BioMetals 1999, **12**: 47–52

Influence of metal ions and temperature on the conformation of *Escherichia coli* K1 capsular polysaccharide

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***Escherichia coli* K1 secretes a homopolymer capsular polysaccharide (CPS) consisting of alpha 2, 8 linked N-acetylneuraminic acid (poly α2,8NeuNAc). Typically poly α2,8NeuNAc is arranged in low and high order alpha helices with carboxyl and hydroxyl groups extending from the helices. Several properties of CPS such as antigenicity and metal binding can be influenced by its structural conformation. We examined the influences of metal ions and temperature on the secondary structure of poly α2,8NeuNAc. Conformation alteration was detected by ultraviolet (UV) spectroscopy and circular dichroism (CD). The majority of metal ions tested had no detectable influence on poly α2,8NeuNAc structure. In contrast, Yb^{3+}, Hg^{2+}, and Cu^{2+} ions greatly altered the UV and CD spectra, which suggests that these ions had disrupted the alpha helical structure of poly α2,8NeuNAc. These changes were influenced by the metal ion concentration. When poly α2,8NeuNAc was incubated at temperatures ranging from 20 – 60°C, alterations in its UV absorption spectra were also seen. The most significant change occurred between 35 and 40°C. In summary, this study suggests that the higher order structure and function of bacterial CPS may be influenced by environmental factors.**

Keywords: capsule secondary structure, exopolysaccharide, bacterial cell surface, copper, mercury, ytterbium

Introduction

Most bacteria, in their natural environments, produce and secrete extracellular polysaccharides (or less commonly polypeptides), thus forming a capsule as their outermost layer. Capsular polysaccharides (CPS) serve several functions for the underlying bacterial cell, most notably protection and adhesion. Numerous studies have illustrated the diversity of CPS primary structure (reviewed in Jann & Jann 1977, Kenne & Lindberg 1983). In *Escherichia coli* alone, there are over 100 strains designated on the basis of CPS structure. With the exception of dextrans and related polymers, bacterial CPS generally have a net anionic charge due to acidic moieties such as carboxylate or phosphate residues (McLean *et al.* 1996). This is well illustrated by the metal ion-binding ability of CPS. Capsule structure has been studied at the ultrastructural level. Using freeze-substitution and transmission electron microscopy, Graham *et al.* (1991) showed diversity in CPS architecture among a number of bacteria including *E. coli*.

Although much previous work has been done characterizing the primary structure of CPS, little work has focused on secondary or tertiary structures of these molecules. In other polymers, notably proteins, higher order structure is fundamentally important to the function of these molecules. One would expect CPS function to be similarly affected. Indeed, Hoyle and Costerton (1989) have shown that elevated concentrations of Ca^{2+} alter the structure of *Pseudomonas aeruginosa* alginate resulting in reduced tobramycin penetration. Using X-ray diffraction of CPS from an *Escherichia coli* M41 mutant, Moorhouse *et al.* (1977) observed limited structural changes due to dehydration, rehydration, and alteration of the CPS salt form from Na to Ca. No other metal ions were tested. Other investigations employing high resolution nmr and monoclonal antibodies have shown that higher order CPS structure are influenced by polymer length (Yamasaki &

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Bacon 1991, Jennings *et al.* 1985, 1989, Brisson *et al.* 1992).

A number of CPS-metal binding studies have been performed. Aside from the application of these studies in bioremediation and biomineralization, metal binding can also be used to investigate the structural properties such as the accessibility of anionic sites in cell surface structures including CPS. *Bacillus licheniformis* and *Klebsiella pneumoniae* K20 capsules are both able to bind metal ions (McLean & Beveridge 1990, McLean *et al.* 1990). While the anionic character of both of these capsules is contributed by carboxylate moieties, the concentration of carboxylate groups is greater in *B. licheniformis* CPS. If metal binding in these polymers were solely a function of anionic moiety concentration, one would anticipate that the relative affinity of each capsule polymer for a given metal would be similar – the only change would be in the total quantity of metal bound. As this is not the case , we assumed that higher order CPS structure influenced metal binding. In this present study, we investigated whether metal ions and other factors could influence the CPS higher order structure.

