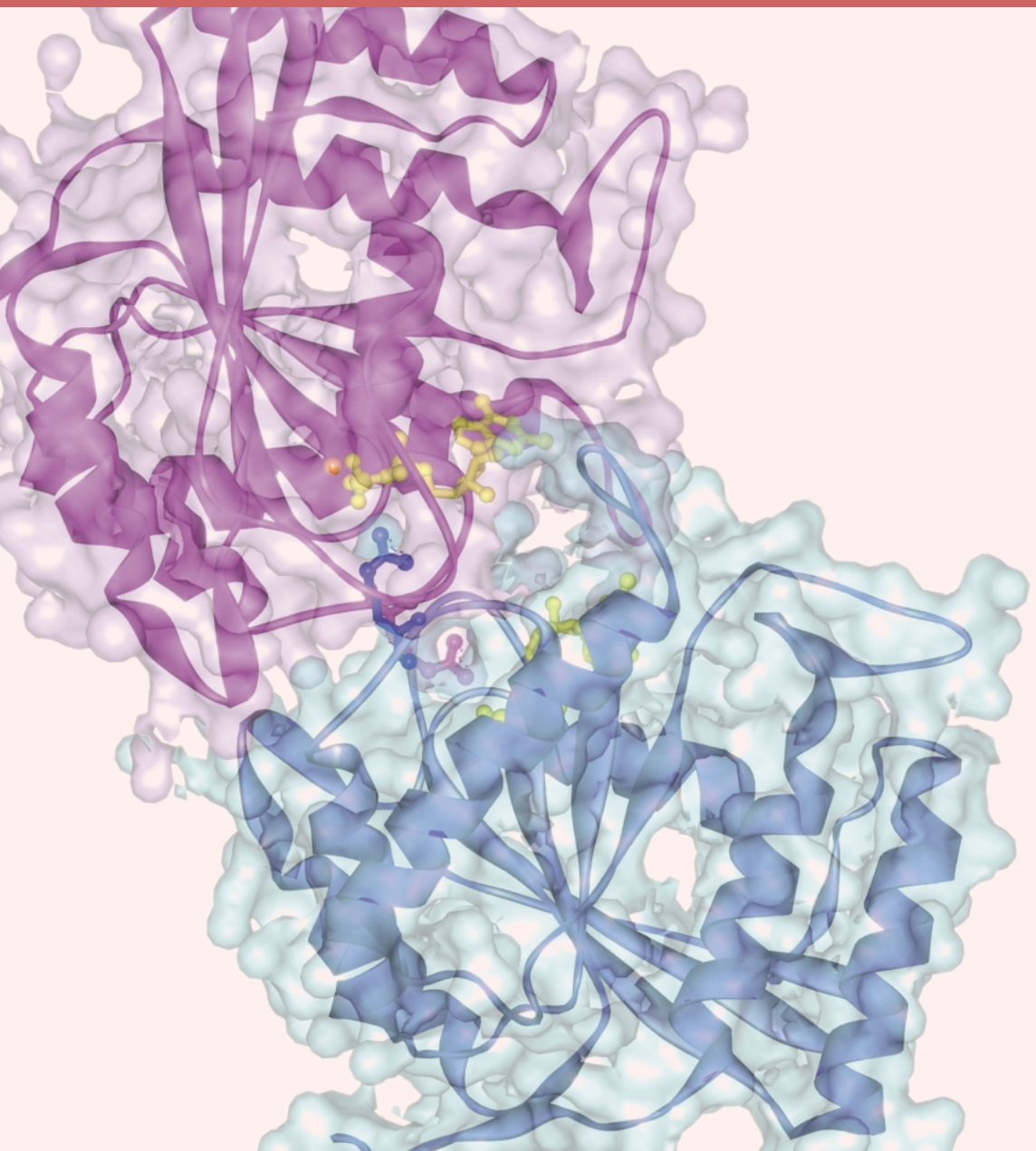
Highlights



7 Biological Science I

7-1 Solution Structure of Signaling Protein Grb2 Composed of Three Domains by Small Angle X-ray Scattering [1]

Small angle X-ray scattering (SAXS) from biological macromolecules in solution yields information on their overall shapes such as a radius of gyration and a distance distribution function, P(r). The P(r) function expresses pair wise distribution of inter-nuclei distances within scattering particles. Because P(r) depends on the geometric shape of scattering materials SAXS data are extremely sensitive to domain orientation and conformational change as well as molecular association in solution [2].

Most of proteins involved in intracellular signal transduction consist of multiple structural and functional domains. Signal transduction is mediated by the molecular recognition through functional domains of these molecules. The growth factor receptor-bound protein 2 (Grb2) is an adapter protein composed of a single SH2 domain flanked by two SH3 domains. Grb2 functions as an important evolutionary conserved link between a variety of cell membrane receptors and the Ras/MAP kinase-signaling cascade [3, 4]. We elucidated the solution structure of Grb2, in its entirety by SAXS.

