Minireview

The allosteric regulation of pyruvate kinase

Andrea Mattevi[a],*, Martino Bolognesi[a,c], Giovanna Valentini[b]

[a]*Department of Genetics & Microbiology, University of Pavia, via Abbiategrasso 207, 27100 Pavia, Italy*
[b]*Department of Biochemistry, University of Pavia, via Taramelli 3b, 27100 Pavia, Italy*
[c]*Department of Physics and IST, Advanced Biotechnology Centre, University of Genova, Largo R. Benzi 10, 16136 Genova, Italy*

Abstract Crystallographic and mutagenesis studies have unravelled the general features of the allosteric transition mechanism in pyruvate kinase. The enzyme displays a dramatic conformational change in going from the T- to the R-state. All three domains forming each subunit of the tetrameric enzyme undergo simultaneous and concerted rotations, in such a way that all subunit and domain interfaces are modified. This mechanism is unprecedented since in all tetrameric allosteric enzymes, characterised at atomic resolution, at least one of the domain or subunit interfaces remains unchanged on the T- to R-state transition. The molecular mechanism of allosteric regulation here proposed provides a rationale for the effect of single site mutations observed in the human erythrocyte pyruvate kinase associated with a congenital anaemia.

Key words: Glycolysis; Metabolism regulation; X-ray crystallography; Pyruvate kinase

1. Introduction

The final step in the glycolytic pathway is the conversion of phosphoenolpyruvate (PEP) to pyruvate with the synthesis of one ATP molecule:

$$PEP + Mg - ADP + H^+ \xrightarrow{Mg^{2+},K^+} Mg - ATP + pyruvate$$
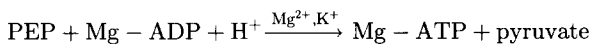
The reaction is catalysed by pyruvate kinase (PK), which requires for its activity both monovalent and divalent cations [1,2]. PK is a typical allosteric enzyme [3], and plays a major role in the control of the metabolic flux from fructose-1,6-bisphosphate (FBP) to pyruvate, in the second section of glycolysis (see Fig. 1). Furthermore, the reaction product pyruvate is involved in such a variety of metabolic pathways that PK may be considered a key enzyme not only for the glycolytic pathway but also for the entire cellular metabolism.

PK from prokaryotes is normally activated homotropically by PEP and heterotropically by sugars bearing either one (i.e. ribose-phosphate) or two (i.e. FBP) phosphate groups. FBP is also the activator of nearly all characterised eukaryotic PK molecules, even though in trypanosomes the enzyme activator is fructose-2,6-bisphosphate [4]. More sophisticated is the control in mammals, where four PK isoenzymes are expressed in a tissue-specific manner [3,5]: M1 PK present mostly in the skeletal muscle; M2 PK in many tissues such as kidney, intestine, lung fibroblasts, testis, and stomach; L PK mostly in the liver; R PK exclusively in the red blood cells. The FBP-dependent M1 and R isoenzymes share allosteric properties similar to those of the other eukaryotic PK molecules,

whereas the L PK is additionally controlled by phosphorylation on a N-terminal Ser residue which causes a decrease in the affinity for the PEP and FBP activators [3]. More unusual is the muscle M1 protein [6] which is the only known PK displaying hyperbolic kinetics and no allosteric control.

While the structure of the cat M1 isoenzyme was determined by Muirhead and coworkers several years ago [6], little information was available about the allosteric transition in PK. Substantial progress in this respect has been observed over the last few years with further refinement of the M1 isoenzyme model [7], and with the X-ray structure determination of the FBP-dependent *E. coli* PK, in the inactive T-state [8]. Furthermore, insight into the enzyme regulatory properties has been provided by mutagenesis studies [9] and by the characterisation of several R PK variants from patients with congenital anaemia [10].

