Molecular shape of palytoxin in aqueous solution†

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Palytoxin, one of the most toxic non-peptide substances, formed an associated dimer of 5 nm length in aqueous solution.

Natural toxins with high specificity to their molecular targets have been contributing greatly to the study of the chemical biology of ion channels. A huge natural product palytoxin [Fig. 1, PTX (**1**): MW 2680, LD_{50} value of 450 ng kg^{-1} against mice],[1] isolated from marine organisms, is a typical example, and has an entirely unique function of converting Na/K pumps into non specific ion channels.[2]

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Conformational analysis generally gives important information on the action mechanism of biologically active natural products. X-Ray crystallographic analysis and NMR are usually good means to obtain the information. However, application of these methods to palytoxin meets difficulties, due to the non-crystalline nature of the compound, and heavy overlaps of signals in the NMR spectra due to its size.[3]

We have investigated the molecular shapes of palytoxin (PTX) and acetylpalytoxin [NAcPTX (**2**)][4] by X-ray small-angle scattering (SAXS). Small-angle X-ray scattering data reflect the time and ensemble average of molecular solution conformations. Palytoxin has an exceptionally large molecular weight for a natural product, but is actually too small to obtain a sufficient scattering intensity. Therefore, there was no precedent that used SAXS for a structural analysis of a natural product in solution. We overcame the problem by use of a synchrotron X-ray source and by a suitably designed SAXS camera.[5] The present paper describes representative conformation models that fit the SAXS data for PTX and NAcPTX.

We started with measurements of NAcPTX. All SAXS measurements were performed at the beamline BL45XU at SPring-8, Hyogo, Japan. The radius of gyration (R_g) and zero-angle scattering intensity ($I(0)/C$) of NAcPTX were estimated based

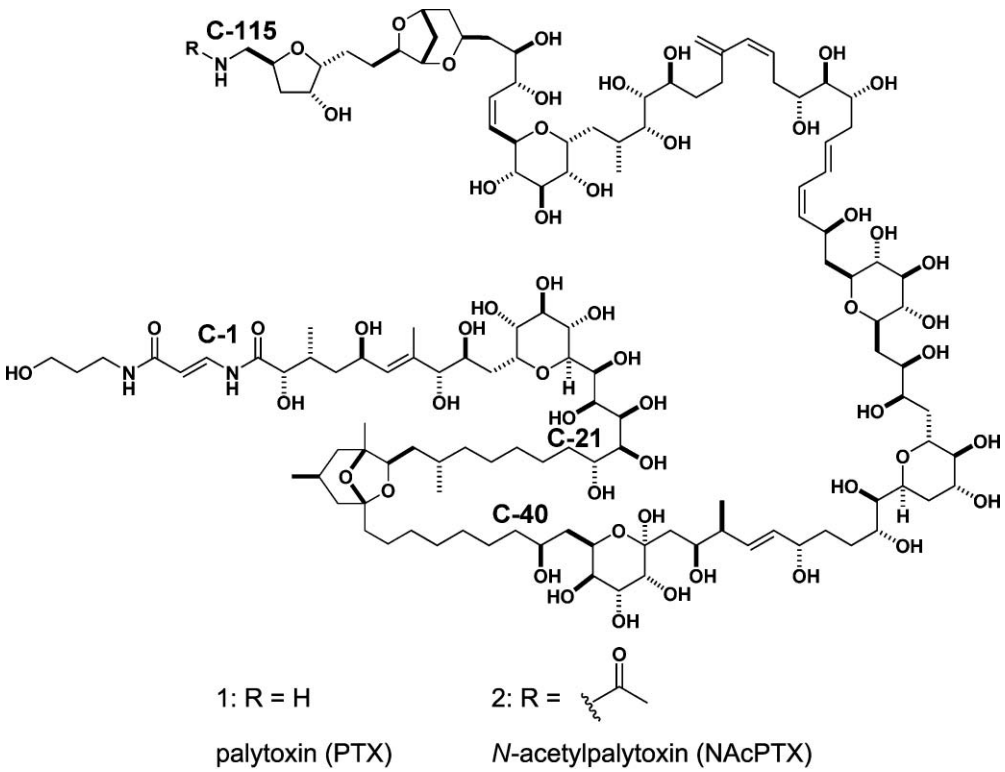

Fig. 1 Structures of PTX (**1**) and NAcPTX (**2**). C-1: Carboxylic acid terminal, C-115: amino terminal C-21–C-40 hydrophobic portion.

on the Guinier approximation (Fig. 2A). The concentration-scaled zero-angle scattering intensity, $I(0)/C$, is proportional to molecular weight. The molecular weight of NAcPTX was estimated to be 2600 based on the $I(0)/C$ values of NAcPTX and cytochrome C[6] (Fig. 2B). Since the molecular weight of NAcPTX ($C_{131}H_{225}N_3O_{55}$) is 2722, NAcPTX was considered to exist as a monomer in solution. The experimental R_g of 11.6 Å was greater than that of a hypothetical random flight chain ($R_g = 6.6$ Å) and much smaller than that of an extended straight chain ($R_g = 49$ Å). The NAcPTX chain appears to be more rigid than a hypothetical random flight chain. Based on the distance distribution function ($P(r)$), the maximum distance (D_{max}) of NAcPTX was about 35 Å (Fig. 3).