The SAXS data of Grb2 was collected with the SAXS diffractometer at BL-10C. The P(r) was calculated from the X-ray scattering data using the indirect Fourier transform method. The X-ray scattering data were extrapolated to zero concentration. The calculated P(r) showed two peaks at about 20 Å and 40 Å and a skirt of the distribution curve extended to 80 Å (Fig. 1(a)). In order to interpret the experimental distribution, an ensemble of a total of 750 structures of Grb2 was

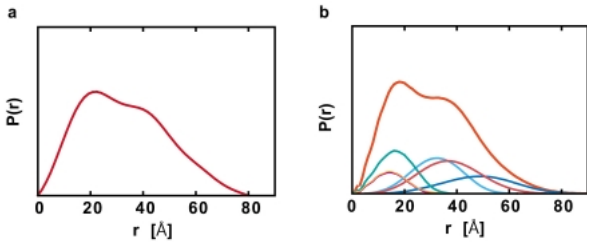


Figure 1
The distance distribution, P(r), of Grb2. (a) The P(r) calculated from X-ray scattering data using the indirect Fourier transform method. (b) The P(r) calculated from the average of the 750 solution structures. The contributions from intra and inter domains were calculated. Intra domain; nSH3 (yellow line), SH2 (green line) and cSH3 (purple line), inter domain; nSH3-SH2 (cyan line), nSH3-cSH3 (red line) and SH2-cSH3 (blue line).

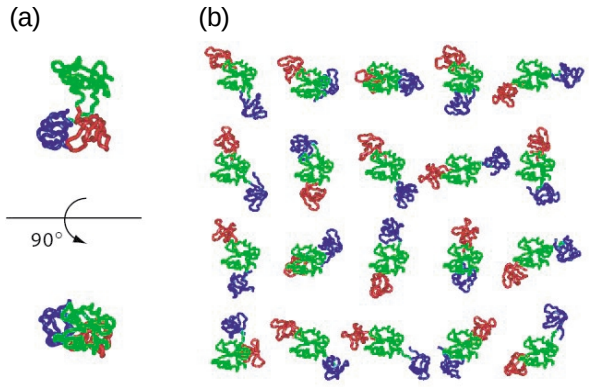


Figure 2
Structures of Grb2 in crystal and in solution. The left panel shows the side and top views of the crystal structure [5]. (a) The right panel shows 20 top view structures of Grb2 randomly selected from the ensemble. (b) The orientation of the SH2 domain is the same for all the structures so that the relative positions and orientations of the nSH3 and cSH3 domains can be compared.

generated based on the assumption that each domain maintained the core structure and the linker regions were flexible (Fig. 2). The average P(r) calculated from this ensemble of models was similar to the P(r) obtained from the solution scattering data. The P(r) calculated from the ensemble has two peaks at 18 Å and 38 Å, and the skirt extended to 80 Å (Fig. 1(b)). Major contribution to the extended skirt was found to be the distance distribution between SH2-cSH3 and nSH3-cSH3 domains. This inter-domain distance distribution calculated from the ensemble has a peak at 50 Å with a half width of 40 Å, indicating that the shape of the skirt is mainly determined by the distance distribution between two SH3 domains and the SH2 and cSH3 domains. On the basis of this analysis, it is concluded that in solution Grb2 exists as the ensemble of extended “open” conformations, not the “closed” conformation seen in the crystal structure [5].

Moreover, the ensemble of such extended “open” conformation is consistent with the model obtained from the NMR data [1]. The “open” conformation may have an advantege over “close” conformation enabling the adapter protein to find and interact with target protein more efficiently. The flexibility of adapter proteins containing some multiple recognition modules may play an important role in the control of their function.

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7-2 Enhancement of X-ray-Induced DNA Damage with DNA Bound Molecules Containing Platinum – Possible Application to Radiotherapy –

High energy photons induce various kind of DNA damage. The damage is derived from ionization and excitation of DNA itself or of surrounding materials such as water molecules. If a photon induces inner-shell ionization, Auger processes follow in the biological molecules, and the ejected electrons and/or multiply-charged ion might induce breakage of chemical bonds. The underlying idea is that the Auger effect could contribute a great deal to radiotherapy when the target molecule is selectively introduced into neoplastic tissues to preferentially kill tumor cells. A compound having a relatively heavy atom would be a candidate target molecule for radiotherapy, because of its large X-ray absorption coefficient. In this study, we adopted one of the platinum-containing molecules, chloroterpyridine platinum II (PtTC) (Fig. 3), which binds to DNA, and examined the possibility of its application to radiotherapy.

Figure 3 shows an absorption spectrum of PtTC around the L_{III} absorption edge of platinum. A resonant structure near the L_{III} edge was observed with its maximum located at E_{res} = 11562 eV. Irradiation with monochromatic X-rays at the resonance energy was performed at BL-27B.

