**Characterization of PatZ from *Escherichia coli*, a multimeric protein regulated by auto-acetylation**

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**Abstract**

Lysine acetylation is an important post-translational modification for metabolic regulation. This study focuses on the structural characterization of PatZ (formerly YfiQ), the only known acetyltransferase in *Escherichia coli* (\(E. coli\)).

To this objective, several techniques have been employed, such as protein HPLC-MS/MS, western blot, GPC (Gel Permeation Chromatography), DLS (Dynamic Light Scattering), AUC (Analytical Ultracentrifuge) and native electrophoresis.

It has been confirmed that PatZ catalyses its own acetylation, being reverted by the deacetylase CobB. Besides, the acetylated PatZ lysines have been identified. With respect to PatZ structural characterization, the results showed that Patz is a stable tetramer which oligomerizes to an octameric form via tetramer acetylation. Finally, an *in silico* PatZ tetramerization model is proposed and validated by 3D hydrodynamic analysis. The results reveal, for the first time, the structural regulation of an acetyltransferase by autoacetylation in a prokaryotic organism.

**Introduction**

Proteins can undergo chemical modifications, known as post-translational modifications. There are a lot of different post-translational modifications (PTMs), such as methylation, phosphorylation, glycosylation or acetylation. Protein lysine acetylation is an important post-translational modification which interest in prokaryotes has increased greatly (Pisithkul, Patel, & Amador-Noguez, 2015). *E. coli* is the main microorganism employed in biotechnology and the study of its metabolism has a great interest. It is known that many metabolic enzymes are acetylated in *E. coli* (Weinert et al., 2013), although the physiological importance of this modification has not been studied in detail. Acetylation has been traditionally described as a reversible PTM catalysed by an acetyltransferase (KAT) with acetyl-CoA as acetyl donor. These enzymes catalyse the transfer of an acetyl group from acetyl-CoA to a primary amine (Dyda, Klein, & Hickman, 2000). Besides, the most of these reactions can be reverted by deacetylases. The only known deacetylase in *E. coli* is the sirtuin CobB.

In *E. coli*, the N-acetyltransferase PatZ is the only enzyme known to be involved in post-translational acetylation of proteins. PatZ is a large acetyltransferase with 886 aminoacids (~98 kDa) belonging to the GCN5-related N-acetyltransferases (GNAT) superfamily. Many members of the GCN5-related N-acetyltransferases superfamilly are oligomeric proteins (Burk, Ghuman, Wybenga-Groot, & Berghuis, 2003).
The main objective of this study is to determine PatZ biochemical characteristics. This characterization will provide a deeper understanding of prokaryotic protein acetylation.

**Material and Methods**

**Construction of PatZ overexpression plasmid.** The 2658-bp patZ gene of *E. coli* BW25113 was PCR-amplified and cloned into the pRSETA plasmid (Invitrogen). CobB protein was overexpressed using the ASKA collection plasmid (Kitagawa, Ara, Arifuzzaman, & Ioka-nakamichi, 2005).

**Overproduction and purification of proteins.** Strains were transformed by heat shock. Cultures were grown overnight at 30°C with orbital shaking (200 rpm). The culture medium used was Terrific Broth (TB). The expression was induced with isopropyl-β-D-thiogalactopyranoside (IPTG). Proteins purification was carried out as described Castaño-Cerezo et al. (2014) (Castaño-Cerezo et al., 2014).

**SDS-PAGE and native electrophoresis.** Proteins were analysed by SDS/PAGE electrophoresis using a Mini-Protean cell (Biorad) or by native electrophoresis, employing NativePAGE Bis-Tris gels (Life Technologies).

**Detection of lysine acetylated proteins by western blot analysis.** Lysine acetylated proteins were detected using a primary rabbit monoclonal anti-acetyl-lysine antibody (ImmuneChem) and a goat anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology).

