**Dichotomic Phylogenetic Tree of the Pyruvate Kinase Family**

**K⁺-DEPENDENT AND -INDEPENDENT ENZYMES**

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K⁺ dependence was assumed to be a feature of all pyruvate kinases until it was discovered that some enzymes express K⁺-independent activity. Almost all the K⁺-independent pyruvate kinases have Lys at position 117, instead of the Glu present in the K⁺-dependent muscle enzyme. Mutagenesis studies show that the internal positive charge substitutes for the K⁺ requirement (Laughlin, L. T. & Reed, G. H. (1997) Arch. Biochem. Biophys. 348, 262–267). In this work a phylogenetic analysis of pyruvate kinase was performed to ascertain the abundance of K⁺-independent activities and to explore whether the K⁺ activating effect is related to the evolutionary history of the enzyme. Of the 230 studied sequences, 46% have Lys at position 117, and the rest have Glu. Pyruvate kinases with Lys¹¹⁷ and Glu¹¹⁷ are separated in two clusters. All of the enzymes of the Glu¹¹⁷ cluster that have been characterized are K⁺-dependent, whereas those of the Lys¹¹⁷ cluster are K⁺-independent. Thus, there is a strict correlation between the dichotomy of the tree and the dependence of activity on K⁺. 77% of the pyruvate kinases that possess Lys¹¹⁷ have Lys¹¹³/Gln¹¹⁴; they also have Ile, Val, or Leu at position 120. These residues are replaced by Glu¹¹⁷ and Thr¹¹³/Lys¹¹⁴/Thr¹²⁰ in 80% of K⁺-dependent pyruvate kinases. Structural analysis indicates that these residues are in a hinge region involved in the acquisition of the catalytic conformation of the enzyme. The route of conversion from K⁺-independent to K⁺-dependent pyruvate kinases is described. A plausible explanation of how enzymes developed K⁺ dependence is put forth. The dependence of enzyme activity on monovalent cations is widespread. Indeed more than 100 enzymes exhibit structural and catalytic requirement for either K⁺ or Na⁺ (1–4). In some enzymes, the monovalent cations participate in the formation of a ternary complex of enzymes and substrates, whereas in other, cations act allosterically or stabilize the protein structure (3–7). Structural information on the molecular origin of these effects has begun to emerge; however, there are still many questions that remain to be solved. Pyruvate kinase, the first enzyme in which an absolute requirement for K⁺ has been documented (8), is an excellent model enzyme for studying the effect of monovalent cations particularly because in the rabbit muscle enzyme the activating effect of K⁺ is about 10,000-fold (9, 10), which, to our knowledge, is the highest reported thus far. In regard to the mechanism of action of K⁺, it is known that this cation changes the ultraviolet and fluorescence spectra of pyruvate kinase (11–13) as well as its immunoelectrophoretic pattern (14). K⁺ also changes the structure of the active site (15–18). In this respect, NMR studies of Ti⁺ bound to pyruvate kinase show that the alkali metal ion binds within 0.8 nm of the Mn²⁺-binding site (16) and that this distance shifts to 0.49 nm upon binding of the substrate phosphoenolpyruvate (19). In addition, it has been shown that K⁺ changes the kinetic mechanism of rabbit muscle pyruvate kinase and is involved in the acquisition of the active conformation of the enzyme (20). The K⁺-binding site is highly conserved in all crystallographic structures reported to date. K⁺ is coordinated by O⁻δ1 of Asn⁷⁴, O⁻γ of Ser⁷⁶, O⁻δ₂ of Asp¹¹², the carbonyl oxygen of Thr¹¹³ (21), a water molecule, and a phosphate oxygen of phosphoenolpyruvate analogous (22), or an oxygen from the γ-phosphate of ATP (5).

For a long time, it was thought that the absolute dependence of K⁺ was a common feature to all pyruvate kinases (8, 23–38). However, as the number of characterized enzymes increased, it became apparent that the activity of several pyruvate kinases was K⁺-independent, for example those from Escherichia coli (Type II) (39), Phycomyces blakesleeanus (40), Corynebacterium glutamicum (41), Zyromonas mobilis (42), Thermoproteus tenax (43), Synchococcus pcc 6301 (44), Archaeoglobus fulgidus, Pyrococcus aerophilum, Aeropyrum pernix, and Thermotoga maritima (45). To explore the molecular basis of this different behavior, Laughlin and Reed (46) compared the amino acid sequence of rabbit muscle pyruvate kinase with those of E. coli type II and of C. glutamicum and found that Glu¹¹⁷ of the rabbit enzyme, which is close to the K⁺-binding site, is a Lys in the bacterial enzymes. The authors constructed the E117K mutant of the rabbit enzyme and found that the mutant is >200-fold more active than the wild type in the absence of K⁺ and exhibits no stimulation by monovalent cations. They also constructed two other mutants, E117A and E117D; these enzymes were activated by K⁺. The data clearly indicate that the positive charge of Lys mimics the action of K⁺ (46). Mimicry of monovalent cation activation by a Lys has also been reported in chaperon HSC70 (47) and H⁺-translocating inorganic pyrophosphatase (48). In consonance with the data of Laughlin and Reed (46), the sequences of five of the character-
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ized K⁺-independent enzymes also contain Lys in position 117 (39, 41, 42, 44, 45). However, pyruvate kinase from T. tenax (43) and P. aerophilum (44, 45) exhibit K⁺-independent activity, albeit with a Ser at position 117. These observations thus indicate that the expression of K⁺-independent activity is not completely explained by the charge of residue 117 and that other residues in the vicinity of the active site may be involved in conversion of the K⁺-independent to K⁺-dependent pyruvate kinase.

