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Comments on the paper by Kumagai, Hibino, Kawano and Sugiyama (1999) FEBS Lett. 450, 227–230

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We have discovered with great surprise in [1], the paper 'Mutation of the N-terminal proline 9 of BLMA from *Streptomyces verticillus* abolishes the binding affinity for bleomycin' by T. Kumagai et al. This work deals with BLMA, a resistance protein against the antibiotic bleomycin (Blm), and its results are the following.

1. The major result, cited in the title, deals with the influence of proline 9 of BLMA on its antibiotic-binding affinity.
2. This proline is necessary to ensure proper dimerization of BLMA.
3. According to the BLMA crystal structure, solved by the same group, but reported as 'submitted' in the present FEBS paper, this proline acts by promoting its mutual amino-end exchange, a strong determinant of the dimer stability.
4. This dimer shows two grooves which should correspond to two binding sites for the antibiotic.
5. Finally, the CD spectrum of a Pro-9-Leu mutant (unable to dimerize and to bind Blm) shows an important change in its secondary structure as compared to the wild-type protein.

We do not contest any of these results. We, on the contrary, fully agree with them because they turn out to be exactly the results that we had obtained and described with great details in two papers.

In the first paper in EMBO J., entitled 'Crystal structure and site-directed mutagenesis of a bleomycin resistance protein and their significance for drug sequestering' and published in 1994 [2], the first crystal structure of such a Blm resistance protein was presented. It is in this paper that (i) the dimeric structure was proven, (ii) that proline 9 was recognized as an essential determinant of dimerization by promoting a mutual arm exchange between the two monomers, (iii) that the dimer does (and not should) bind two Blm molecules and (iv) how these two Blm molecules should be symmetrically bound into the grooves' dimer. It was also clearly stated that the role of proline, as an oligomerization helper, was certainly not confined to this particular case.

In the second paper in Structure, entitled 'Proline-dependent oligomerization with arm exchange' and published in 1997 [3], we effectively showed that many other examples of critical prolines being oligomerization determinants exist. As we came to this generalization after our crystallographic and mutagenesis study on ShBle, we examined in more detail this particular case. We effectively showed in this second paper that a Pro-9-Gly mutant significantly lost its dimerization and Blm sequestering ability. We also showed by CD spectroscopy that, strangely enough, this mutant had lost its original secondary structure.

The only differences between our results and those of Dr M. Sugiyama and his group are two-fold. First, it is true that our work dealt with Shble (from *Streptoalloteichus hindustanus*), not BLMA. However, the two proteins are so much related by:

1. their sequence (58% identity and 73% similarity without gaps),
2. their origin: both are produced by two different *Streptomyces*, i.e. by species as related to each other among bacteria as two rodents among mammals,
3. their structure: compare Fig. 1 of [1] and Fig. 2a of [2], and
4. their function: they both are Blm resistance proteins by their ability of sequestering the antibiotic,

that it cannot be reasonably argued that the five 'new' results in [1] and ours in [2] and [3] are unrelated. Second, it seems that there is some difference between what we have shown in [3] about the Pro-9-Gly mutant and what is reported in [1], where the same mutant, once fused with a maltose-binding protein, retains a significant ability to protect bacteria against Blm. This is certainly a difference that would have been worth discussing in [1]. Otherwise, the only significant difference that can be mentioned is that our work was more complete. Where Dr Sugiyama and his group have looked at the effect of mutation of Pro-9 into Gly, Gln, Leu, Tyr and Ala, we looked, in addition to these, to the effect of Cys, Glu, His, Lys, Phe, Ser and Thr [2] and where they have suggested that BLMA should bind two Blm molecules, we have shown an experimental evidence for that and, also, how they could be bound.

Considering these facts, everybody will understand that we were extremely 'surprised' when we discovered the results in [1] without a single mention, not only, of Gatignol et al. [4], who were the first to characterize and purify such a resistance protein, but also of all our results in [2] and [3]. It is beyond any doubt that such an omission of citation of another's work was totally deliberate. For example, the two sentences 'Proline appears to play a key role in the β-strand of the hinge peptide that in bovine seminal ribonuclease A [5] and tumor necrosis factor [6] links the exchangeable N-terminal segment to its subunit body. Therefore, we hypothesized that the N-terminal proline 9 residue in BLMA may play a role as a hinge to form its dimeric structure' have been written to suggest to somebody really unaware of our work that the authors of [1] have unveiled some new feature of proline. But this fact was clearly

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stated in [2] and was the whole matter of [3] (from which the examples [5,6] have been 'borrowed' by Kumagai et al.). Another example is the following: 'The dimeric form, generated by the alternate arm exchange of the monomeric BLMA molecule, results in a large concavity and a long groove which may trap two molecules of Blm'. Again, this is nothing else then the result we showed in great detail in [2] (see Fig. 3 in particular), except that they mentioned 'a long groove' where we had seen two symmetrical crevices.

Everybody can have a look at the different papers and judge by himself.

References

[1] Kumagai, T., Hibino, R., Kawano, Y. and Sugiyama, M. (1999) FEBS Lett. 450, 227–230.
[2] Dumas, P., Bergdoll, M., Cagnon, C. and Masson, J.M. (1994) EMBO J. 13, 2483–2492.
[3] Bergdoll, M., Remy, M.H., Cagnon, C., Masson, J.M. and Dumas, P. (1997) Structure 5, 391–401.
[4] Gatignol, A., Durand, H. and Tiraby, G. (1988) FEBS Lett. 230, 171–175.
[5] Mazzarella, L., Capasso, S., Demasi, D., Di Lorenzo, G., Mattia, C.A. and Zagari, A. (1993) Acta Cryst. D49, 389–402.
[6] Eck, M.J. and Sprang, S.R. (1989) J. Biol. Chem. 264, 17595–17605.