INVESTIGATION ON THE PHYSICO-CHEMICAL PROPERTIES OF CAPSULAR POLYSACCHARIDES FROM SOME SEROTYPES OF KLEBSIELLA

PLAN AND PROGRESS OF THE WORK

Formation of extracellular polysaccharides[1,2] by most of the gram negative bacteria is the characteristic phenomenon. In the gram-negative bacteria *Klebsiella*, there are a large no. of serologically distinct K-serotypes[3] which produce acidic heteropolysaccharides[4]. These bacterial polysaccharides differ from other naturally occurring polysaccharides by the fact that they have antigenic properties and also they possess a well defined repeating unit varying from trisaccharides to heptasaccharides repeating units. Moreover, in each repeating unit there is a potential anionic sites which is the uronic acid (generally glucuronic acid).

The present investigation deals with the studies on different physico-chemical properties of the capsular polysaccharides of four different serotypes belonging to the same bacterial genus *Klebsiella*. The main objective of this investigation is to study the comparative physico-chemical properties of these polysaccharides and to correlate the primary structures with solution properties and also to evaluate the immunological specificity.

The capsular polysaccharides being acidic in nature will behave as polyelectrolytes in solution. Studies on the interaction of cationic dyes with the polyanions by spectral analysis will reveal very significant results in the field of dye–polymer interaction. Viscometric properties of these biopolymers [5] are expected to yield interesting polyelectrolytic properties. Conductometric experiments will response to the ionic groups of the polymers, enabling determination of equivalent weight. Interaction behavior of the polymers with the suitable fluorescent dyes, measured by excitation and emission spectra with the help of fluorescence spectroscopy, are also expected to give valuable information. Thermodynamic parameters like entropy change, enthalpy change, free energy change etc. can be conveniently determined for the interaction of the polymers with dye cations, which will give a clear picture about the interaction. The results obtained by all these experiments as outlined above will produce valuable comparative studies on their physico-chemical properties.

The work on the project has been running in progress since January, 2006, and the progress of work so far done is summarized below.

*Klebsiella* strain K-51, K-20, K-43, K-34 (kindly supplied by Dr. Ida Orskov of WHO International Escherichia centre, Copenhagen) have been used for the present investigation. The primary structures of all these capsular polysaccharides isolated from k-51, K-20, K-43 and K-34 are now known. All of them have completely different structures.

Primary structures of *Klebsiella* serotype K-51 polysaccharide consists of pentasaccharide repeating units which contain D-Galactose, D-Glucose, D-Glucuronic acid in the molar ratios (2:1:1).

Primary structures of *Klebsiella* serotype K-20 Poly saccharide
consists of Pentasaccharide repeating units which contains D-Galactose, D-Mannose, D-Glucuronic acid in molar ratios (2:1:1).

Primary structures of Klebsiella serotype K43 [ ] Polysaccharide consists of Pentasaccharide repeating units which contains D-Mannose, D-Galactose, D-glucuronic acid in molar ratios (3:1:1).

Primary structures of Klebsiella serotype K-3 4 [ ] Polysaccharide consists of Pentasaccharide repeating units which contains L-Rhamnose, D-Glucose, D-Glucuronic acid in molar ratios (3:1:1).

Polyelectrolytic properties were investigated with respect to binding constant of cationic dye pinacyanol chloride with the K-51 polymer. Induction of metachromasy [19] in the dye molecules by the polymer was studied by spectral measurements. A metachromatic blue shift of about 100nm was observed in the visible range (600-500 nm) during the dye–polymer interaction [20-22]. Spectral studies of other dyes like Pseudosocyanine, Methylene Blue, Toluidine Blue, Crystal Violet, Safranine T, Acridine Orange with K-51 were also studied but no significant blue shift was observed. Metachromatic titration [23], centrifugation [24] and McIntosh [25] methods indicated Stoichiometry of the dye pinacyanol chloride and K51 and K20 polymer have been studied by spectral measurements which suggested interaction of the polymer with the dye cations as 1:1, suggesting stacking conformation [26] of the polyanions. The complete reversal of metachromasy has also been observed by the addition of Methanol, n-Propanol.

Thermodynamic parameters [27, 28] like entropy change, free energy change and enthalpy change for the interaction of dye pinacyanol chloride and K51, K20 have been also studied by spectral measurements which suggested interaction of the polymer with the dye cations.

Dye–Polymer interaction was also studied by flurometric measurements [29-32]. Excitation and emission spectra of the dye Acridine Orange and dye-polymer complex was measured. Quenching of emission peak of the dye by the K51, K20 polymer at increasing polymer/dye (P/D) molar ratios was observed.

Effect of solvents like ethanol, methanol and pure & mixed surfactant on fluorescence spectra was performed.

Similar work on the interaction of the polysaccharides i.e, K43, K34 with the dye pinacyanol chloride have also been studied. The polysaccharides K43, K34 induced a metachromatic blue shift 600-500 nm and 600 to 495 respectively. Stoichiometry of the dye pinacyanol chloride and K-43 polyanion and also K-34 were studied and showed 1:1 stoichiometry in both the cases. Reversal of metachromasy has also been studied.

Thermodynamic properties [27-28] for the interaction of dye and other polymers K20, K43, K34 will be carried out.

Fluorescence spectral studies [29-32] of the various dye molecules and the metachromatic compounds of the capsular polysaccharides of K20, K43, K34 are now in progress.

Viscometric and conductometric [5] experiments will also be carried out to study the polyelectrolytic behavior and the micellar properties of the macroions in
solutions.

Some of the important references are quoted below. The present investigation is being carried out at the Chemistry Department Tripura University, Agartala under the supervision of Dr. R.K. Nath and co-supervisor Dr. A.K. Panda, Department of Chemistry, Behala College.

REFERENCES