2. Overall architecture

The X-ray diffraction studies on cat M1 PK [6] have revealed the complex molecular architecture of the enzyme, which is a tetramer of four identical subunits, each encompassing three domains (see Fig. 2A,B): (i) the A domain, with the classic $(\beta/\alpha)_8$ barrel topology observed in several unrelated proteins; (ii) the small B domain, characterised by an irregular
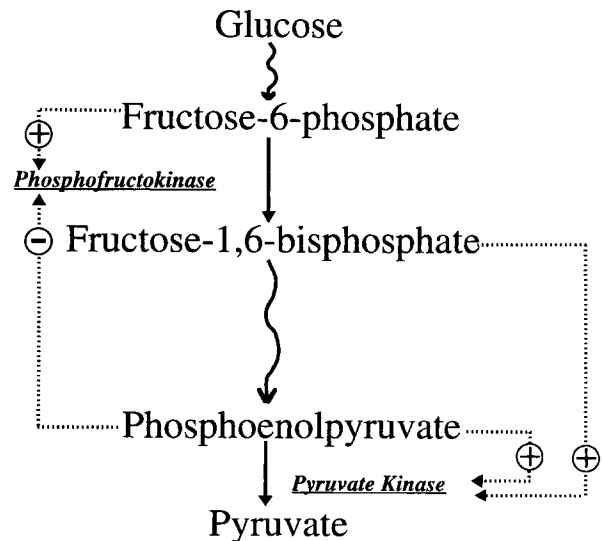

Fig. 1. The role of pyruvate kinase in the regulation of glycolysis. PK controls the second part of the pathway being activated by phosphoenolpyruvate and fructose-1,6-bisphosphate. This metabolite is produced in the reaction catalysed by phosphofructokinase, the other major regulatory glycolytic enzyme, whose activity is inhibited by phosphoenolpyruvate and activated by fructose-6-phosphate.

*Corresponding author. Fax: (39) (382) 528496.

β barrel; (iii) the C-terminal C domain with a α/β topology. A fourth small N-terminal domain, absent in PK from bacteria, is formed by a helix-turn-helix motif. The recent X-ray analysis of the rabbit M1 protein in complex with pyruvate based on crystals grown from polyethylene glycol [7] has identified

the residues forming the PEP and cation binding sites. These are located in the cleft between the A and B domains, and are mostly provided by the sixth and eighth loops of the A domain $(\beta/\alpha)_8$ barrel (Fig. 2A).

In the functional enzyme the four subunits are intimately

A

A243 (A336)
I221 (I314)
L65 (L155)
I47 (I131)
L50 (V134)
I275 (V368)
G239 (G332)
K266 (R359)

Catalytic site
Loop 6

T291 (T384)
M328 (Q421)
A299 (A392)
E333 (R426)

