Volume 252, number 1,2, 88–90 FEB 07446 July 1989

Homologous sequences in cholera toxin A and B subunits to peptide domains in myelin basic protein

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Received 29 May 1989

Recent reports that myelin basic protein (MBP) can be ADP-ribosylated and contains specific sites that bind GTP and G_{M1} ganglioside, have suggested an analogy to the properties of cholera toxin. Comparisons of pairs of sequences between these two proteins yielded two regions of homology between MBP and the cholera toxin B (chol B) subunit, and one region of homology with the cholera toxin A (chol A) subunit. The matching sites within chol B consisted of a 17 amino acid residue sequence (residues 30–46 in chol B and residues 102–118 in human-MBP, hMBP, $p < 0.0007$) and an 11 residue span (residues 31–41 in chol B and sequence 29–39 in hMBP, $p < 0.0004$). The homologous site within chol A corresponded to an 11 residue span (residues 130–140 in chol A and 67–77 in hMBP sequence, $p < 0.00007$). Since portions of the cholera toxin sequence are virtually identical to sections of the sequence in *E. coli* toxin, the homology is also valid for the same sequences in this toxin. The highly antigenic behavior of MBP that is related to the induction of experimental allergic encephalomyelitis may be paralleled by comparable neural pathology from the homologous regions of cholera toxin.

Myelin basic protein; Cholera toxin; Sequence homology

Central nervous system myelin basic protein (MBP) is a peripheral membrane protein that possesses some unique properties not common to other peripheral membrane proteins. The isoelectric point is greater than 10.6 [1], it binds G_{M1} ganglioside [2], GTP [3], and it can be ADP-ribosylated [4]. The protein and some of its peptides are highly antigenic, and are capable of inducing experimental allergic encephalomyelitis, EAE, in a number of laboratory animals. This protein has been the focus of innumerable investigations because of its possible role in the etiology of multiple sclerosis and other demyelinating conditions.

In the mid 1960s, Tomasi and Kornguth [5] suggested that MBP and histone were related proteins. However, it was subsequently shown that the protein these investigators had isolated was, in fact, MBP and not histone [6]. Jahnke et al. [7] searched the protein sequence database, version 3.0, for sequence homologies between MBP and viral proteins. This resulted in the identification of 23 peptide sequences from different viruses which could be matched with appropriate sequences in MBP. Quite recently it was suggested that the 21.5 kDa human MBP and the protein from visna virus contained a region of sequence homology [8].

Cholera toxin is a complex consisting of a single A (chol A) subunit and five B (chol B) subunits. The B subunits bind to cell membranes by an interaction with the G_{M1} ganglioside ($K_D = 1 \times 10^{-9}$ M) whereas the A subunit facilitates the ADP-ribosylation of signal transducing G-type proteins. The similarity between MBP and cholera toxin is strengthened by the reports that MBP exhibits binding properties that are similar to those of cholera toxin.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

The binding of G_{M1} ganglioside by chol B is paralleled by the MBP binding of G_{M1}. The investigations by Yu and his collaborators [2] showed that the interaction of G_{M1} ganglioside with MBP occurred in the region of the molecule comprising amino acid residues in proximity to tryptophan 116. The interaction of the single methylated arginine 107 residue with the G_{M1} molecule was also confirmed. It has been reported [9] that in chol B the chemical modification of Arg 35 blocked the binding of G_{M1}. In addition, the binding of G_{M1} also encompassed a region of hydrophobicity in the toxin suggesting an accommodation of the hydrophobic chain of the glycolipid [9].

The analogy to chol A behavior is strengthened by recent reports from the Moscarello laboratory [3], that GTP is bound to the N-terminal region of human MBP and that MBP can also be ADP-ribosylated [4].

These reports on MBP behavior led us to compare sequences of MBP for possible structural similarity with that of chol A and B. The primary structure for chol A has been reported [10,11] and the amino acid sequence for chol B is also known [13,14]. The amino acid sequence of the *E. coli* LT enterotoxin is very similar to that of chol A and is reported to show a homology of approximately 81% [12]. Thus this toxin will have the same homology to MBP as does chol A.