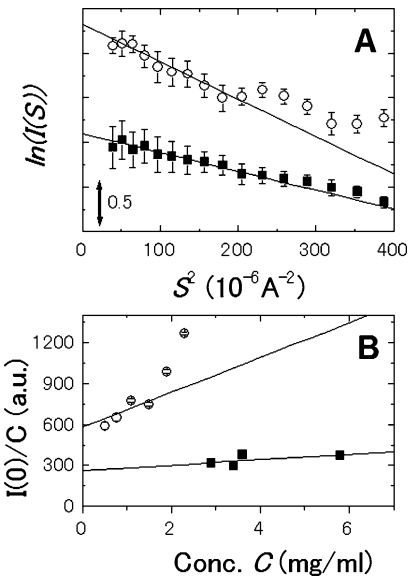

Fig. 2 (A) S^2 *vs.* $\ln(I(S))$ plot and $I(0)/C$ of PTX and NAcPTX. S is the function of the scattering angle, $S = (2\sin\theta)/\lambda$; 2θ is the scattering angle; λ is the wavelength of the incident X-ray. PTX and NAcPTX are indicated as open circles and closed squares, respectively. The plot of PTX was shifted by 0.5 for clarity. (B) Concentration-dependence of forward scattering, $I(0)/C$. C is the concentration of the solute (mg mL^{-1}). The figure legends are the same as above.

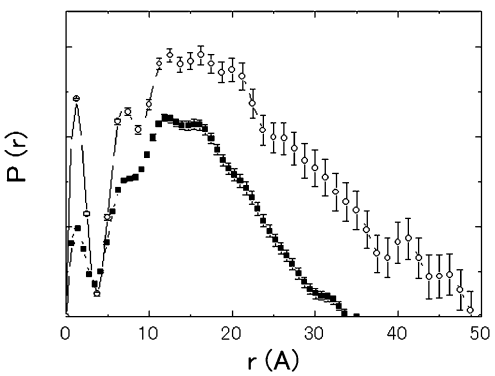

Fig. 3 Distance distribution functions, $P(r)$, of PTX (circles) and NAcPTX (squares) were calculated with *GNOM*.[7]

To investigate how NAcPTX extended in solution, we simulated low-resolution models of NAcPTX from SAXS profiles by using a modeling method with a chain-like ensemble of dummy residues.[7] The models that showed a good fit to the SAXS data were collected, and a representative model is shown in Fig. 4. These models should represent an average character of the solution structure of NAcPTX. The model (Fig. 4) measured 30.6 × 23.4 × 13.0 Å. It had an overall horseshoe-like shape, and half of the mass of the model was concentrated on one edge. This model is consistent with the chemical structure: the hydrophobic portion and the carboxylic acid terminus of PTX were expected to be folded in part[3] and the mass-concentrated bottom edge (Fig. 4) of the bead model would correspond to these regions.

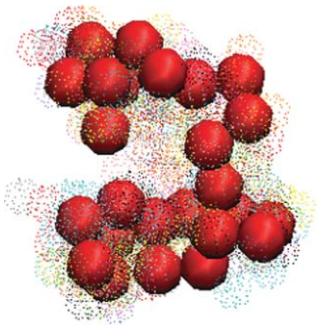

Fig. 4 A low-resolution model of NAcPTX in solution based on dummy-residues modeling using *GASBOR*.[7] Among the 10 best-fit models (dotted with different colors), the model with the most common feature was taken as the representative model (solid red). (see Supporting Information†) The figure was prepared using *VMD*.[7] The mass is concentrated along the bottom edge.

Similarly, SAXS measurements were performed for PTX. The molecular formula of PTX was determined to be 5700 from its $I(0)/C$ value[7] (Fig. 2B). Since the molecular weight of PTX ($C_{129}H_{223}N_3O_{54}$) is 2680, PTX was considered to exist as a dimer in solution. The R_g and D_{max} values were 17.7 Å and 50 Å, respectively (Fig. 2A, Fig. 3).

A low-resolution model was also simulated for PTX, and the result is shown in Fig. 5. This representative model had an overall shape of a prolonged oblate. It measured 52.3 × 22.0 × 15.1 Å. The dimension of the PTX model only differed from that of NAcPTX with regard to the long axis. This model appeared to indicate an associated dimer, in which each PTX units exists in a similar shape with NAcPTX.

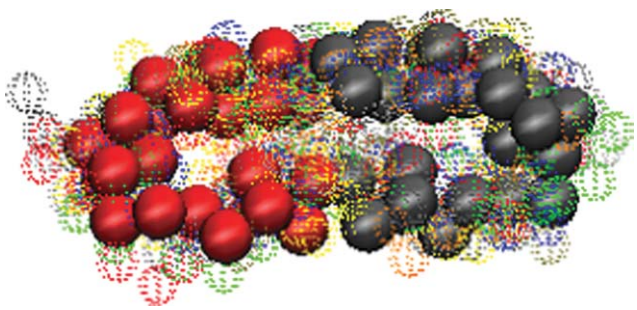

Fig. 5 Low-resolution models of PTX in solution based on dummy residue modeling using *GASBOR*.[7] The visualization method used is the same as that in Fig. 4.

Due to the limited resolution of SAXS, we could not determine the mode of interactions between the two PTX molecules at the

interface in the dimer. However, the only chemical difference between PTX and NAcPTX exists at a terminus, and thus electrostatic interaction through the amino group would be involved in the association at the interface.

In conclusion, we discovered that palytoxin formed an associated dimer in aqueous solution, and that a single site acetylation of a terminal unbinds the associated dimer. At the same time, the acetylation of PTX's amino terminus has been known to decrease its bioactivity to *ca.* 1/100.[8] Correlation between the dimer formation in water and the biological activity of palytoxin remains to be investigated, however, the results obtained from this study should be useful for elucidation of the molecular mechanism of action of palytoxin.

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6 We were not able to determine reliable partial specific volumes of either PTX or NAcPTX, due to the scarcity of the natural products. Thus, the partial specific volumes of PTX and NAcPTX were assumed to be identical to that of cytochrome C.

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