E2-32: Structural studies on the NADP$^+$-linked glutamate dehydrogenase from *E. coli*

I S B Abeysinghe
(*Biochem Div, Tea Research Institute, Talawakelle*)

The structure of GDH from *E. coli* has been determined to 3Å resolution using the molecular replacement method with 2 different conformations of *C. symbiosum* GDH as a starting model. The structure has been refined by conventional least squares procedures to a R factor of 27.4%. Though the data used to determine the *E. coli* GDH structure was collected from the crystals grown in the presence of α-ketoglutarate, there was no clear density for α-ketoglutarate in the active site pocket, indicating that the structure represented that of an apoenzyme. The GDH structure is divided into an approximately N-terminal and C-terminal domain separated by a deep cleft and in the *E. coli* GDH structure relative orientation of the C-terminal domain in the 6 subunits in the asymmetric unit were distinctively different due to crystal contacts. Overall the clostridial and *E. coli* GDH structures are very similar and all the insertions and deletions lie on the exterior of the molecude. The data collected to 4.5Å resolution on the *E.coli* GDH-NADP$^+$ complex has led to the determination of the NADP$^+$ binding site of the *E. coli* GDH and the binding site which is clearly closely related to the *C. symbiosum* NAD$^+$ binding site. The position of the 2' phosphate can clearly be seen and preliminary analysis of this suggests direct interaction of carboxyl group of Asp 261 with one of the phosphate oxygens, implying that the phosphate group of NADP$^+$ is monobasic. The work on the structure determination of the two different forms of a potential glutamate complex of *E.coli* GDH has failed to trap an equivalent closed conformation of *E.coli* GDH to that seen in the clostridial enzyme. The widely different domain orientations seen in these studies suggest that the energy difference between the open and closed conformations of GDH is small and that in solution the two domains undergo at least partial change in domain orientation on a continuous basis.

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