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A low resolution study of the *Bacillus subtilis* Ku protein: deciphering the DNA double strand break repair by the bacterial NHEJ pathway

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▶ KEYWORDS

DNA repair, Non Homologous end joining, DNA double strand break, SAXS, Ku, LigD.

The genome of each cell is constantly exposed to a variety of damaging factors. The DNA double strand breaks (DSB) is the most genotoxic DNA lesion and is a well-known causative event in several human diseases [1]. To survive such deleterious damages, cells have evolved two main DSB repair pathways: Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ). Importantly, the NHEJ is essential for some bacteria to survive under genotoxic conditions. Moreover, NHEJ is active in some important human pathogens, such as the causative agent of tuberculosis. Therefore it could be a target of choice for the development of new antibiotics, providing that the molecular mechanism of the NHEJ is understood.

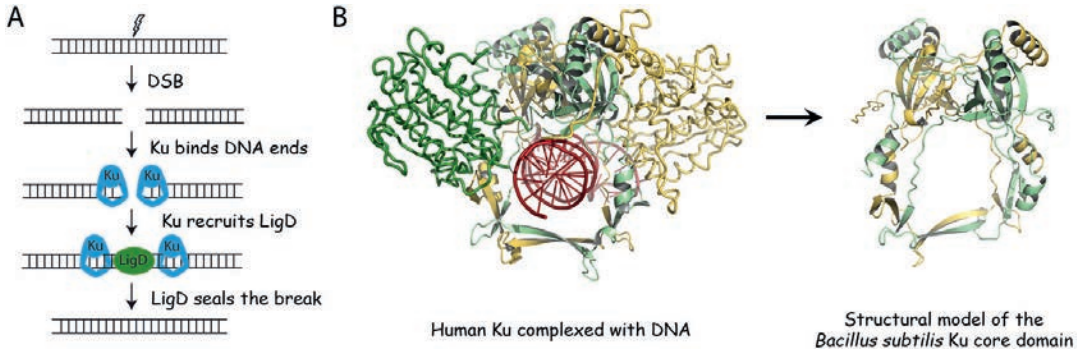
NHEJ is a ligation process requiring two proteins in bacteria: Ku and LigD [2]. A simple mechanistic model has been proposed so far: Ku homodimers bind DNA ends and recruit the ligase LigD to seal the lesion (fig. 1A). To test this model, we characterized the *Bacillus subtilis* Ku protein. We demonstrated that Ku binds to DNA ends and stimulates LigD, as expected, but is also able to thread inward linear DNA molecules after binding. We searched for Ku mutants to evaluate the role of this threading ability on the NHEJ efficiency. Bacterial Ku are composed of three domains (fig. 2A). The Ku core domain (fig. 1B) is conserved and required for DSB binding. The minimal Cter domain is also conserved in bacterial Ku and an extended C-terminal part added to this minimal Cter domain is frequently found in the gene sequences. Roles of the minimal and extended Cter domains remained unknown. We therefore characterized mutant Ku proteins truncated for the extended Cter (KuΔexCter) and for the complete C-terminal region (Ku core, fig. 2A). To ascertain that the different biochemical properties of these mutants compared to the full length protein were due to the different truncations and not to global structural rearrangements of the proteins, we characterized their low resolution structures by Small Angle X-ray Scattering (SAXS).

