Ion Spray Mass Spectra of Native and Recombinant Streptococcal Antitumor Proteins

Masaharu KANAOKA,* Yasuko OGAWA,† and Yoshihisa UMEDA†

Takarazuka Research Center, Sumitomo Chemical Co., Ltd., 4–2–1 Takatsukasa, Takarazuka, Hyogo 665, Japan
†Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., 2257 Noji-cho Sumaikai, Kusatsu, Shiga 525, Japan

Received April 13, 1992

Streptococcus pyogenes, the pathogen of erysipelas, has long been known to have antitumor activity.1–3) An antitumor substance, streptococcal acid glycoprotein (SAGP), was isolated from the cells of a strain (Su) of low virulence by Yoshida et al.4) We have found that the antitumor substance has in vitro and in vivo antitumor activity through direct cytotoxicity against tumor cells with selectivity among tumor cell lines. The growth of sensitive cell lines, HeLa, Meth A, Ehrlich carcinoma, and sarcoma 180, is suppressed by a low dose of the substance, and the growth of others is not affected by higher doses.5) We have cloned and expressed the SAGP gene in Escherichia coli,6) and found that the antitumor activity of the substance produced in the recombinant cells is about one-quarter that from S. pyogenes Su cells.7) A difference in the quaternary structure accounts for the lower activity of the recombinant product; it is a dimer whereas the native form is a tetramer.8) In this study, we determined the molecular weights of the both forms with ion spray mass spectrometry,9) 10) to find if the difference in the quaternary structure arises from post-translational modification of the protein.

The mass spectra were measured on an API III triple quadrupole mass spectrometer (Perkin Elmer ScieX) equipped with an atmospheric pressure ionization ion source (ion spray). The mass spectrometer was operated in the positive mode. The ion spray voltage was 4000 V, the interface voltage was 650 V, and the orifice voltage was 180 V. Nebulizing gas (compressed air) was used at a pressure of 30 psi and a flow rate of 0.8 liter/min. Mass

Fig. 1. Ion Spray Mass Spectra of the SAGP Samples.
A, native SAGP; B, recombinant SAGP. The numbers above the peaks are the m/z and the predicted charges.

* To whom correspondence should be addressed.

Abbreviation: SAGP, streptococcal acid glycoprotein.
calibration was done with a solution of polypropylene glycol 1000 and 2000 (1 × 10^{-4} M in an 80% aqueous solution of acetonitrile containing 1 M ammonium acetate) before analysis.

The samples of SAGP (200 μg in 1 ml of phosphate buffered saline) were prepared as described elsewhere,7-11 desalted on a Sephadex PD-10 column (Pharmacia PL Biochemicals), and concentrated with a Centricon 10 membrane filter (Amicon). Desalting and concentrating steps were repeated once and the volumes were adjusted to 200 μl. A 50 μl portion of each sample was diluted with 20 μl of a 20% aqueous solution of acetonitrile containing 1% formic acid and introduced into the ion spray ion source by flow injection. A Waters 625 LC pump was used to deliver a solvent (80% aqueous solution of acetonitrile containing 0.1% trifluoroacetic acid) at the flow rate of 20 μl/min, and the sample (5 μl) was injected through a Rheodyne injector. During analysis, the mass spectrum was scanned from 1300 to 2000 amu in 0.1-amu steps. Molecular weights were calculated on the basis of the deconvolution mass spectra with a Macintosh IIx computer.

Figure 1 shows the ion spray mass spectra, and Fig. 2 shows the deconvolution mass spectra obtained by data processing from the spectra of Fig. 1. The molecular weights found were 46,164 for both the native and the recombinant substances, so the two forms had identical primary structures. The molecular weights were in accord with those calculated from the amino acid sequence, indicating that neither substance was post-translationally modified as was suggested by Yoshida et al.7 The precision of ion spray mass spectrometry is, in general, 0.01% or better.10 Thus, the difference in the quaternary structure between the two kinds of SAGP probably arises from a difference in their conformation. The conditions under which the proteins were synthesized must be responsible for the structural diversity.

The molecular mass of a protein is generally determined by methods such as SDS-polyacrylamide gel electrophoresis and gel filtration, the accuracies of which are not sufficient to uncover small modifications of proteins. The ion spray mass spectrometry used here had enough accuracy to show if a carbohydrate moiety was attached to the protein.

Our findings suggest that the name 'streptococcal antitumor protein' might be more appropriate than the current name 'streptococcal acid glycoprotein.'

References