Materials and methods

Polysaccharide, chemicals, and organisms

Poly α2,8NeuNAc was purchased from Sigma Scientific Products (St. Louis, MO) at a purity of 99% and a molecular weight of 100 kDa. K1F bacteriophage, having neuraminidase activity (Petter & Vimr 1993), was kindly provided by E. Vimr (University of Illinois, Urbana, IL). *E. coli* K1 (ATCC 23503) was purchased from the American Type Culture Collection (Rockville, MD). All metal ions were purchased as analytical grade chloride salts from local suppliers. Ultrapure water (18.2 Ω cm^1 resistance) was used in all experiments to prevent contamination with trace metal ions (McLean *et al.* 1990).

Effects of metal ions on polysaccharide structure

The chloride salts of the following metal ions were tested for interations with poly α2,8NeuNAc: Hg^{2+}, Cd^{2+}, Sr^{2+}, Cu^{2+}, Zn^{2+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Yb^{3+}. These metal ions were chosen for their charge and location on the periodic table. Other metal ions, notably Al^{3+}, Fe^{3+} and Cr^{3+}, were not used because they precipitated in the presence of poly α2,8NeuNAc. In most instances, poly α2,8NeuNAc was dissolved (10 mg/ml) in 100 mM metal solutions and equilibrated at room temperature (22°C) for 30 minutes before measurements with CD or UV spectroscopy. As Cu^{2+}, Hg^{2+}, and Yb^{3+} showed significant effects on poly α2,8NeuNAc structure, the effects of these ions, and two ions showing little effect on CPS structure (Mg^{2+} and Ca^{2+}) were also tested at 10, 1, and 0.1 mM concentrations.

Effects of temperature on polysaccharide structure

Poly α2,8NeuNAc (10 mg/ml) was dissolved in ultrapure water and equilibrated in water baths at 25, 30, 35, 40, and 80°C for a minimum of 24 h before measurements with UV spectroscopy.

Absorbance scans

Ultraviolet spectroscopy absorbance scans were performed on a Beckman 640C spectrophotometer. Absorbance was read from 190 nm to 400 nm. 1 scan was taken per sample at an interval of 35.00 seconds and a scan speed of 1200 nm per minute. The data was smoothed using the least squares protocol of Savitzky and Golay (1964). Unless otherwise stated, all samples were at a concentration of 10 mg/ml poly α2,8NeuNAc and 100 mM metal chloride solutions. The corresponding CPS-free metal solutions or ultrapure water were used as a blank.

Circular dichroism

CD measurements were performed on a Jasco J600 spectropolarimeter (Japan Spectroscopic Co. Ltd., Tokyo, Japan) from 350 nm to 190 nm at a bandwidth of 1.0 nm. The slit width was automatic, sensitivity was 20 mdeg, time constant was 1 second, scan speed was 20 nm/minute, step resolution was 1.0 nm, PMT range was short, and accumulator was set to 1. The samples were prepared by dilution of the spectrophotometer samples in water by a factor of 10, giving a final concentration of 1 mg/ml poly α2,8NeuNAc and 10 mM metal chloride as this reflected the detection range of this instrument. The corresponding CPS-free metal solutions or ultrapure water were used as a blank. Due to restricted access to the spectropolarimeter, no other metal chloride concentrations were tested.

