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Unique sodium-caged structure of a potent endothelin-1 inhibitor: crystal structure of BQ123 sodium salt, *cyclo*(-D-Trp-D-Asp$^-$-Pro-D-Val-Leu-)·Na$^+$

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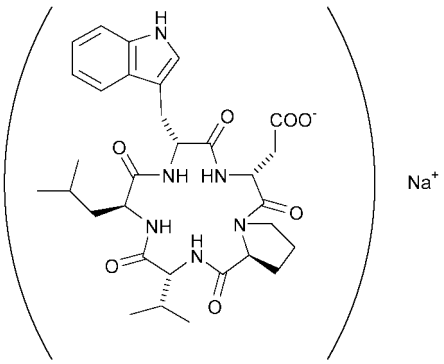
The crystal structure of *cyclo*(D-Trp-D-Asp$^-$-Pro-D-Val-Leu-)·Na$^+$, a potent endothelin-1 antagonist, showed four conformational isomers and two Na$^+$-caged structures in pairs.

Endothelin (ET) is an endogenous vasoconstrictor peptide with 21 amino acid residues.[1] Two cyclic peptapeptides of BE18257A and B, isolated from *Streptomyces misakiensis*,[2,3] antagonize the ET induced vasoconstriction by binding to the ET-A receptor subtype.[4] The peptide BQ123, *cyclo*(-D-Trp-D-Asp-Pro-D-Val-Leu-), was designed from these leading peptides (Scheme 1).[5,6] Many NMR studies have been carried out on ET,[7–11] and it was recently suggested[11] that BQ123 partially resembles the structure of ET-1 determined by X-ray diffraction.[12,13] In spite of such intensive approaches, no structures of ET antagonists have been elucidated in the solid state. We reported here, the crystal structure of the sodium salt of BQ123.† This is the first X-ray structural analysis of an ET antagonist.



Scheme 1

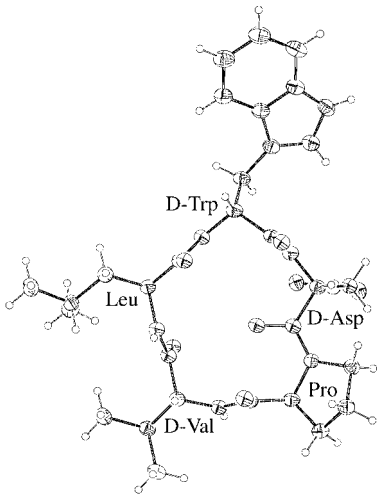


Fig. 1 Molecular conformation of BQ123. Since the conformations of four independent molecules are very similar to each other, only molecule 1 is drawn.

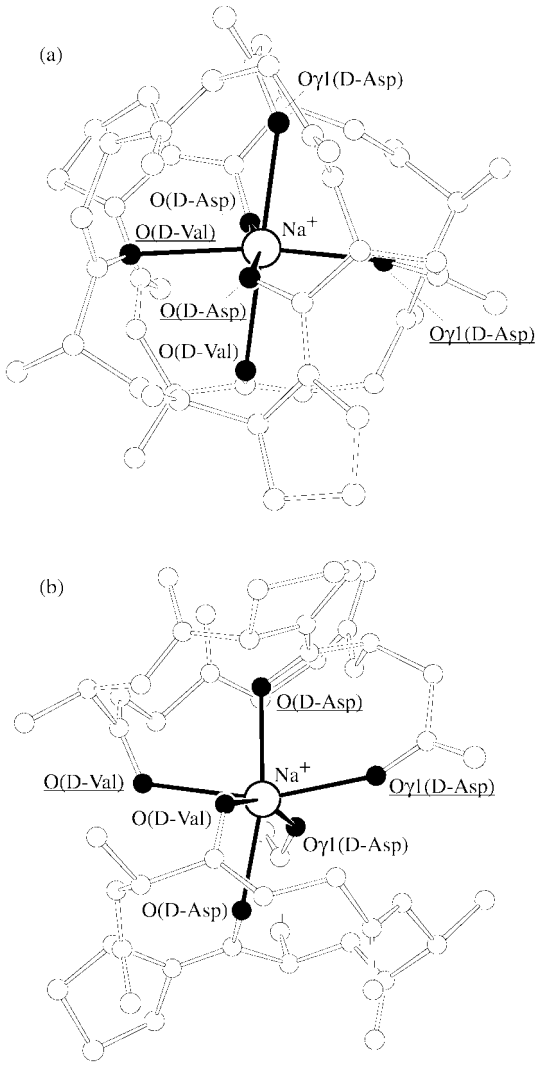


Fig. 2 A caged structure composed of two peptides and a Na$^+$ ion. (a) Top and (b) orthogonal side (rotated −90° for horizontal axis) views. Hydrogens and side chains of D-Val, Leu and D-Trp are omitted for clarity. Filled bonds and balls represent Na–O bonds and coordinated oxygen atoms, respectively. Oxygen atoms of independent molecules 1 and 2 are distinguished by underlined labels. This structure corresponds to one of two similar caged structures in the crystal.

