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Regulation of colominic acid biosynthesis by temperature: role of cytidine 5'-monophosphate *N*-acetylneuraminic acid synthetase

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Synthesis of colominic acid in *Escherichia coli* K-235 is strictly regulated by temperature. Evidence for the role of cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) synthetase in this regulation was obtained by measuring its level in *E. coli* grown at 20 and 37°C. No activity was found in *E. coli* grown at 20°C. CMP-Neu5Ac started to be quickly synthesized when bacteria grown at 20°C were transferred to 37°C and was halted when cells grown at 37°C were transferred to 20°C. These findings suggest that temperature regulates the synthesis of this enzyme and therefore the concentration of CMP-Neu5Ac necessary for the biosynthesis of colominic acid.

Colominic acid; CMP-*N*-acetylneuraminic acid synthetase; (*E. coli*)

1. INTRODUCTION

Colominic acid (CA) or polysialic acid is a capsular homopolymer of *N*-acetylneuraminic acid (Neu5Ac) with $\alpha(2-8)$ ketosidic linkages or a mixture of $\alpha(2-8)$ and $\alpha(2-9)$ linkages [1–6]. This polymer has been shown to be a pathogenic determinant in capsular antigens of *Neisseria meningitidis, Escherichia coli* and several strains of *Salmonella* [1] and is also a component of high molecular mass animal glycoproteins (cell adhesion molecules) [2]. In *E. coli* K-235, CA biosynthesis involves the following enzymatic steps: (i) synthesis of Neu5Ac by the enzyme *N*-acetylneuraminate pyruvate-lyase, (ii) synthesis of CMP-Neu5Ac by the enzyme CMP-Neu5Ac synthetase and (iii) incorporation of Neu5Ac into an endogenous acceptor and transfer of the native chain to a final acceptor allowing the polymer to grow. These last reactions are catalyzed by a membrane-bound sialyltransferase complex [3].

It has been shown [4–6] that biosynthesis of CA is strictly regulated by temperature, however, the precise molecular mechanism of this control is unknown. Here, we describe the effect of temperature on synthesis of CMP-Neu5Ac synthetase, which explains the lack of production of CA by *E. coli* grown at temperatures below 20°C.

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2. MATERIALS AND METHODS

E. coli K-235 (ATCC 13025) was obtained from the American Type Culture Collection and a culture was also kindly supplied by Professor F.A. Troy (Department of Biological Chemistry, University of California, Davis, CA). Strains were kept freeze-dried or in liquid N_2 (gas phase). Bacteria were grown in a chemically defined medium, ideal for CA production, as in [6].

Cell-free extracts and soluble enzyme were obtained as described by Vann et al. [7]. CA was determined as described [6]. CMP-Neu5Ac synthetase activity was assayed by a modification of the method of Kean and Roseman [8]. The assay mixture contained 0.18 M Tris-HCl (pH 9.0), 4 mM $MgCl_2$, 0.5 mM CTP and 0.5 mM Neu5Ac in 250 μl; incubation was at 37°C for 30 min. After incubation, excess Neu5Ac was reduced with 50 μl $NaBH_4$ (100 μg/ml) (20°C, 15 min). $NaBH_4$ was destroyed using three drops of glacial acetic acid for 15 min at room temperature, and CMP-Neu5Ac synthesized was measured by the resorcinol method [6].

Protein was determined by the method of Lowry et al. [9] using bovine serum albumin as standard.

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3. RESULTS AND DISCUSSION

The role of growth temperature in the expression of some capsular products in Enterobacteriaceae has long been known [10]. This effect has also been described in the development of certain *E. coli* capsular K antigens [11].

Previous studies have shown that when *E. coli* K-235 is grown at temperatures below 20°C the quantity of CA synthesized is negligible, whereas synthesis increases markedly after raising the temperature to 37°C [4–6]. Vijay and Troy [4] showed that the fluidity of the lipid phase in the membrane is important for the proper functioning of the sialyltransferase complex (the last enzyme in the pathway of CA biosynthesis), suggesting that this could be the cause of the lack of CA synthesis at low temperatures. They also reported that the addition of undecaprenyl phosphate to intact membrane preparations, at temperatures above the transition temperature of the bulk membrane lipids, greatly stimulated sialyl polymer synthesis (120%) [5]. However, at 20°C (at which the lipid bilayer is essentially immobile) addition of exogenous undecaprenyl phosphate had no effect.