Results

Effects of metal ions on polysaccharide structure

UV spectroscopy of poly α2,8NeuNAc (Figure 1) revealed changes in absorption spectra in the presence of some metal ions. UV absorption spectra for poly α2,8NeuNAc suspended in Mg^{2+} and Ca^{2+} closely resembled that of the control spectra (poly α2,8NeuNAc suspended in H_2O). Similar spectra were seen when poly α2,8NeuNAc was suspended in Cd^{2+}, Sr^{2+}, Zn^{2+}, Na^{+}, K^{+}, Mn^{2+}, Co^{2+}, and Ni^{2+} (data not shown). In contrast, notable differences were seen when poly α2,8NeuNAc was suspended in Cu^{2+}, Hg^{2+}, or Yb^{3+}. To further investigate these spectral changes, poly α2,8NeuNAc was suspended in differing concentrations of Mg^{2+}, Ca^{2+}, Cu^{2+}, Hg^{2+}, and Yb^{3+}. In all cases, the most significant deviations from the control spectra were seen at high (100 mM) metal ion concentrations. As the metal ion concen-

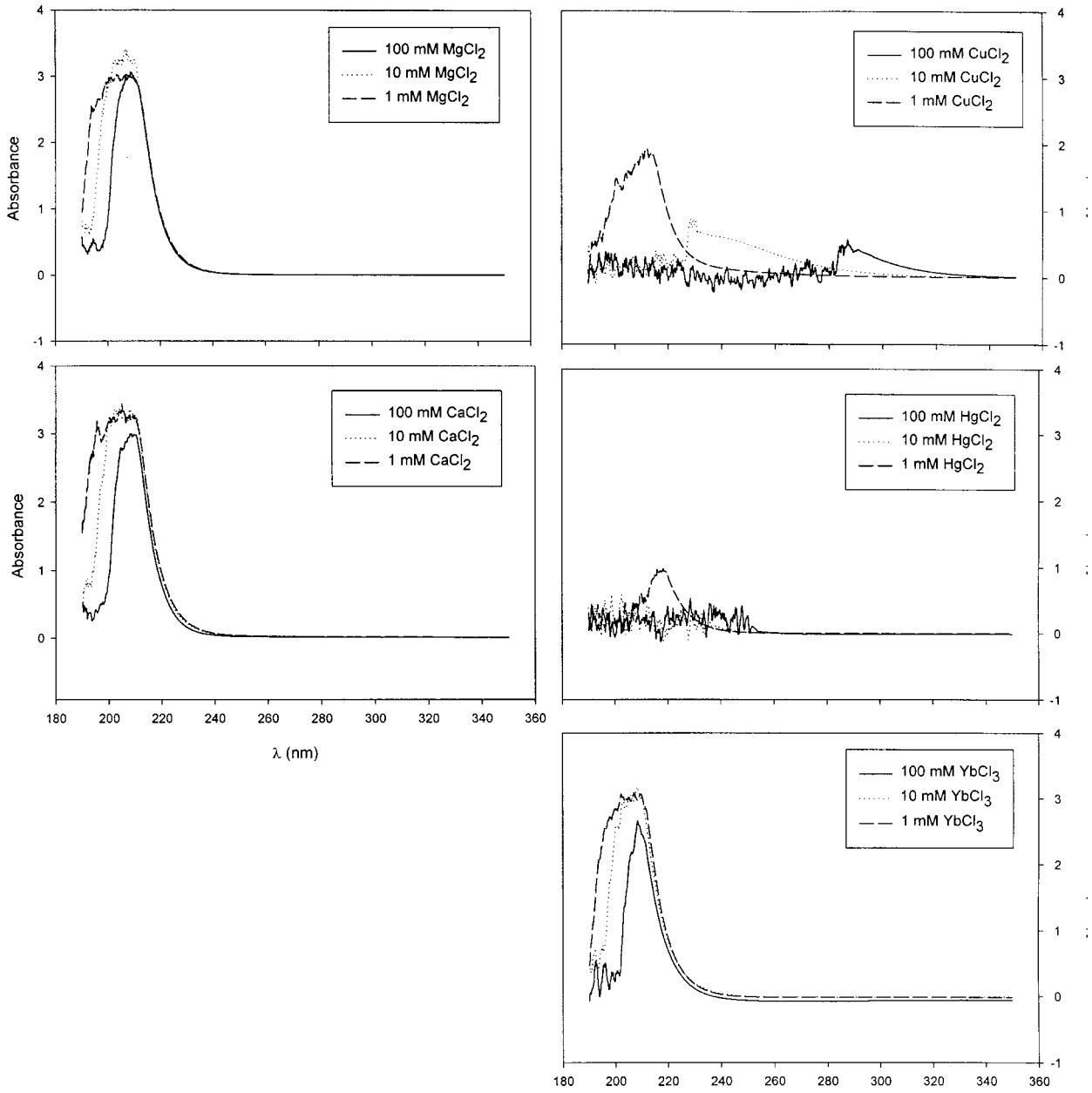

Figure 1. UV absorbance scans of 1 mg/ml poly α-2,8-NeuNAc dissolved in water (control), and varying concentrations of $CaCl_2$, $MgCl_2$, $CuCl_2$, $HgCl_2$, and $YbCl_3$. Absorbance profiles with H_2O (control) or other metal ions were similar to those of $CaCl_2$ and $MgCl_2$. Note the influence of $CuCl_2$ concentrations on UV absorption maxima.

trations were lowered to 1 mM, the spectra more closely resembled the control spectra, suggesting the metal ion effects on poly α2,8NeuNAc are concentration dependent. With the exception of the Cu^{2+} spectra, the wavelength of the absorption maxima for each metal ion concentration tested, remained