The Da Vinci Code of TAP

Decoding the conformation of antigenic peptides bound to TAP by EPR spectroscopy

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The transporter associated with antigen processing (TAP) plays a key role in the adaptive immune defense against infected or malignantly transformed cells by translocating cytosolic peptides into the ER lumen for subsequent loading of major histocompatibility complex class I molecules. A prerequisite for peptide transport is peptide binding to TAP. TAP preferentially binds peptides with a length of 8–16 amino acids. However, peptides with a length up to 40 amino acids as well as sterically restricted peptides are recognized by TAP. This broad substrate spectrum of TAP suggests a structural flexibility of the substrate binding pocket.

In this study, we used electron paramagnetic resonance (EPR) spectroscopy to reveal conformational details of spin-labeled 9-mer and 15-mer peptides bound to TAP. The side chain information was derived from PROXYL spin probes covalently bound to engineered cysteines, whereas TOAC spin labeled peptides were introduced to detect the backbone flexibility and environment. Dependent on the spin probe's position in the peptide, relevant differences in affinity, side chain dynamics and micro-environment polarity were identified. The mobility of the side chains was strongly restricted at the termini of the peptide, while spin probes attached in the central region of the peptide were distinctly more mobile. In the case of 15-mer peptides, flexibility of side chains next to the anchoring sites was higher than for the 9-mer peptides. It seems that this increase in flexibility at the anchor positions of the 15-mer peptides explains the lower affinities for long peptides.

To explain the mobility pattern, double spin-labeled variants of the 9-mer and 15-mer peptides were engineered in order to determine interspin distances in the free and bound states and reveal the topology of the bound peptide by Double Electron Electron Resonance (DEER). The DEER traces deciphered an extended kink conformation of bound 9-mer and 15-mer peptides. The N- and the C-terminus of the bound peptide are separated by 2.2 nm, which can explain the length restriction of TAP to minimum 8-mer peptides and the possibility that a single transporter can recognize a broad peptide spectrum highly diverse in sequence and length.