I268 (N361)
K413 (R510)
K382 (R479)
K389 (R486)
T401 (R498)
A398 (A495)
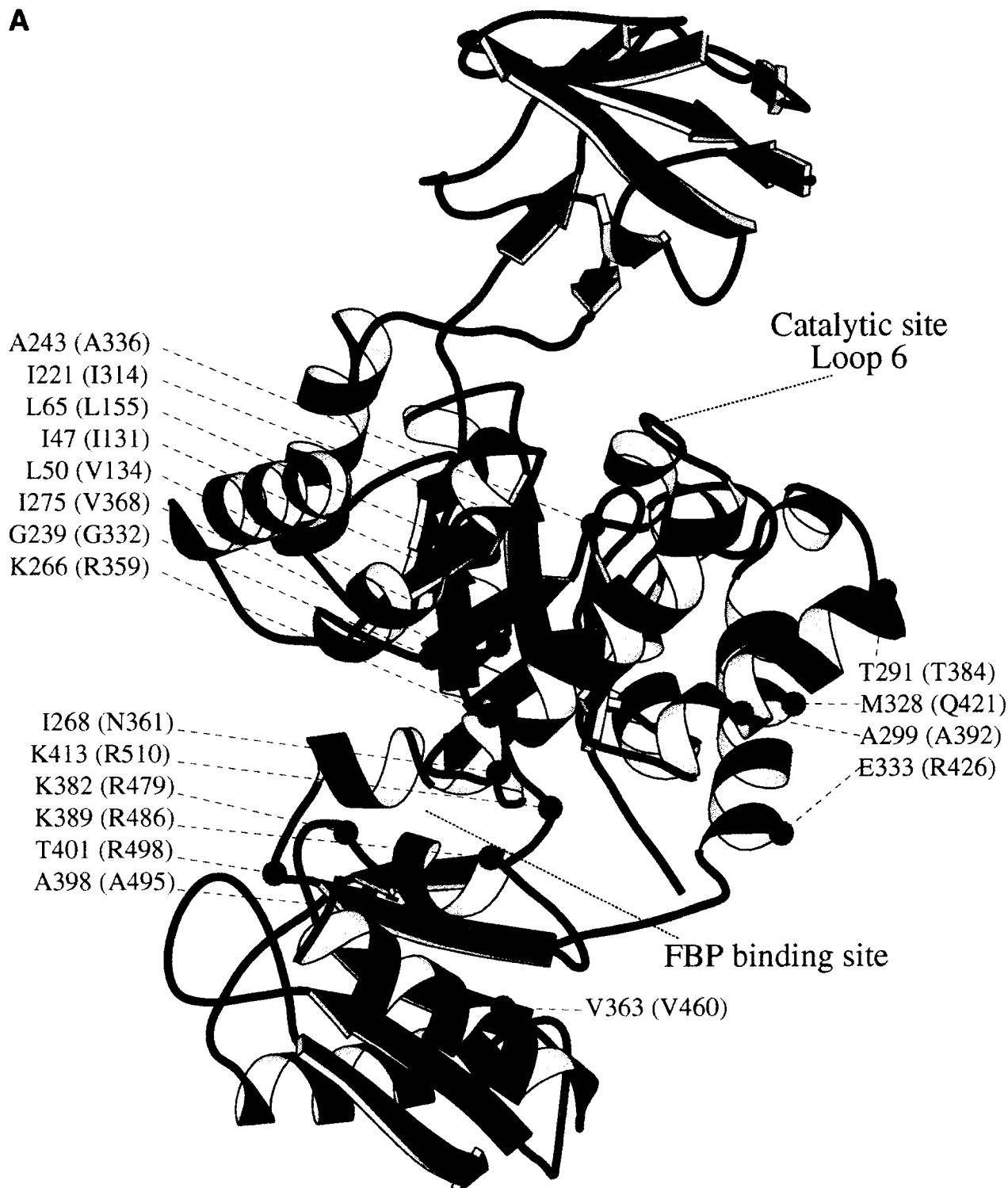
FBP binding site

V363 (V460)