In this work we performed an extensive phylogenetic study of the pyruvate kinase family. The purpose of the study was to ascertain the abundance of K⁺-independent activities and to explore whether the K⁺ activating effect is related to the evolutionary history of the enzyme. Phylogenetic analyses of pyruvate kinase family have been reported previously (43, 45, 49). Schramm et al. (43) and Johnsen et al. (45) found a dichotomic structure of the phylogenetic tree that does not coincide with the universal tree topology (Bacteria, Archaea, and Eukarya) (50, 51); this unusual topology may be the result of ancient gene duplication or lateral transfer events (43). The latter studies were an attempt to correlate the major subfamilies or clusters to the type of allosteric effector of the enzymes. According to Schramm et al. (43), cluster I mostly includes enzymes activated by sugar phosphates, whereas cluster II comprises enzymes regulated by nucleotides. Given that some enzymes of cluster I are regulated by effectors of cluster II and vice versa and some even lack heterotropic allosteric regulation, a classification based on allosteric effectors of the enzymes may be more complex.

Our phylogenetic data show that there are numerous enzymes that have Lys at position 117 (the numbering is that of rabbit muscle pyruvate kinase). Because pyruvate kinases that contain Lys₁¹⁷ and the E117K mutant of the rabbit enzyme are K⁺-independent, it may be safely assumed that K⁺-independent pyruvate kinases are broadly present in nature (106 sequences from 230 have Lys). In accordance with Schramm et al. (43), we found a dichotomic topology of the pyruvate kinase phylogenetic tree. However, in our case, cluster I groups sequences that include Glu₁¹⁷ correspond to K⁺-dependent enzymes, whereas cluster II comprise sequences that have Lys₁¹⁷ and are therefore K⁺-independent enzymes. In addition, we found that changes in the residue at position 117 are consistently accompanied by conservative changes in residues 113, 114, and 120. In all likelihood these residues are mechanistically important, because they localize in a hinge bending region that participates in the acquisition of the active catalytic conformation of the enzyme.

**RESULTS AND DISCUSSION**

Phylogenetic Analysis—The search for sequences of pyruvate kinase comprised the data reported up to August 2004. A total of 230 sequences were retrieved: 69 eukarya (21 animals, 17 fungi, 16 plants and 15 protists), 143 bacteria, and 18 archaea (Fig. 1). Position 117 is occupied by Glu in 121 sequences and by Lys, Ser, and Arg in 106, 2, and 1 sequence, respectively. The requirement for K⁺ by 21 pyruvate kinases that have Glu at position 117 has been examined, and the activity of all of them is K⁺-dependent (8, 23–38). The activities of eight pyruvate kinases that do not have Glu at that position have been characterized; in all cases the activity is K⁺-independent. Therefore, there is an excellent correlation between the dependence of activity on K⁺ and the existence of Glu at position 117. About half of all the sequences analyzed (46%) have Lys¹¹⁷; thus, the K⁺-independent activity is as frequent as the K⁺-dependent activity.

Our phylogenetic analysis shows a dichotomic tree structure (Fig. 1) that, similar to a previous phylogenetic study (43), does not coincide with the universal tree topology (Bacteria, Archaea, and Eukarya) (50, 51). As discussed by Schramm et al. (43), this unusual topology may be caused by an early gene duplication event. However, a remarkable feature of our phylogenetic data is the high correlation between the dichotomic nature of the tree and the dependence on K⁺. As shown in Fig. 2, the red branch corresponds to the enzymes that contain Glu¹¹⁷, which include the 21 experimentally characterized K⁺-dependent enzymes. The only exception found in this branch is pyruvate kinase from the anaerobic bacteria *Clostridium perfringens*, (GenBank™ accession number Q46289) that has Lys at position 117. To gain insight into the peculiar position of pyruvate kinase of *C. perfringens*, we examined the sequences that had been deposited (in GenBank™) in the last year. We found four new sequences belonging to the genus *Clostridium*. Translated nucleotide sequences were retrieved from the GenBank™ of the National Center for Biotechnology Information.