The amino acid sequence of human MBP was compared with that of chol A and B subunits using the Staden program DIAGON [15,16] run on a VAX computer. Initial matching was based on 11-residue spans using a score threshold at the 0.1% level of expectation ($p < 0.001$), as calculated by the McLachlan [17] double matching probability. After a sequence match had been recognized, the residue span and the score threshold were increased to find the lowest p value compatible with that particular match. These sequence matching determinations revealed that a portion of the chol B unit, consisting of amino acid residues 30–46, was homologous to a region of the MBP molecule comprising amino acid residues 102–118. The p value for the validity of this correlation was lower than 0.0007. This 17 amino acid sequence was the largest homology match that could be validated. A smaller 11 amino acid segment encompassing hMBP residues 29–39 was also matched to the same region (31–41 in the chol B sequence) with a p value of 0.0004. The numbering of the MBP sequence is the one used for bovine MBP as given by Martenson et al. [6].

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S Q G K G R G L S L S R F S W G A   human MBP
102                             118 p < 0.0007

S L A G K R E M A I I T F K N G A   cholera toxin B
 30                              46

  L P R H R D T G I L D             human MBP
   29                 39            p < 0.0004
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The homology found between chol A and MBP involved the chol A residues 130–140 and 53–64, that matched residues 67–77 and 8–20 in the MBP sequence, respectively.

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V H F G V L D E Q L H          cholera toxin A
130                 140

A H Y G S L P Q K S H          human MBP
 67                  77        p < 0.00007

V R H D D G Y V S T S I        cholera toxin A
 53                  64

Q R H G S K Y L A T A S        human MBP
  8                  20        p < 0.0001
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Sequence alignment and homology reveal several noteworthy features. The primary feature is the alignment of arginine 35 in cholera B with arginine 107 in MBP. Each of these respective residues has been reported to be a participant in the binding of G_{M1} ganglioside [2,9]. The second feature is the closeness of the tryptophan residue to this arginine in MBP. The NMR study [2] also implicated this residue in the binding of the G_{M1} ganglioside. Although the attachment of azido GTP is reported to occur in MBP at glutamine residue 3 [3], the peptide sequence homology with chol A occurs from residues 8 to 20. This small sequence displacement does not detract from the homology that has been identified, since elements of secondary and tertiary structure could influence the placement of the GTP on equivalent glutamines in the region. All four MBP segments that were found to be homologous to cholera toxin correlate with conserved sequence regions [26] of the MBP molecule. This would be expected if they were essential for myelin structure and function. These features of parallel properties, that can be

related to homologies in peptide sequences, may also be reflected in the antigenic properties of the cholera toxin homologous peptides. In human MBP the sequence from residue 43 to 88 is a dominant antigenic form present in cerebrospinal fluid after myelin injury [18] and contains the major encephalitogenic determinant reported to be responsible for EAE in Lewis rat [19], rabbit [20] and monkey [21]. This sequence contains the epitope residues 64–75 [22], which are part of the MBP sequence 67–77 that in turn is homologous to the chol A segment of amino acids 130 to 140. Incidents of neural pathology that were correlated with the subcutaneous administration of cholera vaccines are reported in the literature [23]. Neurological complications resulting from vaccines are not uncommon [24]. These vaccines may have contained small amounts of the toxin [24] and, in the most severe responses elicited a strong antibody response accompanied by encephalomyelitis [25]. It has been claimed that only six amino acids appear to be necessary to define an antigenic determinant [8] so that the delineated homology may be sufficient to elicit the neurological complication. However, the X-ray crystal structure of the lysozyme antibody complex from the Poljak group [27] reveals that there is an approximately 700 $Å^2$ interface of accessible surface area of the antibody combing site. Thus, there are many more contacts than just the antigenic determinants and the question of what defines an antigenic determinant is much more complicated.

Since pertussis toxin shares some common properties with cholera toxin we are now examining this system for homologies with MBP.

Acknowledgement: This work was supported in part by a grant from NIH NS023474.

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