fairly constant at all metal ion concentrations. With Cu^{2+}, the absorption maximum wavelength decreased from 291 nm to 210 nm as the Cu^{2+} concentration was reduced from 100 mM to 1 mM, suggesting that copper may have unique effects on poly α2,8NeuNAc structure.

CD scans of poly α2,8NeuNAc (Figure 2) also showed differences due to the presence of some metal ions. When poly α2,8NeuNAc dissolved in water was examined (control spectra in Figure 2), a

single peak was observed at 222 nm. Poly α2,8NeuNAc has been reported to have an alpha helical conformation (Brisson *et al.* 1992). Since the shape of the CD spectrum of this molecule resembles peptides having a known alpha helical conformations (Sreerama & Woody 1993, Yang *et al.* 1986) we interpret the control spectra in Figure 2 to indicate the alpha helical conformation of poly α-2,8-NeuNAc in water. Similar peaks and CD spectra were also observed in the presence of Mg^{2+} Hg^{2+}, Cd^{2+}, Sr^{2+}, Zn^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} (data not shown). The 222-nm peak disappeared in the presence of Cu^{2+} and Hg^{2+} that suggests a loss of the alpha helical structure of poly α-2,8-NeuNAc, and the development of a random coil conformation (Yang *et al.* 1986). When Yb^{3+} was present, the peak was replaced by a valley, and the overall Yb^{3+} – poly α-2,8-NeuNAc spectrum resembled a mirror image of the control spectrum. One possible interpretation is that Yb^{3+} induced a reversal of the alpha helical conformation of poly α-2,8-NeuNAc from a left-handed helix to a right-handed helix.