Fig.1 shows that in *E. coli* grown at 37°C, CMP-Neu5Ac synthetase began to be synthesized during the early logarithmic phase of growth (5–10 h). The level of the enzyme increases linearly during the exponential growth phase, reaching a maximum after 25 h incubation and from this time until 70 h it decreased continuously. This kind of kinetic behaviour, which parallels that of bacterial growth, is very similar to that observed for the sialyltransferase complex, a key enzyme in the biosynthesis of this capsular polymer [12]. However, at 20°C no CMP-Neu5Ac synthetase activity was detected even after 70 h of growth, sug-

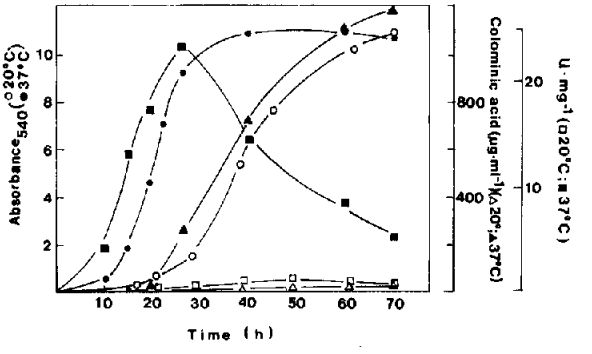

Fig.1. Time course of appearance of CMP-Neu5Ac synthetase activity, colominic acid production and growth in *E. coli* grown at 20°C (□, △, ○) and 37°C (■, ▲, ●).

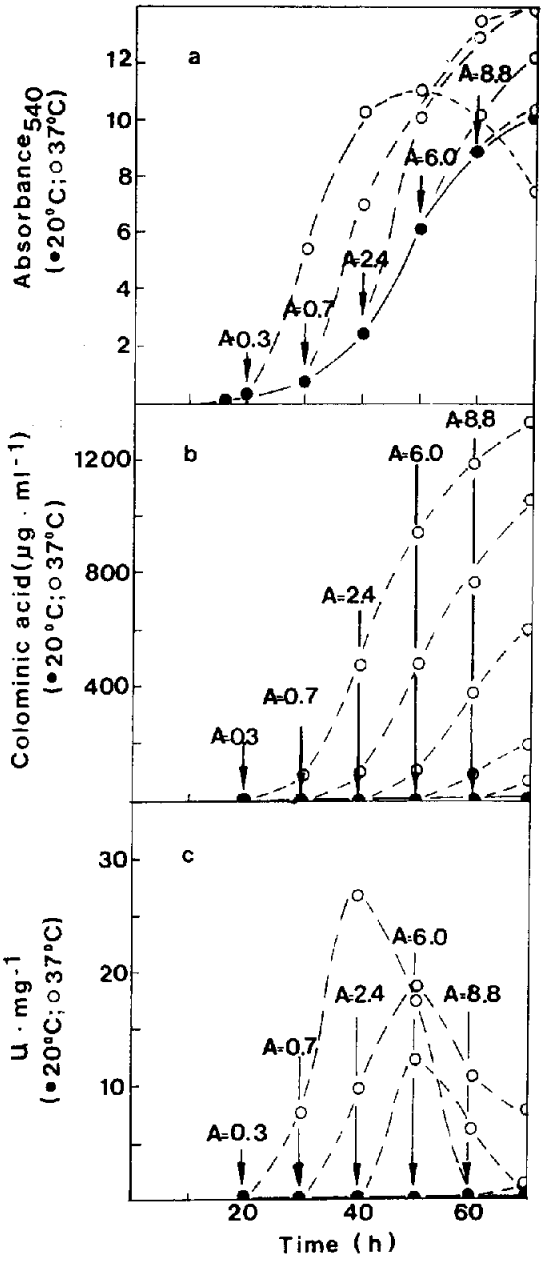

Fig.2. Effect of temperature for *E. coli* cultures transferred from 20°C (●) to 37°C (○) on: (a) bacterial growth, (b) colominic acid production, (c) CMP-Neu5Ac synthetase activity. Arrows indicate the time of transfer. A, absorbance 540 nm.

gesting that either the gene that codes for the enzyme is not transcribed or the corresponding mRNA is not translated. Further experiments demonstrated that synthesis of CMP-Neu5Ac synthetase began when cells grown at 20°C were transferred to 37°C (fig.2) whereas it ceased in those grown at 37°C and then switched to 20°C (fig.3).

