experiments show that the adsorption of toxin to insolubilized ganglioside can be reversed: a possible method of purification from crude material. Since isolated heavy chain is also adsorbed but isolated light chain is not, it appears that the ganglioside-binding site must lie almost exclusively in the heavy chain.

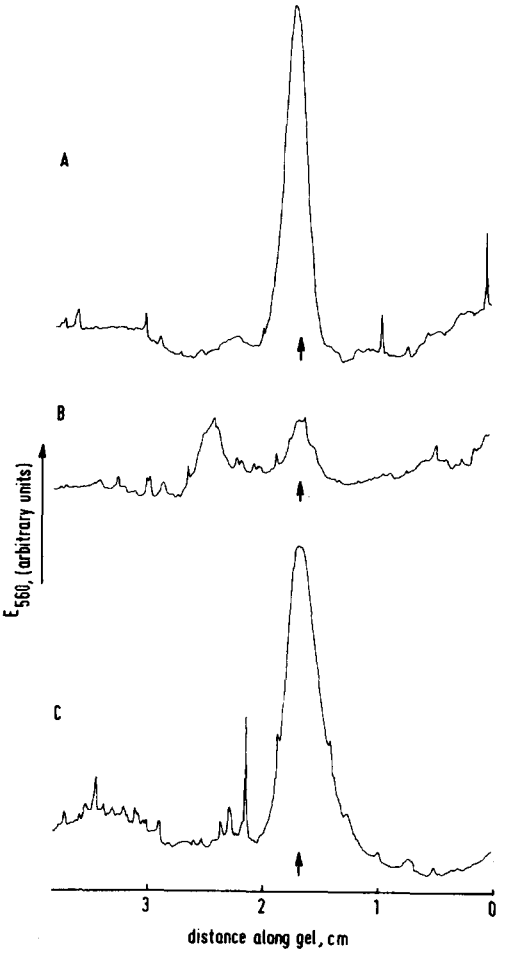

Fig.1. Spectrophotometric traces of 7.5% polyacrylamide gels run in sodium dodecylsulphate [11], and stained with Coomassie Brilliant Blue R.

There are interesting analogies here with other toxins. For example, cholera toxin (see [9]) also binds a ganglioside (though a different one: GGnSLC). The toxin is made up of two different kinds of subunit: A and B, of which only B can bind to ganglioside. Indeed, the subunit-B to ganglioside binding is so strong that 8 M urea elutes only subunit A from an insoluble toxin-ganglioside-cerebroside complex (prepared in a similar way to the tetanus toxin complex described above) because the A–B bonds are broken but the B-ganglioside bonds are not. A similar experiment does not work with tetanus toxin because conditions strong enough to separate the two chains also separate the toxin from the ganglioside.

The A subunit of cholera toxin is the one that has toxic activity, while the B subunits bind to gangliosides in the outer membrane of susceptible cells. Similarly, diphtheria toxin has two polypeptide chains (like those of tetanus toxin, the product of proteolysis of a single polypeptide) one of which (fragment B) binds to cells while the other (fragment A) has the enzymic activity that is the cause of toxicity [10].

These analogies suggest that tetanus toxin might work in the same sort of way. The heavy chain may bind to susceptible tissues, while the light chain has the activity responsible for toxicity. This idea is certainly compatible with the observation that binding to ganglioside does not inhibit activity of whole toxin [4]: it must surely have a marked effect on the heavy chain but may leave the light chain unaltered. Neither purified chain is toxic towards whole animals by itself [7], but then nor are the subunits of cholera toxin or the fragments of diphtheria toxin. It may be necessary to find some simpler system in which to investigate the activity of the chains of tetanus toxin, as it was with the other toxins.

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