Volume 332, number 3, 260–262 FEBS 13116 October 1993

The effect of sugars on the morphology of the bacterial flagellum

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Received 14 July 1993; revised version received 31 August 1993

Using dark-field microscopy, we have found that certain sugars cause the normal-to-curly helical transition of bacterial flagella. Titration of flagella isolated from *Salmonella typhimurium* with 16 different carbohydrates showed that: (i) only certain sugars cause the transition. There is no obvious relationship between the simple physico-chemical properties of the sugar and whether the sugar causes the transition or not; (ii) the efficacies of sugars that do cause the transition differ markedly. For these sugars there is a relationship between efficacy and molecular size. These results suggest that the specific, though weak, binding of sugars to sites on flagella cause the morphological transition.

Flagellum; Carbohydrates; Dark-field microscopy; Morphology

1. INTRODUCTION

The filament of the bacterial flagellum is a helical structure that can reach over 5 μm in length and can adopt one of several helical morphologies. The actual morphology adopted is dependent upon the primary sequence of flagellin [1] as well as such environmental conditions as pH [2,3], temperature [4], and the presence of organic solvents [5]. The helix type is also determined by the torque applied to the rod [6]. This latter effect appears to be an important component of the tumbling behavior of motile cells.

We report here that flagella undergo the morphological transition from the 'normal' helical form (left-handed helix, pitch = 2.3 μm, diameter = 0.45 μm) to the 'curly' helical form (right-handed helix, pitch = 1.14 μm, diameter = 0.30 μm) in the presence of only certain sugars. The specificity of the effect is sufficient to discriminate between 1,2- and 1,3-linked disaccharides. This is the first report of a molecular specificity associated with a morphological transition. It is also the first report of the involvement of biologically relevant molecules in the morphological transition.

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Abbreviations: MA, morphological agent; $K_{0.5}$, the sugar concentration at which 50% of the flagella have the normal helical morphology and 50% the curly.

2. EXPERIMENTAL

Flagella were prepared from *Salmonella typhimurium* SJW1103 as reported previously [7].

Sugar solutions contained 10 mM sodium phosphate and 150 mM sodium chloride. The final pH of all solutions was brought to 7.1. All procedures were performed at 22°C.

The number of normal and curly flagella in the various samples was determined by dark-field microscopy [8]. (Photomicrographs of these 2 forms can be found in reference [3].) The flagella/sugar mixtures were made by adding a small aliquot of flagella to the desired sugar solution and subjecting the mixture to 45 s agitation on a Vortex mixer. A 5 μl sample was then removed, placed under the microscope and the normal and curly morphologies scored in several randomly chosen regions of the slide glass. At least 100 flagella were counted for each scoring.

Sucrose was chosen as representative of those sugars that did not cause the morphological transition (see below) for determination of the effects of prolonged exposure on flagella morphology. Flagella were incubated for 60 min in a 30% sucrose solution with continuous stirring using a magnetic stir-bar and then incubated overnight at room temperature. After each period, a sample of flagella was analyzed by dark-field microscopy.

3. RESULTS AND DISCUSSION

The structures of the 16 sugars utilized in this study are given in Table I together with their effect upon the normal-to-curly transition. The sugars can be divided into 2 broad categories: (i) those that generate a complete normal-to-curly transition of the flagella, and (ii) those that have no effect or only have a minimal effect on morphology where more than 70% of the flagella remain unchanged. The former class of sugars will be referred to as morphological agents (MAs) and the latter as non-MAs.

The extended treatment of flagella with sucrose (a non-MA), described above, did not result in any further morphological transformation of the flagella than that

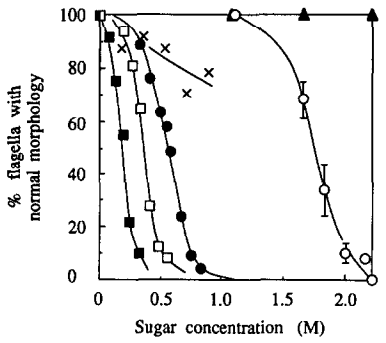

Fig. 1. The normal-to-curly morphological transition of flagella in response to the addition of various sugars. For clarity, titration curves are shown for only 4 of the total of 9 sugars that caused the normal-to-curly transition: fructose (○); raffinose (□); melezitose (■); turanose (●). In addition, the minimal effects of sucrose (X) and sorbose (▲) are also shown. The error bars on the fructose titration curve are provided to indicate the typical extent of the variation between separate titrations.