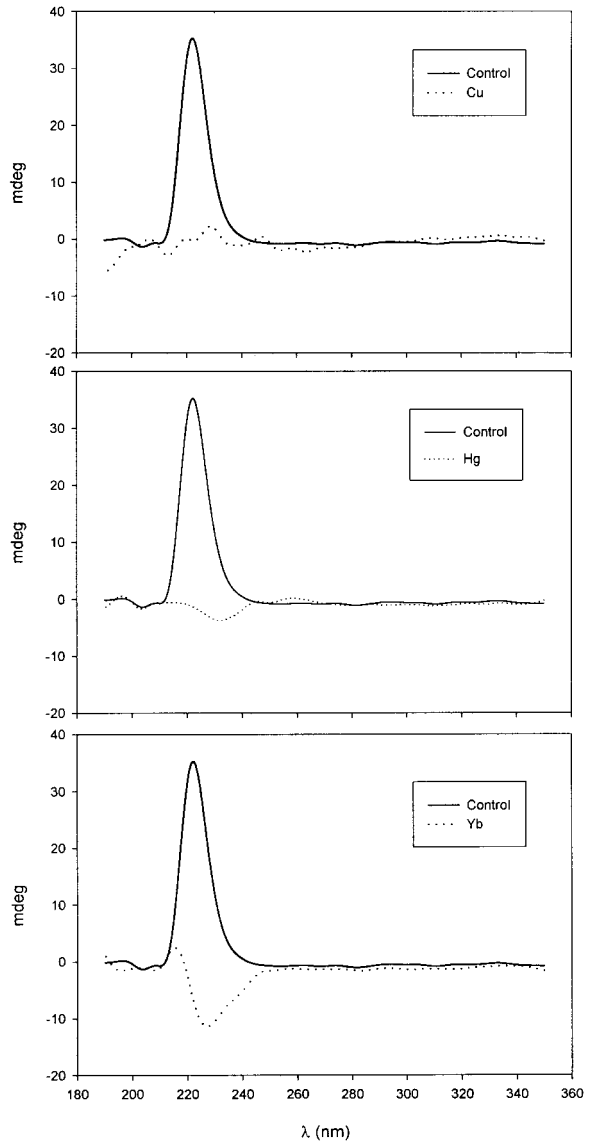

Figure 2. CD scans of 1 mg/ml poly α-2,8-NeuNAc dissolved in water (control) and 10 mM $CuCl_2$, $HgCl_2$, and $YbCl_3$. Absorbance profiles with other metal ions were similar to the control scan.

Effects of temperature on polysaccharide structure

As poly α-2,8-NeuNAc, dissolved in H_2O was heated in 5°C increments from 25°C to 40°C the UV absorption spectra of the molecule shifted irreversibly (Figure 3). The large absorbance peak, which is found from 190–230 nm at 25°C broadened as the temperature, increased. A second peak around 275 nm was also observed at higher temperatures. The most dramatic increase in size of this second peak occurred between 35°C and 40°C suggesting that changes to the structure of poly α-2,8-NeuNAc occur around human body temperature (37°C). Due to equipment limitations, CD measurements of temperature effects on poly α-2,8-NeuNAc were not performed.

Discussion

Poly α-2,8-NeuNAc is a homopolymer found as CPS in *Neisseria meningitidis* group B and *E. coli* K1 (Yamasaki & Bacon 1991). Structural analysis has

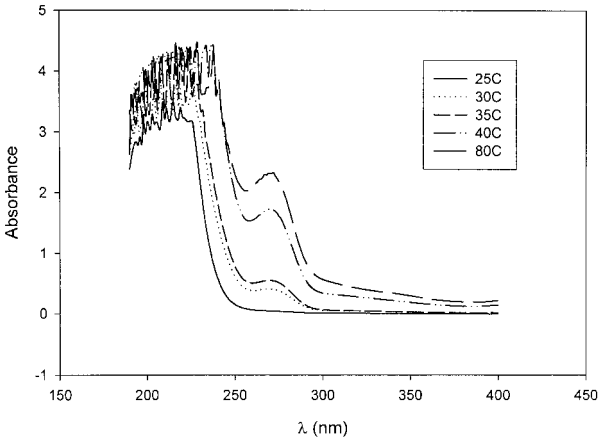

Figure 3. Effects of temperature on UV absorption spectra of 10 mg/ml poly α-2,8-NeuNAc dissolved in water. Note the appearance of a secondary peak at 275 nm at temperatures above 25°C.

revealed that the molecule contains regions of alpha helices, with carboxyl and hydroxyl groups extending out from the helices (Brisson *et al.* 1992, Yamasaki & Bacon 1991). At neutral or alkaline pH, the carboxyl and hydroxyl groups are partially ionized, which creates an anionic environment in the capsular matrix (Geesey & Jang 1990). Studies of CPS structure have traditionally employed a number of procedures including nmr, spectroscopy, chromatography, and a wide variety of other chemical procedures (Aspinall 1982, Rees *et al.* 1982). CD has also been employed in some studies of CPS structure (Daoust & St-Cyr 1982, Manning 1970). While commonly used in studies of protein structure, UV spectroscopy is not considered to be a sensitive probe of CPS structure due to the lack of CPS amino groups (Aspinall 1982, Rees *et al.* 1982). As amino groups are present in poly α-2,8-NeuNAc, the use of UV spectroscopy will indicate some changes in structural information. In the present study, CD confirmed any changes noted by UV spectroscopy.

