STUDIES ON CHEMICALLY MODIFIED FORMS OF THE MYELIN BASIC PROTEIN: REQUIREMENTS FOR ENCEPHALITOGENICITY

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1. Introduction

The basic protein of myelin is one of the major protein constituents isolated from this membrane. This protein is responsible for the induction of experimental allergic encephalomyelitis (EAE) in a variety of animals including the guinea pig. A nonapeptide (residues 114–122 inclusive) derived from this basic protein is encephalitogenic in the guinea pig [1]. It has been shown that substitution of amino acid residues 3, 8 or 9 in this nonapeptide destroys its encephalitogenic activity [2,3]. However, it has not been shown that this amino acid region in the intact protein is the sole requirement for encephalitogenic activity although chemical modification of the sole tryptophan residue 116 greatly lowers this activity [4].

The modified proteins were tested for their ability to induce EAE in the guinea pig. In the present work, we show that although this peptide sequence may be necessary, it is not a sufficient condition to determine encephalitogenic activity.

In support of the view that the linear sequence Trp - - - - Gln, Lys is not a sufficient condition for EAE activity, the results with gastric inhibitory polypeptide (GIP) are pertinent. This sequence, rarely found in proteins has been demonstrated in GIP [5]. The lack of activity of GIP in the guinea pig has given the impetus to a study of other factors reported here.

2. Materials and methods

The basic protein was isolated from the myelin fraction of normal human white matter by the method of Lowden et al. [6]. A sample of the bovine basic protein (Al) was kindly provided by Dr E. H. Eylar (Playfair Neurosciences Unit, 1 Spadina Crescent, Toronto) and the gastric inhibitory peptide [5] was a gift from Dr J. C. Brown (Dept. of Physiology, University of British Columbia). The synthetic encephalitogenic nonapeptide was purchased from Beckman Inst. Co.

The bovine basic protein was reacted with nitromalonyl dialdehyde by the method of Signor et al. [7] as previously described for the human basic protein [8]. The resulting δ-(5-nitro-2-pyrimidyl) ornithine derivative of the basic protein (NP-Al) was reduced with sodium borohydride to the 1,4,5,6,-tetrahydropyrimidyl derivative (THP-Al) [7]. NP-Al (10 mg) was suspended in 0.8 ml absolute ethanol; 0.2 ml of 0.1 N NaOH was added followed by 10 mg sodium borohydride which was added as a dry powder with stirring. Most of the yellow colour disappeared from the solution after a few minutes. After 10 min, the reaction was stopped by the addition of 2 ml of 0.2 M acetic acid. The resulting turbid suspension was dialyzed against several changes of distilled water and the final product recovered by lyophilization.

There are 10 histidine and 4 tyrosine residues in the basic protein of normal human myelin which can

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react with iodine to form mono- or di-iodo derivatives. Ten mg of the basic protein from human myelin was suspended in 0.01 M sodium phosphate buffer, pH 9.25. A seven-fold molar excess of iodine (with respect to the basic protein) dissolved in 1 ml of chloroform was shaken with the suspension of basic protein. The iodine colour disappeared and the chloroform layer was removed and discarded. This procedure was repeated with the same amount of iodine. The protein was recovered as a precipitate at the chloroform–water interface, washed with acetone and dried under vacuum. Normal human basic protein was modified with citraconic anhydride according to the method of Gibbons et al. [9]. Ten mg of basic protein was suspended in 4.0 ml of 0.1 M $(NH_4)_2CO_3$, pH 8.8. A 100-fold excess of citraconic anhydride (with respect to the basic protein) was dissolved in 0.7 ml of $(NH_4)_2CO_3$ and this was added to the basic protein solution. The reaction was carried out at 4°C. The pH was maintained at pH 8.8 by the addition of concentrated NH_4OH until the reaction was complete. The solution was dialysed against distilled water which had been adjusted to pH 8.8 with NH_4OH using Thomas tubing 3787-H45, with a molecular weight cut-off of 3500.

For enzymatic hydrolysis, 150 μg of gastric inhibitory polypeptide was suspended in 500 μl of NH_4CO_3 containing 0.001 M Ca^{++}. The concentration of trypsin used was 1% of that of the protein concentration. Hydrolysis was allowed to proceed for 24 h at 37°C. The sample was lyophilized and then suspended in 200 μl of physiological saline combined with Freund's adjuvant and injected into guinea pigs.