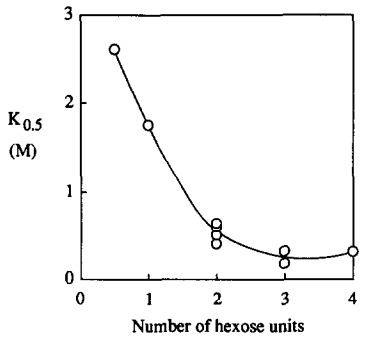

Fig. 2. The relationship between sugar size and $K_{0.5}$ for all the sugars that generated the normal-to-curly morphological transition. ($K_{0.5}$ is the sugar concentration at which 50% of the flagella have the normal helical morphology and 50% the curly.)

obtained after 45 s of mixing (Fig. 1). We conclude that we were not observing a kinetic effect of the sugar's ability (or lack of it) to induce the normal-to-curly transition.

It should be pointed out that we are unable to say that sucrose, and those other sugars that we classify from this study as non-MAs, are absolutely unable to effect the complete transformation of flagella morphology: at sugar concentrations of 30–40%, the flagella were no longer visible under the dark-field microscope and, consequently, the effect of higher sugar concentrations remains unknown. In particular, sucrose, maltose and maltohexaose caused the appearance of small percentages (<30%) of curly flagella at the highest attainable concentrations. The other sugars classified as non-MAs (ribose, glucose, galactose and sorbose) had no affect whatsoever on flagella morphology over their useable concentration ranges.

Table I

The sugars tested for their ability to act as morphological agents (MA)

Sugar	$K_{0.5}$ (M)	Structure
Dihydroxyacetone	2.6	
Ribose	–	
Glucose	–	
Galactose	–	
Fructose	1.75	
Sorbose	–	
Maltose	–	α-Glu-1,4-Glu
Gentiobiose	0.55	β-Glu-1,6-Glu
Sucrose	–	α-Glu-1,2-β-Fru
Turanose	0.59	α-Glu-1,3-Fru
Lactulose	0.41	β-Gal-1,4-Fru
Melibiose	0.62	α-Gal-1,6-Glu
Raffinose	0.31	α-Gal-1,6-α-Glu-1,2-β-Fru
Melezitose	0.18	α-Glu-1,2-β-Fru-3,1-α-Glu
Stachyose	0.29	α-Gal-1,6-α-Gal-1,6-α-Glu-1,2-α-Fru
Maltohexaose	–	$(\alpha\text{-Glu-1,4-Glu})_3$

The values of $K_{0.5}$ for those sugars that acted as MAs are given. If a sugar did not act as an MA, a dash is given.

The reversibility of the reactions was not determined. However, no other morphological forms, and only a minute number (<1%) of intermediate forms, or disrupted forms were observed during the titrations. This indicates that the flagella were not damaged by exposure to the various sugars.

Is there a simple structural determinant of whether a sugar acts as a MA or not? From an examination of Table I it is evident that the ability to act as an MA is not a simple function of oligosaccharide length. Therefore, the ability to cause the normal-to-curly transition does not appear to be a function of simple physicochemical properties of the sugars, such as molecular mass or hydroxyl group content.

Further examination of Table I reveals that there is no specific monosaccharide chemical unit, combination of chemical units, or chemical property, such as the presence of a reducing group, that endows the ability to act as an MA.

Instead, the MA effect appears to be dependent, at least in part, on the conformation of the sugar. Thus, for example, whilst turanose (glucose-1,3-fructose) is an MA, sucrose (glucose-1,2-fructose) is not. This suggests that the MA effect is best explained by the existence on the flagella of stereo-specific binding sites for sugar ligands.

Consider next those sugars which fall into the MA category (Fig. 1). In this case, there is a broad relationship between the size of the sugar and its potency as an MA (Fig. 2). We have quantitated the potency of the various sugars in terms of the quantity '$K_{0.5}$', that is, the sugar concentration at which 50% of the flagella have the normal helical morphology and 50% the curly. The plot of $K_{0.5}$ vs. sugar size (Fig. 2) suggests that the

putative sugar binding sites on flagella are of sufficient size to accommodate small oligosaccharides.

The sites bind sugars weakly ($K_{0.5} > 0.18$ M) and are unlikely to be of physiological significance. However, the ability of bacterial flagella to discriminate between closely related molecules, and respond with a micrometer-scale structural change, is of potential interest in the biosensor industry. Furthermore, the fact that the effectors are naturally occurring molecules suggests that a search be made for other biological molecules which may play a physiological role in the morphological transition. It can be speculated that these molecules will contain sugar components as, for example, found in certain antibiotics.

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