Fig. 2. (A) The subunit of the *E. coli* T-state PK [8]. Domains A, B and C are shown in green, blue and red, respectively. The spheres outline the residues whose mutation in the human R PK causes the non-spherocytic haemolytic anaemia ([16–18] and references therein). The amino acid sequence identity between human R and *E. coli* PK is 51%. The sequence number of the R PK residues is shown in brackets. (B) Subunit arrangement in the tetrameric *E. coli* T-state PK (left) and the M1 R-like PK (right). The domains are coloured as in (A). The small helix-turn-helix N-terminal domain of the M1 protein is depicted in yellow. Produced with MOLSCRIPT [23].
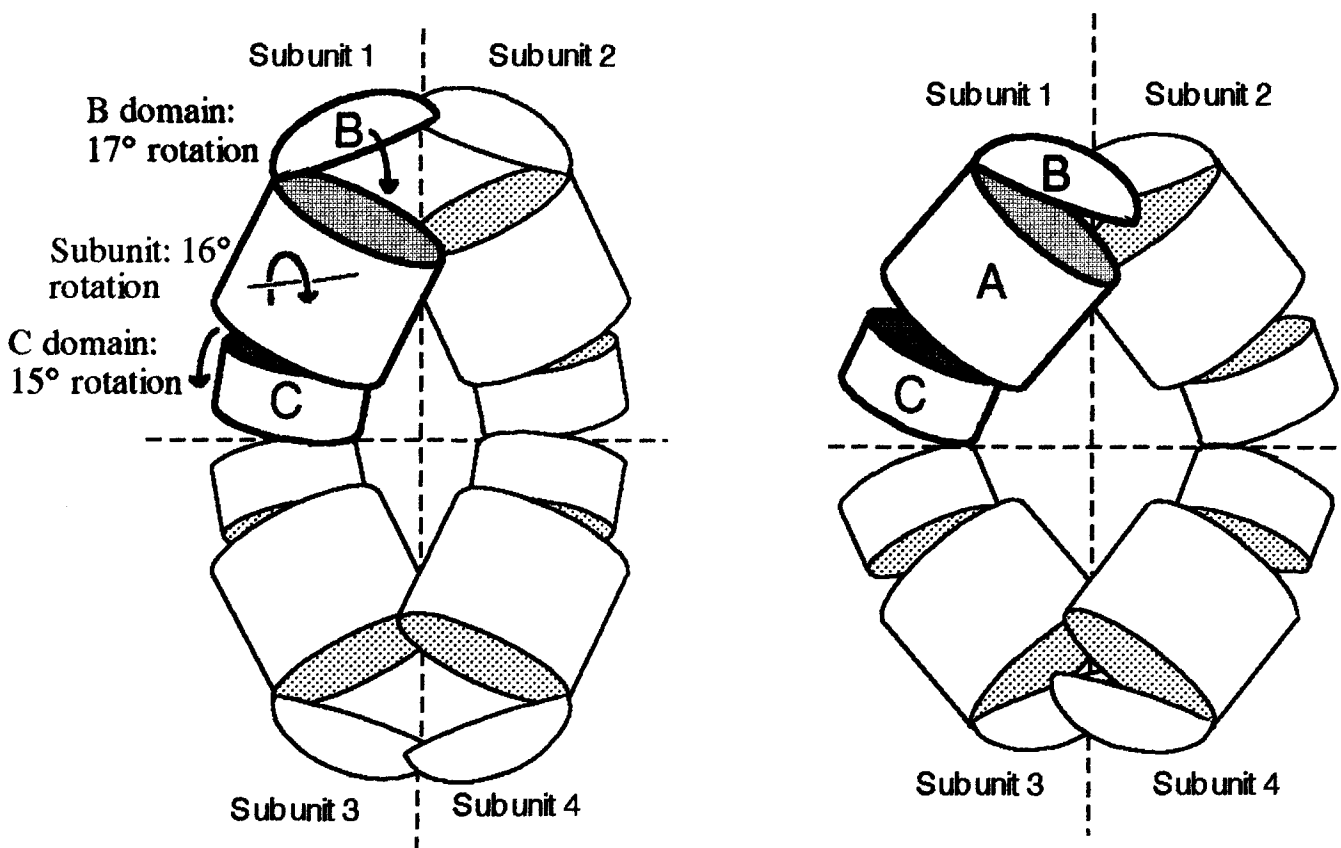
B

Fig. 2. (continued).

Fig. 3. Schematic representation of the domain and subunit rotations occurring on the T- to R-state PK transition. The T-state structure is on the left.

associated to form a tetramer of 222 symmetry. Extensive interactions mainly between the A and C domains of two opposite subunits stabilise the oligomeric assembly (Fig. 2A,B).

3. The allosteric transition

M1 PK is not allosterically regulated such that the enzyme is thought to be a sort of allosterically locked 'natural' mutant adopting an active R-like conformation [11]. This is supported by the fact that the non-allosteric M1 and the allosteric M2 isoenzymes, which derive from alternative splicing of the same precursor RNA [12], differ in their primary structures only for a stretch of 45 amino acids, 21 of which are substituted. On this basis, examination of the recently determined T-state *E. coli* PK structure, and its comparison with the M1 R-state conformation [8], has allowed us to analyse the general features of the allosteric transition mechanism. Particularly, it has revealed that PK undergoes a dramatic conformational change on switching from the low affinity T-state to the high affinity R-state conformation (Fig. 2B). A combination of two kinds of movements is observed: (i) the rotation of the B and C (17° and 15°, respectively) domains within every subunit, and (ii) a 16° rotation of every subunit within the tetramer (Fig. 3). The enzyme activation process, therefore, appears to involve a combination of domain and subunit rotations, suggesting that the PK tetramer can be portrayed as a modular protein in which all 12 domains are capable of undergoing highly concerted motions. This notion is further supported by the observation that the structure of the individual domains is not significantly altered by their movements, im-

plying that they rotate as rigid bodies and are connected to each other by highly flexible hinges.