▶ MAIN RESULTS

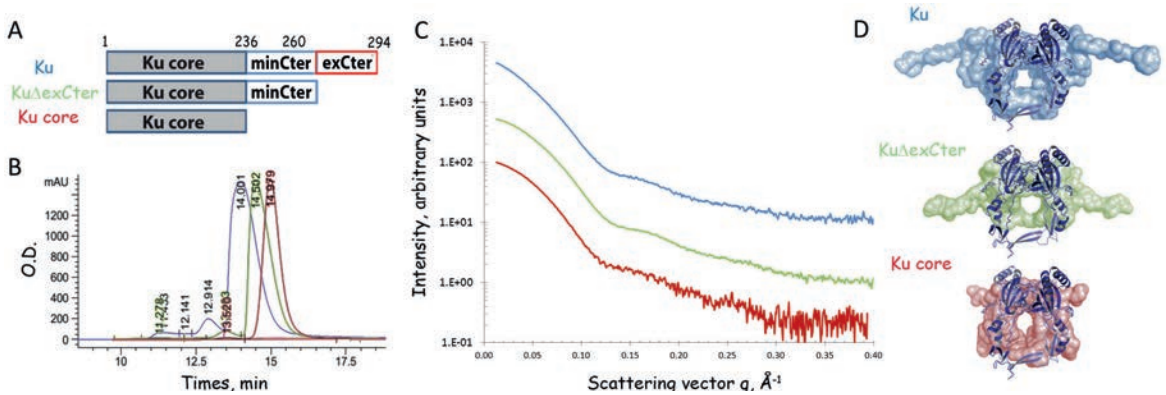
Three Ku proteins have been analysed by SAXS: the full length protein Ku and the two C-terminal truncated mutants. They were analyzed by size exclusion chromatography (fig. 2B) with an HPLC coupled to the SWING line to remove aggregates or other contaminants and to obtain a perfect subtraction of the solvent. SAXS data have been obtained for all Ku proteins (fig. 2C). These results, combined with the structural data available for the human Ku homolog, allowed us to propose a structural model for Ku and its derivatives in solution (fig. 1B and 2D). The conclusions of this study [3] were:

- all these proteins are dimers,
- the Ku protein displays a non globular shape with extended arms,
- the C-terminal domain of the homodimeric Ku maps in these extended arms, since these arms decreased or are absent in the truncated Ku mutants, and seems clearly available for interaction with DNA and/or protein in solution,
- the Ku structural model, based on the human Ku 3D structure, fits well with the globular shape of the bacterial Ku core suggesting a conserved core structure. This Ku core structure is not altered in the truncated mutants.

Since the two truncated mutants conserved a shape similar to the wild type Ku protein except the extended arms, we characterized further these mutants and demonstrated that the minimal Cter domain of Ku is required for its interaction with LigD and that the extended Cter domain i) is required for an efficient stimulation of the ligase activity, ii) decreases the ability of Ku to thread inward a DNA molecule and iii) allows the bridging of DNA molecules together [3].



▶ *Figure 1: A. The bacterial NHEJ model. Ku is in blue and LigD in green. DSB stands for DNA double strand break. B. The structural model of the* B. subtilis *Ku core domain. This model was built from the available 3D structure of the human Ku [4] and our experimental SAXS data obtained on the SWING beamline.*



▶ *Figure 2: Low resolution structures of the* B. subtilis *Ku and C-terminal truncated mutants derived from SAXS. A. Proteins used in this study and their domain composition are indicated. minCter and exCter stand for minimal and extended C-terminal domains, respectively. B. Elution patterns of Ku (blue), KuΔexCter (green) and Ku core (red) were obtained after loading on a size exclusion HPLC column coupled to the SWING beamline. C. Experimental scattering curves for Ku and its C-terminal mutants (colors as in B). D. Low resolution shapes of Ku, KuΔexCter and Ku core proteins. Shapes were generated by GASBOR (transparency sphere). The all atom model was calculated with Dadimodo (shown in cartoon) and are superimposed on the GASBOR calculated shapes.*

▶ CONCLUSION

Based on our observations, we propose that Ku binds DSBs via its Ku core domain and recruits LigD to its site of action via its minimal Cter domain. This recruitment would be aided by the high local concentration of Ku at the ends due to its ability to thread and accumulate preferentially at this site thanks to its extended Cter domain. Moreover the ability of this extended C-terminal part to promote bridging of DNA molecules should contribute to increase the efficiency of the ligase activity. Interestingly, the threading of Ku on DNA could also reveal new roles of Ku in the bacterial DNA repair. In conclusion a large set-up of biochemical and biophysical approaches was crucial for the progress of our understanding of the NHEJ molecular mechanism in bacteria. We currently used structural approaches, including the SAXS methodology, focused on the 3D structure and dynamic assembly of a whole NHEJ machinery.