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Four independent peptide molecules are located in an asymmetric unit. Conformational differences are observed in the side chain rotation of Trp and the puckering of Pro among the independent molecules, although their overall conformations are similar to each other; the rms deviations are 0.10–0.17 Å in the main chains. Fig. 1 shows the conformation of a single peptide molecule. The conformation of BQ123 is similar to the solution structure of the Na^+-free form in methanol and chloroform,[10] though some local differences are induced *via* the interactions with ions. The most prominent feature in the crystal is the Na^+-coordinated form sandwiched between two independent peptides in pairs, as shown in Fig. 2. The carbonyl oxygens (C=O) of D-Asp and D-Val and the carboxyl oxygens (COO^-) of D-Asp are coordinated to the Na^+ ion, forming an octahedral structure. In this form, the carboxyl and the carbonyl oxygens of the Asp residues are coordinated at the *cis* and *trans* positions, respectively, and these six Na–O bonds range in distance from 2.2 to 2.5 Å. Intermolecular hydrogen bonds are formed between the two BQ123 molecules wrapping the Na^+ ion (not shown in Fig. 2), and thus the peptide molecules form a unique caged structure specific for the Na^+ ion. Two similar caged structures are formed in the crystal, and are distinguished based on interaction modes with the solvents and the two remaining Na^+ ions forming either five- or six-coordinate bonds.

Although we surveyed the characteristics of the structures of ET-1 and the Na^+-ion and BQ123 complexes, no similarities were found, except for a number of amino acids (ET-1 is composed of 21 amino acids, and the total residue number of the four independent cyclic pentapeptides is 20). However, considering that both peptides selectively bind to the ET-A receptor, such differences and the unique structure of the sodium salt of BQ123 are important information for identifying the stereospecificity of the ET-A receptor.

Notes and references

† *Crystal data* for BQ123·Na: $4C_{31}H_{41}N_6O_7Na{\cdot}10H_2O{\cdot}8C_3H_7OH$, M = 3192.6, orthorhombic, space group $P2_12_12_1$, a = 25.3987(3), b = 34.7167(3), c = 25.7365(3) Å, V = 22693.4(24) Å^3, Z = 4, D_c = 0.934 g cm^{-3}, $F(000)$ = 6864, μ = 0.076 mm^{-1}, λ = 0.834 Å, T = 100 °K, 35804 independent reflections were used, θ_{max} = 31.42° (0.80 Å resolution). The molecular weight of BQ123·Na is > 3000 Da, and all attempts to solve the structure using the intensity data collected using commercial X-ray generators (Rigaku RU200/300) failed. The data were, therefore, measured using a Rigaku RAXIS-4 on a synchrotron, SPring-8/BL24XU-A (the figure-eight undulator and double-crystal monochromator system), with the approval of Hyogo prefecture and the Japan Synchrotron Radiation Research Institute (Approval No. C99A24XU-005N and 006N). The structure was finally solved by the electron density modification method using LODEM[14] and refined using SHELXL-97.[15] The solvent molecules were located by difference Fourier syntheses, and 8 isopropyl alcohol and 16 water molecules were found between the peptides. Some solvent sites were disordered: the calculated occupancies of two isopropyl alcohol molecules were 0.55 and 0.75, and those of 8 water molecules ranged from 0.16 to 0.36. Hydrogens of peptides were calculated in geometrically ideal positions by the 'ride on' method, and the hydrogens of solvent molecules were found from the Fourier map considering hydrogen-bonding networks. All hydrogens were included in the calculation of structure factors with isotropic temperature factors. A total of 2088 refinement parameters were divided into three blocks, and the positions and temperature factors were refined in a single refinement cycle. The $R1$ and wR values converged to 0.0845 and 0.2146, respectively, and goodness of fit = 1.044, $\Delta\rho_{max}$ = 0.572 e Å^3 and $\Delta\rho_{min}$ = −0.383 e Å^3.

CCDC 182/1579. See http://www.rsc.org/suppdata/cc/a9/a909413j/ for crystallographic files in .cif format.

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