AFFINITY PURIFICATION OF TWO POPULATIONS OF ANTIBODIES AGAINST FORMAT DETERMINANTS OF SYNTHETIC MYELIN BASIC PROTEIN PEPTIDE S82 FROM S82-AH- and S82-CH-SEPHAROSE 4B COLUMNS

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Two different kinds of immunosorbents were prepared that contained the synthetic myelin basic protein didecapeptide S82 (TTHYGSLPQKAQGHRDQDEG)—one coupled with AH-Sepharose 4B through hexanoate spacers to the C-terminal glycy1 residue; the other, with CH-Sepharose 4B through hexanoate spacers to the N-terminal threonine residue. An antiserum rich in antibodies to a format determinant of S82 was passed through each column, and, by means of affinity purification, two homogeneous populations of anti-format antibodies were obtained, each with a binding affinity of $1 \times 10^5 \text{M}^{-1}$ for S82. The population recovered from S82-AH-Sepharose 4B cross-reacted to a considerable extent with synthetic peptide S8 (GSLPQKAQGHRPQDEG) but only to a limited extent with S79 (AQGHRPQDEG). The population recovered from S82-CH-Sepharose 4B cross-reacted poorly, if at all, with S8. An equimolar mixture of S8 + S79, however, reacted well with either population of anti-format antibodies, thus showing that...
the mixture could mimic the format of S82. It was concluded that secondary structural conformation of S82 could be preserved during the coupling procedure and that the resulting immunosorbents could be used for the affinity purification of anti-S82 antibodies to the format determinants.

INTRODUCTION

A model for the purification of myelin basic protein peptides in picomole quantities was recently developed (1) in which antibodies to the C-terminal determinant of S82 (TTHYGSLPQKAQGHRPQDEG) were affinity-purified from an adsorbent prepared from S79 (AQGHRPQDEG). The S79 peptide had been coupled through its N-terminal end to the hexanoate spacer groups of CH-Sepharose-4B™ in order to preserve the integrity of the glycine end and to make it readily available. The rabbit-anti-S82 antiserum that had been used in the development of that study was unusually rich in antibodies to the C-terminal determinant, but most rabbit and rat anti-S82 antisera had been found to contain as their major populations antibody fractions specific for other S8 determinants (2).

One such major determinant appeared to depend on the conformation of residues common to and no smaller than the S80 family of peptides (GSLPQKAQGHRPQDEG) or to synthetic peptide S8 (GSLPQKAQGHRPQDENG). In the present experiments a coupling method was sought that would not destroy the format determinant, and to that end synthetic peptide S82 was used in the preparation of two different adsorbents, not only S82-CH-Sepharose 4B, coupled to the hexanoate spacers primarily through its N-terminal threonine residue, but also S82-AH-Sepharose 4B, coupled to hexanoate spacers through its C-terminal glycyl residue. It was hoped that format determinants indifferent to changes to at least one end of the peptide would thus be preserved. This overall result was obtained, and, in addition, an additional format determinant was uncovered.

EXPERIMENTAL PROCEDURE

The S82, S81, S8, and S79 Peptides. The peptides were synthesized by the general procedure of the Merrifield solid-phase method (3) and have been described (4). The peptide S82 sequence is a copy of bovine MBP residues 65-83 plus a C-terminal glycine substitution for asparagine at position 84; S81, a copy of residues 68-83 plus glycine; S80, residues 69-83 plus glycine; S8, residues 69-84 plus glycine; and S79, residues 74-83 plus glycine.

S82 TTHYGSLPQKAQGHRPQDEG
S81 YGSLPQKAQGHRPQDEG