The determination of the *E. coli* PK T-state structure has been hampered by an intrinsic non-isomorphism of the crystals [8], which, even when grown in the same experiment, may exhibit large variability in their X-ray diffraction pattern. Analysis of the numerous X-ray diffraction data sets collected during structure determination has revealed that the B-domain varies its orientation from crystal to crystal, thereby causing non-isomorphism. In this respect, variability of the B domain orientation has been observed also in the cat [6] and rabbit [7] M1 PK structures, further emphasising the enzyme flexibility and modularity, two key properties which are at the heart of the regulatory mechanism. Moreover, these independent observations suggest that the T- and R-states probably represent, in PK, an ensemble of conformations rather than a single well defined structure. In keeping with this hypothesis, fluorescence quenching experiments on yeast PK have led to a model for cooperativity which incorporates an intermediate state R', with properties different from those of the T- and R-states [13].

4. Catalytic centre and effector binding site

The comparison of the M1 R-state and *E. coli* T-state PK structures [8] shows that domain and subunit rotations are coupled to a defined conformational change in the enzyme active site. Upon R- to T-state transition, loop 6 of the A domain $(\beta/\alpha)_8$ barrel (Fig. 2A) shifts by about 1 Å, triggering a distortion of the PEP binding site, which is the likely cause

of the low substrate affinity of the T-state enzyme (for details see [8]). In a similar way, the effector binding pocket undergoes a structural transition. Mutagenesis and chemical modification [9,14] studies have revealed that the enzyme activator binds in a cleft between the A and C domains, characterised by a cluster of positively charged residues. In the low affinity T-state, upon rotation of the C domain, the cleft shrinks preventing effector binding. A better characterisation of the active and allosteric site conformational changes, and of their coupling to the domain rotations, must of course await the structure determination of the R-state PK in complex with activator and with the PEP and ADP substrates. These structural studies will be complemented by site-directed mutagenesis experiments aimed at the creation of mutants with altered allosteric properties [15]. The genes of PK from *E. coli* (Valentini et al., unpublished), yeast [13] and *B. stearothermophilus* [9] have been cloned and overexpressed, paving the way for this approach.

5. Molecular abnormalities and anaemia

Deficiency of the human erythrocyte R PK is the most common cause of the hereditary non-spherocytic haemolytic anaemia [10], a congenital disease with severe clinical manifestations, leading to occasional death in the neonatal period. Over the past years, the PK gene of many patients has been sequenced, revealing that in most cases a single site mutation is causing the enzyme deficiency ([16–18] and references therein). The effect of many of these pathological PK mutations can now be rationalised on the basis of the described allosteric transition mechanism. In fact, the amino acid substitutions so far detected appear to cluster in three well defined regions of the structure (Fig. 2A): (i) the hydrophobic core of the A domain $(\beta/\alpha)_8$ barrel; (ii) the A domain helices 6, 7 and 8, which are involved in the quaternary structure intersubunit association; and (iii) the interface between the A and C domains, where the effector binding site is located. The most frequent pathological mutation $(Arg^{510} \rightarrow Gln, R$ PK numbering) occurs in this third region and causes an affinity decrease for FBP and PEP, but not for the non-allosteric ADP substrate, suggesting that the mutation primarily affects the equilibrium between the R and T forms. In this context, it is remarkable that the severity of the anaemia appears to be correlated with the level of impairment in the allosteric response, rather than with the decreased catalytic efficiency of the mutated enzyme [17,18]. These data clearly emphasise the centrality and pivotal role of PK in the regulation of the cellular metabolism.

6. Conclusions

The molecular mechanism for the allosteric transition mechanism of PK is unique in that it involves the simultaneous rotation of 12 domains and the consequent alteration of all domain and subunit interfaces. Such a mechanism is unprecedented since in all tetrameric cooperative proteins, of known three-dimensional structure, at least one of these interfaces remains unmodified on allosteric activation [19]. In most cases, like tetrameric fructose-1,6-bisphosphatase [20] or phosphofructokinase [21], the transition is based on the rotation of one dimer with respect to the other, such that the intersubunit contacts within each of the two rotating bodies are preserved.