Progressive multiple sequence alignment was calculated with the ClustalX package (54), using secondary structure-based penalties. The alignment was manually corrected according to the results of gapped BLASTP and three-dimensional alignments obtained from the three-dimensional structure data base of Entrez (55). Phylogenetic analyses were performed with MEGA 3.1 software (56) using maximum parsimony, minimum evolution, and neighbor-joining methods with the aid of the empirical Jones-Taylor-Thornton amino acid substitution model. Differences between amino acid sequences were corrected for multiple substitutions assuming gamma distribution for rate variations among sites. The gamma-shaped parameter (a = 1.0) was estimated with the Whelan-Goldman matrix of substitutions and the eight-category discrete-gamma model using TREE-PUZZLE 5.2 (57). Confidence limits of branch points were estimated by 500 bootstrap replications.

Structural analysis of domain movements and hinge bending regions of pyruvate kinase were performed with DynDom software. DynDom is a program that determines dynamic protein domains, hinge axes, and amino acid residues involved in hinge bending (58).
One of these sequences corresponds to a pyruvate kinase of *C. perfringens* that surprisingly has Glu in position 117. We also found that *Clostridium acetobutylicum* has K117 pyruvate kinase, in addition to the E117 enzyme included in the phylogenetic tree. This property is also shared by *Clostridium beijerinckii* where both Glu and Lys pyruvate kinases were found. The data on the *Clostridium* genus are highly suggestive that the two enzymes resulted from recent gene duplication.
fact, the identity between the Glu and Lys enzymes of \textit{C. perfringens} is 52.6\%, whereas identity between early duplicated pyruvate kinases of \textit{E. coli} in cluster I and cluster II is 36.5\%.

The \textit{blue branch} (Fig. 1) contains enzymes with Lys\textsuperscript{117} that comprise the eight experimentally studied K\textsuperscript{+}-independent enzymes; it also includes the K\textsuperscript{+}-independent enzymes with Ser and Arg at position 117 (indicated in \textit{black}) and three archaeal enzymes that contain Glu\textsuperscript{117}. Thus the branching of the phylogenetic tree concurs with the residue at position 117. Accordingly, the existing pyruvate kinases may be divided into two
The phylogenetic tree and the presence of Leu113 suggest that enzymes have not been characterized, but their localization in position 117 have Leu in position 113 (see Fig. 2). These groups: group I is formed by the K⁺-dependent enzymes and Group II by the K⁺-independent enzymes. As this classification is based on an all-or-none property, i.e. K⁺-independent or -dependent activities, it has advantages over classification based on allosteric effectors (43, 45). It is relevant to acknowledge that K⁺ dependence has been used as a criterion to explain the dichotomous topology of the phylogenetic tree of the H⁺-translocating inorganic pyrophosphatase family (48). However the lack of structural information available on these enzymes does not allow the establishment of structural correlations between amino acid substitutions and the K⁺-binding site.

Co-evolving Amino Acids with Residue 117—The phylogenetic analysis also shows that there is a correlation between the residue that exists at position 117 and some of its neighboring residues (Table I). When Glu is in position 117, in 99% of the cases the residue in position 113 is Thr. Likewise, when Lys is in position 117, position 113 is occupied by Leu in 98% of the enzymes. A similar, but slightly less strict pattern was observed between residue 117 and those at positions 114 and 120. The data show that: 1) pyruvate kinases that have Glu117 have Lys114 in 94% of the cases; 2) when Lys is in position 117, position 114 is occupied by Glu in 83% of the enzymes; 3) enzymes with Glu in position 117 have Thr in position 120 in 83% of the cases; 4) enzymes with Lys117 have a hydrophobic residue in position 120 (Leu, Val, or Ile) in 93% of the cases.

The main chain carbonyl oxygen of Thr113 participates in K⁺ binding. As shown in Table I, this residue is almost invariably present when Glu is in position 117 (cluster I). It is likely that the conservation of Thr113 is the result of the evolutionary pressure to keep the integrity of the K⁺-binding site intact. In the K⁺-independent enzymes (cluster II), Leu113 is practically invariant (98%). This is rather atypical because there is no obvious evolutionary benefit for the conservation of this residue. Leu113 is associated to a positive charged residue in position 117 (Lys, Arg) or to serine. The constancy of Leu113 in the enzymes of cluster II suggests that it is involved in the expression of the K⁺-independent activity. It is also noteworthy that in the K⁺-independent activity cluster, aside from the enzymes that contain Ser117, three archaeal enzymes that have Glu in position 117 have Leu in position 113 (see Fig. 2). These enzymes have not been characterized, but their localization in the phylogenetic tree and the presence of Leu113 suggest that they are K⁺-independent enzymes.