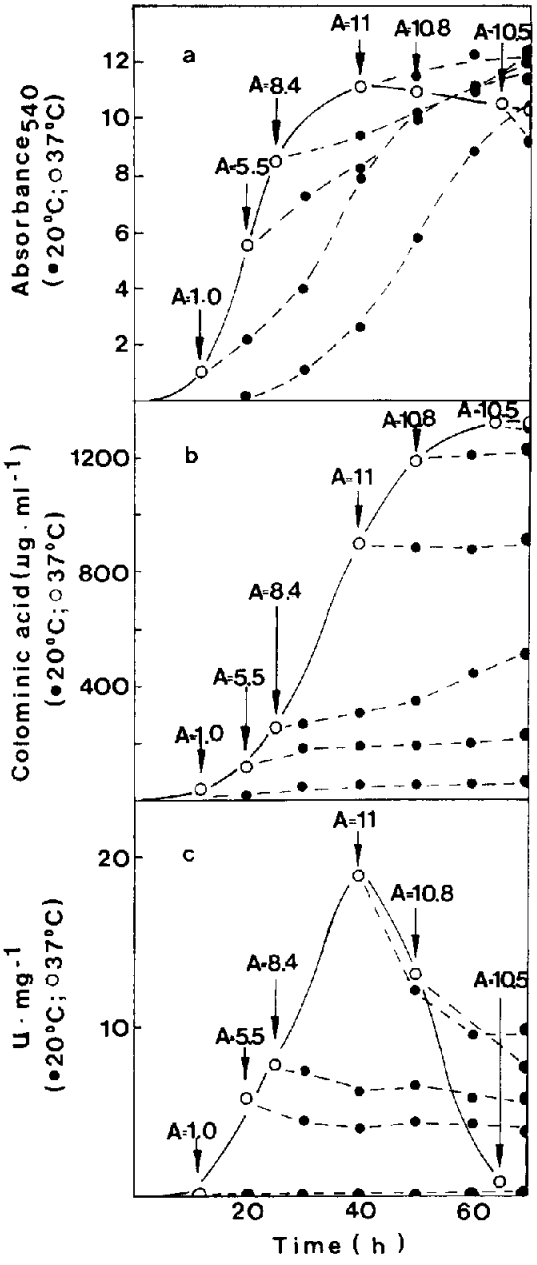

Fig.3. Effect of temperature for transfer of *E. coli* cultures from 37°C (○) to 20°C (•) on: (a) bacterial growth, (b) colominic acid production, (c) CMP-Neu5Ac synthetase activity. Arrows indicate the time of transfer. A, absorbance 540 nm.

Whether CMP-Neu5Ac synthetase is a critical target point in the regulation of CA by temperature or whether the lack of this enzyme is caused by a block in the polymerization process is not yet clear. Thus, at least two different hypotheses can be presented for explaining the regulation of CMP-Neu5Ac synthetase by temperature:

(i) The target point of CA control by temperature is the polymerization process. If this were the case a permanent or transitory block in the polymerization process (caused for example by a fall of the growth temperature) would lead to a transitory accumulation of CMP-Neu5Ac that could stop, by feedback control, its own synthesis.

(ii) CMP-Neu5Ac synthetase is an independent critical regulatory point in the control of CA biosynthesis by temperature. If this were the case, when the growth temperature shifts down to a certain value (lower than 20°C) the biosynthetic pathway of CA might be rapidly stopped at the enzymatic step responsible of CMP-Neu5Ac synthesis.

The fact that mutants of *E. coli* K-235 blocked in the terminal steps of CA biosynthesis accumulate large amounts of CMP-Neu5Ac in spite of the extreme toxicity of this product [13] suggests that the second hypothesis must be correct.

4. CONCLUSIONS

The present results are the first description of the regulation of CMP-Neu5Ac synthetase by low temperature. This fact may account for the absence of CA biosynthesis at temperatures below 20°C.

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