**Gel permeation chromatography (GPC).** Gel permeation analysis was performed with an HPLC (Agilent Technologies) using a Supelco Discovery Bio GFC 300 column (Sigma Aldrich). The elution of proteins was monitored by absorbance at 280 nm.

**Liquid chromatography tandem mass spectrometry assay (LC-MS).** Proteins were digested with 0.5-1 µg of Trypsin Gold Proteomics Grade (Promega). Tryptic peptides were separated and analysed by LC/MS system with an Agilent 1100 equipped with a Zorbax SB-C18 HPLC column and connected to an Agilent Ion Trap XCT Plus mass spectrometer (Agilent Technologies).

**Analytical ultracentrifugation (AUC).** Analytical ultracentrifugation experiments were performed in a Beckman Coulter Optima XL-I analytical ultracentrifuge (Beckman-Coulter), employing the UV–visible detection system. A wavelength of 280 nm was used in the absorbance optics. Sedimentation velocity (SV) was carried out at a rotor speed of 40000 rpm.

**Results and Discussion**

**Reversible PatZ auto-acetylation.** Recently, PatZ in vivo acetylation has been described (Castaño-Cerezo et al., 2014; Weinert et al., 2013). In this study, employing western blot, PatZ autoacetylation and deacetylation by CobB has been established (Fig. 1). Acetyltransferases auto-acetylation is a well-known phenomenon in eukaryotes, but PatZ autoacetylation is the first confirmed in prokaryotes.

To know the acetylated lysines in PatZ, a mass spectrometry assay of PatZ incubated with a saturating acetyl-CoA concentration (1 mM) was conducted. Six acetylated lysines were identified, five of which lie on the surface of the protein core (146, 149, 391, 446, and 635) and one in the catalytic (GNAT) domain (819).
With the purpose of knowing the changes induced in PatZ by acetylation, the molecular weight and sedimentation coefficient of the native protein was determined by analytical ultracentrifugation (Fig. 2A). The graph showed a main peak with $M \approx 446$ kDa. The results also showed two other minor but significant peaks, one with $M \approx 800$ kDa, and another with $M \approx 97$ kDa. These molecular weights corresponded with a PatZ tetramer, octamer and monomer, respectively.

To test PatZ structure when it was acetylated, a gel permeation chromatography was used (Fig 2B). In the absence of acetyl-CoA, PatZ eluted mainly at 8.2 min with an apparent molecular weight of 410 kDa which corresponds with a tetrameric form. In contrast, at a saturating acetyl-CoA concentration (1.5 mM) the main peak eluted at 7.3 min, which corresponds with an octamer.

Interconversion between PatZ oligomeric forms was monitored by native PAGE at different acetyl-CoA concentrations. In the absence of acetyl-CoA the tetramer was mainly observed but, when the acetyl-CoA concentration was increased, an octameric structure was formed. A western blot with an anti-acetyl-Lys antibody revealed that only the octameric form was acetylated (Fig. 3). These results confirm that the formation of the octamer occurred via tetramer acetylation and not from the monomer.
Figure 3. PatZ native western blot at different acetyl-CoA concentrations (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 1 mM).

Three-dimensional PatZ tetramer model. In order to propose a tetramer PatZ model, Phyre 2 server (Kelley & Sternberg, 2009) was used to predict the PatZ monomer structure, and ClusPro 2.0 server (Comeau, Gatchell, Vajda, & Camacho, 2004) to generate dimeric and tetrameric structures. The final tetramer model is shown in Figure 4. The tetramer model was employed to calculate, with the HYDROPRO version 10 (Ortega, Amorós, & García de la Torre, 2011), the theoretical sedimentation coefficient and the hydrodynamic radius. The results matched greatly with the experimental data determined by analytical ultracentrifugation (data not shown).

Figure 4. Proposed PatZ tetramer model. PatZ monomer is in dark.

In conclusion, this study demonstrates for the first time the structural regulation of an acetyltransferase by auto-acetylation in a prokaryotic organism, which represents a breakthrough in the prokaryotic protein modification studies.

References


