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Molecular Weights of Antineoplastic Glycans From BCG Vaccine Using A Diffusion Technique

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BCG vaccine, developed originally for the prophylaxis of tuberculosis, is a potent immunostimulant approved for the treatment of superficial bladder carcinoma. Antineoplastic glycans have been extracted from the Tice® substrain of BCG by boiling and by urea treatment. The aim of this study was to determine the mean molecular weight of two extracts of BCG, denoted as PS1 and PS1A1, using a diffuison technique. The experiments were performed using Valia-Chien side-by-side diffusion cells with dextran standards of different molecular weights (range: 9.3 kDa to 73 kDa). A semi-empirical relationship between the effective diffusion coefficients, D_p, and the weight average molecular weights, M_w, of these dextrans followed the equation $D_p = K_D M_w^b$, with $K_D = 2.233 \times 10^{-6}$ and b = -0.66. Based on this relationship, the molecular weights of PS1, a mixed polysaccharide complex, and PS1A1, a glucan component of PS1, were found to be 22.8 kDa and 57.4 kDa respectively. Previous studies involving gel filtration chromatography with protein standards demonstrated PS1 to have a molecular weight of 22.4 kDa in agreement with the value obtained through measurement of diffusion coefficients. In the case of PS1A1, size-exclusion chromatography had previously estimated the molar mass to be between 65 and 87 kDa. The discrepancy between these values may be attributed to the fact that diffusion through membrane pores is dependent on both molecular size and configuration, as well as on charge interactions with the membrane. The conformation of PS1A1 molecules may differ from that of dextrans, especially in view of the known differences in the branching frequency and branch length of the two entities. In addition, PS1A1 is believed to contain small numbers of charged phosphate groups which may lead to differences in membrane interactions relative to neutral dextrans.

The Effect of Ethanol And Sodium Sulfate On The Molecular Behavior of Gelatin

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The stability of the native structure of gelatin in solution is affected by the presence of desolvating agents, temperature and pH of the solution. When sufficient desolvation is obtained, the gelatin molecules begin to aggregate, resulting in phase separation, forming a coacervate or, if excessive desolvation is applied, a precipitate. The objective of this study was to determine the effect of the desolvating agents ethanol and sodium sulfate on the distribution of molecular weight species between the two phases. Solutions of B225 gelatin (0.2 % w/w) containing increasing amounts of ethanol (40% to 75% w/w) or sodium sulfate (0% to 12.5% w/w) were adjusted to pHs 3,5 and 7 and incubated at 20°C, 37°C and 56°C. Aliquots from the solutions were filtered through a 0.2-μm filter, and the filtrate analyzed by size exclusion HPLC, using a Waters Ultrahydrogel Linear mixed-bed column. The HPLC chromatograms were divided into the different molecular weight classes previously described and the areas under the curve (AUC) calculated for each species. The degree of turbidity of the system was assessed using a Malvern Zetasizer, and correlated with the total AUC readings obtained from the HPLC chromatograms. We observed that the higher molecular weight fractions, in particular the microgel and delta fractions, are more readily desolvated at lower ethanol concentrations than the lower molecular weight species. This is particularly evident in case of ethanol, which acts as a weaker desolvating agent than sodium sulfate. In both cases, increases in temperature resulted in an increase in the amount of desolvating agent necessary to produce coacervate formation. The pH of the system was also critical. However, while desolvation was greatest at pH 5 for ethanol (close to the measured isoelectric potential of the gelatin) and least at pH 3, systems desolvated with sodium sulfate showed greatest coacervate formation at pH 3, implying the presence of a charge interaction. We believe that the characterization of this behavior is crucial in order to optimize the conditions required for the formation of gelatin nanoparticles using the desolvation technique as originally described by Marty et al. (1978)*.

Marty I.I., Oppenheim R.C. and Spesier P. (1978): Pharm. Acta Helv. 53:17-23.

Isolation, Characteristic And Study Proteins of Vipera Lebetina Venom And Products Their Metabolism

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For isolation, identification and studying of proteins of Vipera lebetina obtusa vemon and products of their metabolism, methods of polyacrylamide gel electrophoresis, gel-filtration and liquid-liquid extraction method were applied.

The venom of snake was subjected to polyacrylamide gel electrophoresis and it was found that there are 34 protein spots on the plate. Using the method of gel chromatography by elution with 0.1 M sodium phosphate buffer we have been found the optimal conditions for fractionation of snake venom proteins and products of their metabolism. At the separation of venom of Vipera lebetina and the products of its biotransformation by gel filtration on the Sephadex G-75 20 protein fractions and 19 products of their metabolism were obtained. The absorption spectra of proteins of zootoxin and the products of their biotransformation of snake venom have been investigated and optical density of protein fractions of the Vipera lebetina venom and products of its metabolism was determined. The outcome of this study might be useful for pharmacy.