

## COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS

This application is a continuation-in-part of U.S. Ser. No. 08/344,248, filed Nov. 23, 1994, now abandoned, which is a continuation of U.S. Ser. No. 08/248,037, filed May 24, 1994, now abandoned.

### BACKGROUND OF THE INVENTION

Copolymer-1 is a synthetic polypeptide analog of myelin basic protein (MBP), which is a natural component of the myelin sheath. It has been suggested as a potential therapeutic agent for multiple sclerosis (Eur. J. Immunol. [1971] 1:242; and J. Neurol. Sci. [1977] 31:433). All references cited herein are hereby incorporated by reference in their entirety. Interest in copolymer-1 as an immunotherapy for multiple sclerosis stems from observations first made in the 1950's that myelin components such as MBP prevent or arrest experimental autoimmune encephalomyelitis (EAE). EAE is a disease resembling multiple sclerosis that can be induced in susceptible animals.

Copolymer-1 was developed by Drs. Sela, Arnon, and their co-workers at the Weizmann Institute (Rehovot, Israel). It was shown to suppress EAE (Eur. J. Immunol. [1971] 1:242; U.S. Pat. No. 3,849,550). More recently, copolymer-1 was shown to be beneficial for patients with the exacerbating-remitting form of multiple sclerosis (N. Engl. J. Med. [1987] 317:408). Patients treated with daily injections of copolymer-1 had fewer exacerbations and smaller increases in their disability status than the control patients.

Copolymer-1 is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 6:2:5:1, respectively. It is synthesized by chemically polymerizing the four amino acids forming products with average molecular weights of 23,000 daltons (U.S. Pat. No. 3,849,550).

It is an object of the present invention to provide an improved composition of copolymer-1.

### SUMMARY OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa).

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa.

Moreover, the invention relates to a pharmaceutical composition and a method for the treatment of multiple sclerosis, using the above-discussed copolymer-1.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 displays the molecular weight distribution of three batches of copolymer-1, showing the proportion of species with molecular weight above 40 KDa. FIG. 2 shows similar data relating to the molar fraction.

### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa). Preferably, the composition contains less than 5% of species

of copolymer-1 having a molecular weight of 40 KDa or more. More preferably, the composition contains less than 2.5% of species of copolymer-1 having a molecular weight of 40 KDa, or more.

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa. In particular, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8 KDa and a copolymer-1 having an average molecular weight of about 6.25 to about 8.4 KDa.

Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. No. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine,  $\gamma$ -benzyl glutamate and E-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the  $\gamma$ -carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20° to about 26° C.

The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for sub-cutaneous injection. Alternatively, copolymer-1 may be formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs.

Typically, copolymer-1 is administered daily to patients suffering from multiple sclerosis at a dosage of 20 mg.

The invention will be exemplified but not necessarily limited by the following Examples.

#### EXAMPLE 1

Chromatographic method of preparation of low-toxicity copolymer-1 Two batches of copolymer-1 were prepared according to the methods known in the art, for example, U.S. Pat. No. 3,849,550.

One batch was then subjected to chromatographic separation, as described below.

A column for gel filtration, FRACTOGEL TSK HW55 (600×26 mm) was prepared in a Superformance 26 Merck cartridge according to the manufacturer's instructions. The column was equilibrated with water and acetone solution was injected for total volume determination. The column was equilibrated with 0.2M ammonium acetate buffer pH 5.0. 30 ml copolymer-1 samples (20 mg/ml, in 0.2M ammonium acetate pH 5.0) were loaded on the column and fractions were collected every 10 minutes. A fraction having