The structures of T6, T3R3 and R6 bovine insulin: combining X-ray diffraction and absorption spectroscopy

The crystal structures of three conformations, T6, T3R3 and R6, of bovine insulin were solved at 1.40, 1.30 and 1.80 Å resolution, respectively. All conformations crystallized in space group R3. In contrast to the T6 and T3R3 structures, different conformations of the N-terminal B-chain residue PheB1 were observed in the R6 insulin structure, resulting in an eightfold doubling of the unit-cell volume upon cooling. The zinc coordination in each conformation was studied by X-ray absorption spectroscopy (XAS), including both EXAFS and XANES. Zinc adopts a tetrahedral coordination in all R3 sites and an octahedral coordination in T3 sites. The coordination distances were refined from XAS with a standard deviation of <0.01 Å. In contrast to the distances determined from the medium-resolution crystal structures, the XAS results were in good agreement with similar coordination geometries found in small molecules, as well as in other high-resolution insulin structures. As the radiation dose for XRD experiments is two orders of magnitude higher compared with that of XAS experiments, the single crystals were exposed to a higher degree of radiation damage that affected the zinc coordination in the T3 sites in particular. Furthermore, XANES spectra for the zinc sites in T6 and R6 insulin were successfully calculated using finite difference methods and the bond distances and angles were optimized from a quantitative XANES analysis.