The intact and modified basic proteins were suspended in equal volumes of physiological saline and Freund's complete adjuvant to give a final concentration of either 50 μg protein or 100 μg protein per 100 μl. 100 μl of protein was injected intradermally into the nape of the neck of each guinea pig.

3. Results and discussion

It can be seen from table 1 that the intact basic protein from either bovine or human myelin was encephalitogenic in the guinea pig. The commercially available synthetic nonapeptide corresponding to residues 114–122 of the intact bovine basic protein was considerably less encephalitogenic than the intact protein. A number of laboratories have reported the nonapeptide to be highly encephalitogenic [10,11]

The important amino acids for the encephalitogenic activity of this nonapeptide in the guinea pig was Trp- - -Gln, Lys. The terminal Lys could be replaced

Table 1
The encephalitogenic activity of human and bovine myelin basic protein, gastric inhibitory polypeptide, and chemically modified human and bovine basic protein

Protein	Amino acid(s) modified	Protein concentration (μg per guinea pig)	Number of guinea pigs which developed clinical EAE
Human	None	50	7/8
	Arginine	50	0/4
	Lysine	100	0/4
		50	0/8
	Tyrosine Histidine	50	5/5
Bovine	None	50	7/8
	Arginine (NP-Al)	50	0/8
	Arginine modified then reduced (THP-Al)	50	5/8
Synthetic nonapeptide	None	25	2/16
GIP	None	50	0/8
Tryptic digestion of GIP	None	50	0/4

by Arg [2]. These three amino acids (Trp, Gln, Lys) have been found to occur in the sequence of gastric inhibitory polypeptide and in one fragment of a tryptic digest [5]. Neither the intact GIP nor the fragments obtained by tryptic digestion were found to be encephalitogenic in the guinea pig (table 1). From this we concluded that the positioning of the three 'essential' amino acids in the nonapeptide from bovine basic protein may be necessary but not the sole requirement for encephalitogenic activity. This result supports those of Hashim and Sharpe [3] who used synthetic peptides substituted at positions other than 3, 8 and 9 of the nonapeptide and found a loss of encephalitogenic activity.

The region around tryptophan residue 116 of the intact protein is known to be important for the encephalitogenic activity. Swanborg [12] and Chao et al. [13] have shown that chemical modification of this sole Trp residue results in a loss of encephalitogenic activity. Lindl et al. [4] modified tryptophan residue 116 in a fragment of the basic protein containing residues 89–169 and found that some encephalitogenic activity remained.

We have prepared chemical derivatives of the basic protein which are not encephalitogenic although the tryptophan region of residues 114–122 is unaltered. The human basic protein has an arginine residue at position 122 of the intact protein while the bovine protein has a lysine residue in this position. Chemical modification with citraconic anhydride, a reagent specific for amino groups (Lys), should not effect the encephalitogenic determinant of the human basic protein while nitromalonyl dialdehyde, a reagent specific for guanidyl groups should not effect the encephalitogenic determinant of the bovine basic protein. However, both of these chemical modifications resulted in the abolition of encephalitogenic activity (table 1). The cause for this loss of activity has not been determined but could have resulted from a large change in the overall charge of the protein resulting in a conformational change. Other derivatives of the lysine and arginine residues of the protein resulting in changes in charge retain their encephalogenicity [12] and reaction of the amino groups with imidates which do not result in a change of charge also retains activity even though the encephalitogenic determinant may be modified [14]. In the case of the arginine modified derivative, we showed that the conditions of chemical modifications itself did not cause loss of activity since borohydride reduction of this derivative resulted in the regeneration of the positive charge as well as EAE activity, although the arginyl residues remained altered as the 1,4,5,6-tetrahydropyramidyl derivatives. Iodination of the basic protein did not effect the tryptophan residue in the protein but decreased the EAE activity (table 1) although the protein was extensively modified.

Thus extensive chemical modification of the basic protein in regions outside of the encephalitogenic determinant does not guarantee the destruction of EAE activity but at the same time, the presence of the unaltered encephalitogenic determinant (residues 114–122) is not a sufficient condition for the presence of EAE activity as is shown by the inactive NP-Al and citraconylated basic proteins.

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