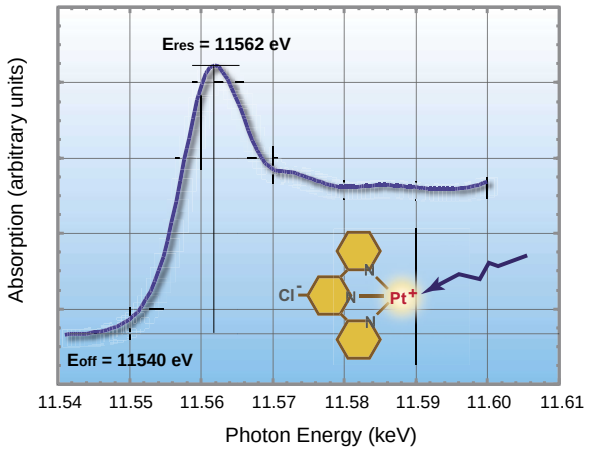

Figure 3
Molecular structure and X-ray absorption spectrum of PtTC.

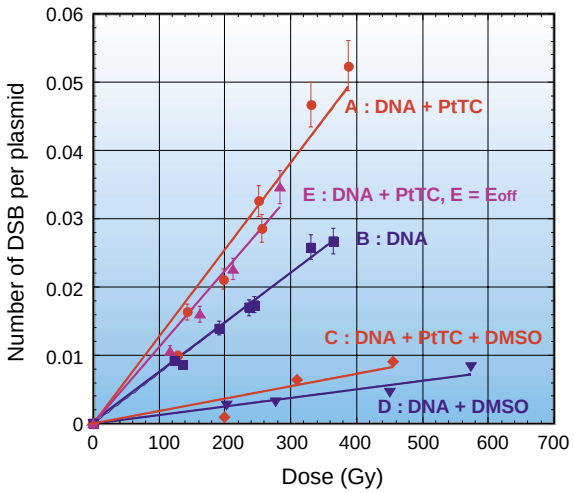

Figure 4
DSBs per plasmid induced with monochromatic X-rays.

Covalently closed circular DNA, pBR322 plasmid DNA, was used as a biological sample. The DNA solution was mixed with the PtTC solution to the ratio of the number of Pt atoms to the number of phosphorus atoms in DNA of 1/10, namely, one platinum atom in every 5 base-pairs in DNA. After irradiation, the numbers of single- and double-strand breaks were measured by a gel electrophoresis method. In this report, we focused mainly on DNA double strand breaks (DSBs), one of the most crucial kinds of DNA damage induced by ionizing radiation.

The numbers of DSBs per DNA molecule were plotted (Fig. 4) as a function of radiation dose under different conditions as below;

(1) in the presence and absence of PtTC (curves A and B) irradiated at the platinum L_{III} resonance absorption energy (E_{res} = 11562 eV)
(2) as above but in the presence of a radical scavenger, dimethyl sulfoxide (DMSO), to estimate the contribution of radical mediated damage (curves C and D)
(3) in the presence of PtTC irradiated off-resonance (E_{off} = 11540 eV) (curve E)

Enhancement of the DSB induction was observed when the plasmids contained PtTC. Even when off-resonant X-rays were used, DSB yields were higher than expected based upon the absorption cross section (for detail of the quantitative analysis, see ref. [1]). In addition, more than 85% of DSBs were scavengable by DMSO, which means more than 85% of DSBs were mediated by water radicals in the presence of PtTC. From these results, a mechanism could be suggested that the photoelectrons generated from the ionization of water efficiently ionize platinum atoms. Since the heavy atoms have a large collision cross section with electrons, these atoms located close to DNA likely behave as a

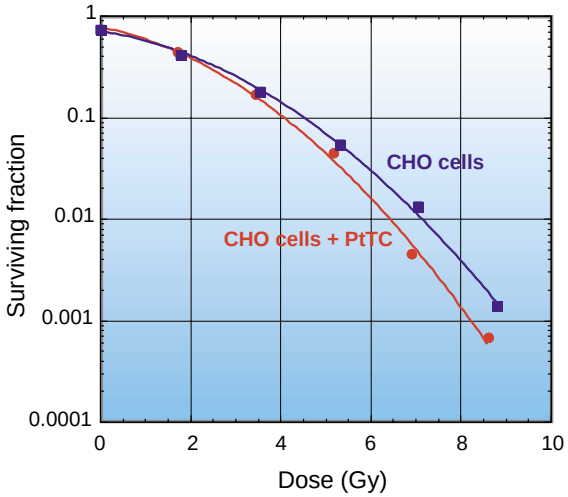

Figure 5
Surviving fraction of CHO cells irradiated at the platinum L_{III} resonance energy.

radiosensitizer, and hence the DNA-binding compounds containing heavy atoms have the potential to be a radiosensitizer in radiotherapy for the treatment of tumors.

Recently, we started an experiment with mammalian cells in order to demonstrate the potentiality of PtTC. Figure 5 displays the surviving fraction of Chinese hamster ovary (CHO) cells cultured in the medium supplemented with and without PtTC, irradiated at the resonant energy. An enhancement was clearly observed. This result indicates that this drug might be applicable to radiotherapy of tumors.

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References

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