Biological Science

10C/2003G144

Low resolution modeled structure of inactive recombinant glutamate dehydrogenase from *Pyrobaculum islandicum* by *ab initio* method

Shuichiro GODA[1], Yoshimi NISHIKAWA[1], Masaki KOJIMA[2], Haruhiko SAKURABA[1], Toshihisa OHSHIMA[1], Yuzuru HIRAGI[*3]
[1] The University of Tokushima, Tokushima 770-8506, Japan.
[2] Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 192-0392, Japan
[3]Kansai Medical University, Hirakata, Osaka 573-1136, Japan.

Introduction

The specific activity of recombinant *Pyrobaculum islandicum* glutamate dehydrogenase (GDH) expressed in *Escherichia coli* is much lower than that of the native GDH. However, upon heating the inactive recombinant GDH (iGDH) at 90˚C, the activity increases to a level comparable to that of the native GDH. Small-angle X-ray scattering (SAXS) measurements revealed that the radius of gyration of the hexameric iGDH was reduced to 47 Å from 55 Å by heat, though the molecular mass of the GDH was unchanged [1, 2]. Although the crystal structure of activated GDH (aGDH) has been described previously [3], the structure of the iGDH remains unknown. We modeled the low-resolution structure of the iGDH in an *ab initio* manner from the scattering curve.

Materials and Methods

Recombinant GDH was expressed in *E. coli*. Expression condition, purification procedure, SAXS measurements and analysis were described elsewhere [1, 2]. The modeling program, DAMMIN represents particle shape as an ensemble of densely packed beads inside a spherical search volume with diameter *Dmax* [4]. Starting from a random distribution of beads, simulated annealing is employed to find a compact configuration minimizing the discrepancy χ between the experimental and calculated scattering curves. Several independent reconstructions were carried out by randomly approximating the initial packing radii of the dummy atoms (r_o = 3-4 Å) to compute the average and the most probable models.

Results and Discussion

As DAMMIN permits modeling using symmetry restrictions on the solution, the low-resolution structure of the aGDH was restored by 32-point symmetry, like crystal structure [3], and was well fitted to the crystal structure (Fig. 1a,b). Then, structure of the iGDH was modeled no symmetry and 32-point symmetry (Fig. 1c,d). The structure of iGDH restored using 32-point symmetry differed substantially from that of the aGDH (Fig. 1c,d, top row), and appeared unlikely in terms of the subunit construction. On the other hand, the overall structure restored with no symmetry was similar to that of the aGDH (Fig. 1b,c). These results suggest that the no symmetry structure is more feasible for iGDH. The structure of iGDH has two features that distinguish it from the aGDH. The first is its lack of molecular symmetry. The aGDH has 32-point symmetry with one 3-fold axis passing through the two identical trimers, which face one another yielding a whole molecule with a cylindrical shape (Fig. 1a,b, bottom row) and one trimer stacking on the second with a symmetrical interface (Fig. 1a,b, top row). On the other hand, the structure of the iGDH has no symmetry; consequently, no subunit from one trimer superimposes on the corresponding subunit of the other (Fig. 1c, top row). The second structural feature of the iGDH is that it is not cylindrical, but is instead more spread out, especially at the surface of the molecule (Fig. 1c, middle row). It thus appears that not only is oligomerization of GDH necessary for enzymatic activity, but the proper arrangement of the subunits is also essential.

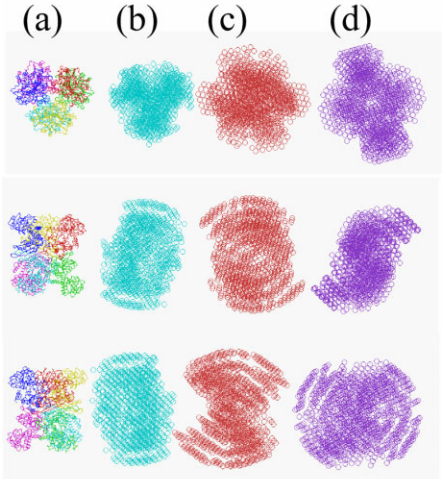

Figure 1. Crystal and low-resolution structures of GDH. (a) Crystal structure of aGDH. (b) Low-resolution structure of the aGDH with 32-point symmetry. Low-resolution structures of the iGDH with no symmetry (c) and 32-point symmetry (d).

References

[1] S. Goda et al., PF activity reprots 252 (2002).
[2] Y. Nishikawa et al., PF activity reports 236 (2003).
[3] M. W. Bhuiya, et al., J. Mol. Biol. 345, 325-337 (2005).
[4] D. I. Svergun, Biophys. J. 76, 2879-2886 (1999).
* yhiragi@yahoo.co.jp