Structures of the type I DNA restriction enzymes

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The article by Liu et al. (1) on the structure of type I DNA restriction and modification enzymes purports to significantly advance our understanding of these enzymes and proposes a model for their operation. While the partial structure of one of these enzymes is interesting and defines the interface between some of the subunits, the article contains many misinterpretations of the literature.

In 1968, these enzymes were the first restriction enzymes to be purified (2). It was soon apparent that they contained three subunits; R, M, and S for restriction, modification, and sequence specificity, respectively, in the complex $R_2M_2S_1$. A M_2S_1 complex could act as a modification methyltransferase. These complexes and partially assembled nonfunctional complexes, notably M_1S_1 and $R_1M_2S_1$, were stable in the absence of bound DNA. The relative proportions of the active and nonfunctional complexes depended on the source of the enzyme, and a set of equilibria for the assembly is therefore determined by thermodynamics (3). The equilibria are further complicated by the addition of DNA and cofactors. The structures of M_2S_1 and $R_2M_2S_1$ were recently presented (4, 5) and showed large conformational changes upon DNA binding (4–7). They also showed R and M interacting before DNA binding.

Liu et al. (1) propose a model requiring M_2S_1 to bind to DNA and only then for the R subunits to bind, an order contradicting all previous experiments. Their model is based upon size-exclusion chromatography and GST pull-down experiments on the well-studied EcoKI enzyme, an enzyme that is purified as $R_2M_2S_1$. Previously, size exclusion showed assembly of R with M_2S_1 in the absence of DNA (3), but Liu et al. (1) do not see this interaction, most likely because their M subunits contain N-terminal histidine tags that could weaken any interaction [it is known that the N terminus of M is in close proximity to R (4)]. It is noteworthy that neither of these experiments are at equilibrium and will easily miss weakened interactions.

The Liu et al. (1) structure does define the interface between the M and S subunits as being a four-helix bundle and they claim that this resolves the "confusing" situation in the literature of having the M subunit interacting with parts of the S subunit that show high sequence variation. The literature has long recognized that the interface between the M and S subunits uses the conserved sequences in the S subunit (e.g., refs. 8 and 9) to interact with the C terminus of the M subunit (10). This new structure simply adds atomic detail to this interface between M and S, which was already identified as a helical bundle in the structure of the EcoR124I enzyme bound to DNA (4).

Thus, the model of Liu et al. (1) is an unnecessarily constrained model based upon the crystallographic structure of part of a completely uncharacterized type I enzyme and a limited reading of the literature. The general model of Kennaway et al. (4) coupled with the variation in assembly due to variability in sequence of these enzymes is still valid.

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