Also the haemoglobin R- to T-state transition proceeds, at least in general terms, through this mechanism [19]. More complex is the case of bacterial lactate dehydrogenase [22], which displays rotation of the four subunits, without, however, any significant mutual reorientation of the two domains forming each monomer.

The large domain and subunit rotations observed in PK represent a striking example of a flexible protein architecture developed by nature for the purpose of complex and strict enzyme regulation. More details of the mechanism by which this large conformational change is coupled to the alteration of the active and allosteric sites remain, however, to be elucidated. Consideration of the available experimental evidences suggests that we may have sampled only few of the possible enzyme conformations: PK may well reserve more twists and turns for the future.

References

[1] Kayne, F.J. (1973) in: The Enzymes (Boyer, P.D., Ed.), 3rd edn., vol. 8, pp. 353–382. Academic Press, New York.
[2] Gupta, R.K., Oesterling, R.M. and Mildvan, A.S. (1976) Biochemistry, 15, 2881–2887.
[3] Fothergill, L.A. and Michels, P.A. (1992) Progr. Mol. Biol. Biophys. 59, 105–227.
[4] Flynn, I.W. and Bowman, I.B.R. (1981) Mol. Biochem. Parasitol. 4, 95–106.
[5] Hall, E.R. and Cottam, G.L. (1978) Int. J. Biochem. 9, 785–793.
[6] Muirhead, H., Clayden, D.A., Barford, D., Lorimer, C.G., Fothergill-Gilmore, L.A., Schiltz, E. and Schmitt, W. (1986) EMBO J. 5, 475–481.
[7] Larsen, T.M., Laughlin, L.T., Holden, H.M., Rayment, I. and Reed, G.H. (1994) Biochemistry 33, 6301–6309.
[8] Mattevi, A., Valentini, G., Rizzi, M., Speranza, M.L., Bolognesi, M. and Coda A. (1995) Structure 3, 729–741.
[9] Walker, D., Chia, W.N. and Muirhead, H. (1992) J. Mol. Biol. 228, 265–276.
[10] Valentine, W.N., Tanaka, K.R., Paglia, D.E. (1989) in: The Metabolic Basis of Inherited Disease (Beaudet, A.L., Sly, W.S., Valle, D., Eds.), p. 2341. McGraw-Hill, New York.
[11] Consler, T.G., Woodard, S.H. and Lee, J.C. (1989) Biochemistry 28, 8756–8764.
[12] Noguchi, T., Inoue, H. and Tanaka, T. (1986) J. Biol. Chem. 261, 13807–13812.
[13] Murcott, Th.L., Gutfreund, H. and Muirhead, H. (1992) EMBO J., 11, 3811–3814.
[14] Speranza, M.L., Valentini, G., Iadarola, P., Stoppini, M., Malcovati, M. and Ferri, G. (1989) Biol. Chem. Hoppe-Seyler 370, 211–216.
[15] Collins, R.A., McNally, T., Fothergill-Gilmore, L.A. and Miurhead, H. (1995) Biochem. J. 310, 117–123.
[16] Lenzner, C., Nurnberg, P., Thiele, B.J., Reis, A., Brabec, V., Sakalova, A. and Jacobasch, G. (1994) Blood 83, 2817–2822.
[17] Kanno, H., Ballas, S.K., Miwa, S., Fujii, H. and Bowman, H.S. (1994) Blood 83, 2311–2316.
[18] Kanno, H., Wei, D.C., Chan, L.C., Mizoguchi, H., Ando, M., Nakahata, T., Narisawa, K., Fujii, H. and Miwa, S. (1994) Blood 84, 3505–3509.
[19] Perutz, M.F (1989) Mechanism of Cooperativity and Allosteric Regulation in Proteins, Cambridge University Press, Cambridge.
[20] Zhang, Y., Liang, J.-Y., Huang, S. and Lipscomb, W.N. (1994) J. Mol. Biol. 244, 609–624.
[21] Schirmer, T. and Evans, P.R. (1990) Nature 343, 140–145.
[22] Iwata S., Kamata, K., Yoshida, S., Minowa, T. and Ohta, T. (1994) Nature Struct. Biol. 1, 176–185.
[23] Kraulis, P.J. (1991) J. Appl. Crystallogr. 24, 946–950.