The influence of metal ions on tertiary structure may be the most significant finding of this study because trace amounts of these ions are present in every natural environment. In most bacteria, CPS are anionic and are thus able to bind metal cations (Geesey & Jang 1990, McLean *et al.* 1996). Several studies have shown that CPS metal binding specificity and the related phenomenon of mineral formation is affected by CPS structure (Dumanski *et al.* 1994, McLean & Beveridge 1990, McLean *et al.* 1990). Although a broad spectrum of metal ions was tested, varying in charge and size, only three metal ions, Cu^{2+}, Hg^{2+}, and Yb^{3+}, significantly affected the tertiary structure of poly α-2,8-NeuNAc. The reason for this is not clear. Previous studies performed in this laboratory have demonstrated that all of the metals tested do actually bind to the poly α-2,8-NeuNAc, but the degree of binding needs to be further elucidated. It is possible that the coordination chemistry of these metals (Magini *et al.* 1988) plays a role in determining CPS tertiary structure. In an X-ray diffraction study of the structure of CPS from an *E. coli* M41 mutant, Moorhouse *et al.* (1977), showed that the Ca^{2+} salt form of CPS differed only marginally from the Na^{+} salt form. No other metal ions were tested. Our observations agree with Moorhouse *et al.* (1977) in that significant effects were not seen with Ca^{2+}. Future studies are needed to investigate binding stoichiometry and coordination complex formation of metal ions with poly α-2,8-NeuNAc and other CPS. Such work may have implications in the use of CPS for metal binding and biomineralization (Mann 1988, McLean *et al.* 1996).

This study demonstrates that even minimal environmental factors can greatly influence the structure of poly α-2,8-NeuNAc. Previous structural analyses of poly α-2,8-NeuNAc were performed at 25°C (Yamasaki 1988, Yamasaki & Bacon 1991) or 15°C (Brisson *et al.* 1992). These temperatures are not representative of temperatures found during an infection (≥37°C). The current study demonstrates that a large spectral shift occurs in ultraviolet wavelength absorbance scans between 35°C and 40°C (Figure 3), indicating that structure is temperature dependent. As stated previously, these changes in structure were irreversible. One possible explanation is that conformation shifts due to slight temperature increases are stabilized by the propensity of adjacent poly α-2,8-NeuNAc strands to form hydrogen bonds. Another possible explanation for the spectral shifts is hydrolysis of poly α-2,8-NeuNAc when suspended in H_2O for 24h. As the absorption spectra for poly α-2,8-NeuNAc at different temperatures (Figure 3) do not resemble the spectra of this molecule when hydrolyzed with neuraminidase from K1F bacteriophage (data not shown), it is not likely that significant hydrolysis occurred. From a medical context, future structural analysis of CPS should be performed at 37°C to demonstrate the structure of these molecules during an infection.

Acknowledgments

This work represents the MS thesis of Mary Kay Koenig (Koenig 1996) and was funded by research grants from the Research Enhancement Program of Southwest Texas State University and the Advanced Research Program of the Texas Higher Education Coordinating Board to RJCM. The spectrophotometer used in this study was purchased with funds from the USDA Strengthening Program to PW Barnes and RJCM, Southwest Texas State University. We thank the Department of Chemistry and Biochemistry at the University of Texas at Austin for the use of their spectropolarimeter. We also thank E. Vimr for the K1F bacteriophage, PA White and E Brown for preliminary work, and ME Bayer, MH Bayer, JW Fitch, HJ Jennings, MM Muir, and MJ Stillman for helpful advice. We dedicate this paper to the memory of Mariel M Muir.

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