Concerning residue 114, Laughlin and Reed (46) found that in the rabbit muscle E117K mutant, the presence of Lys (the most abundant residue found in position 114 in K⁺-dependent enzymes) or Gln (found mostly in K⁺-independent enzymes) did not affect the kinetics of the enzyme. Therefore, it is possible that only the pair Leu113/Gln114 participates in K⁺-independent activity. In fact, there is not a single pyruvate kinase that has the pair Thr113/Gln114.

No functional role has been assigned to residue 120; however the co-evolution between this amino acid and the residue 117 (83% in cluster I and 93% in cluster II) makes it an attractive target to study. Finally, it is noted that in the transition between clusters I and II, there are about 30 sequences that do not show the conserved pattern in residues 114 and/or 120. This suggests that these positions are not as critical as positions 113 and 117 for monovalent cation dependence.

The overall analysis of K⁺-dependent pyruvate kinases (cluster I) shows that the sequence Thr113/Lys114/Glu117 in 93% of the enzymes is conserved in 80% of the cases, whereas in the K⁺-independent enzymes (cluster II) the sequence Leu113/Gln114/Glu117/Thr120 in 77% of the enzymes. The co-evolution of residues 113, 114, and 120 with that in position 117 show that these residues are involved in the activating effect of K⁺ in the K⁺-dependent enzymes or in the expression of the K⁺-independent activity. This is relevant that the data presented of Laughlin and Reed (46) show that in the rabbit muscle enzyme, Lys117 may replace K⁺; however the activity of the mutant is about 10% of the activity of the wild type with K⁺. Therefore, further substitutions are needed to attain full enzymatic activity.

Transition from K⁺ Independence to K⁺ Dependence—The broad phyletic distribution of K⁺-independent pyruvate kinases (cluster II) in the three domains of life (Archea, Bacteria, and Eukarya) in comparison with K⁺-dependent pyruvate kinases (cluster I, found in Bacteria and Eukarya only) suggest that K⁺-independent activity preceded the appearance of the K⁺-sensitive activity. To explain the possible route through which K⁺-independent are converted to K⁺-dependent pyruvate kinases, we analyzed the amino acid codons for positions 113, 114, 117, and 120. As shown in Fig. 3, all of the changes in positions 113, 114, 117, and 120 took place by single nucleotide substitutions, except in the Antarctic hair grass, Deschampsia antarctica (GenBank™ accession number AAM22747), and the sulfate-reducing proteobacteria, Desulfovibrio desulfuricans (GenBank™ accession number ZP_00130421), where more than one substitution was needed. Because the frequency of mutations of amino acid codons occurs in the order of

### Table 1

| Cluster I K⁺-dependent enzymes | Cluster II K⁺-independent enzymes |
|-------------------------------|---------------------------------|
| Residues co-evolving with Glu117 | Residues co-evolving with Lys117 |
| %                             | %                               |
| Thr113                        | Leu113                          |
| 99                            | 98                              |
| Lys114                        | Gln114                          |
| 94                            | 83                              |
| Thr113                        | (Val-Ile-Leu)112                |
| 83                            | 93                              |
| Thr113/Lys114                 | Leu113/Gln114                   |
| 90                            | 82                              |
| Thr113/Thr120                 | Leu113/(Val-Ile-Leu)120         |
| 80                            | 93                              |
| Lys114/Thr120                 | Gln114/(Val-Ile-Leu)120         |
| 81                            | 78                              |
| Thr113/Lys114/Thr120          | Leu113/Gln114/(Val-Ile-Leu)120  |
| 80                            | 77                              |
almost exclusive of the K-dependent and independent enzymes differ in the region of the intestinalis.

The conservation of the K-binding site, the level of conservation of residues that contribute to the coordination of the cation was determined. It is noted, however, that all of the crystallographic structures of pyruvate kinase reported so far are K-dependent. In all of them K$^+$ is coordinated through O-$\delta$1 of Asn$^{74}$, O-$\gamma$ of Ser$^{76}$, O-$\delta$2 of Asp$^{112}$, and by the backbone carbonyl oxygen of Thr$^{113}$ (21). In the 230 sequences that were studied, Asn$^{74}$ and Asp$^{112}$ are conserved except for the pyruvate kinases from Giardia intestinalis and D. antarctica. Ser$^{76}$ is less conserved; it is replaced by Ala in 12 and by Cys in 5 pyruvate kinases sequences. Collectively, the data indicate that the residues that form the K-binding site, with the exception of Thr$^{113}$, which is almost exclusive of the K-dependent pyruvate kinases, are highly conserved even in enzymes that do not require K$^+$ for activity.

Structural Analysis of the Consensus Sequence—To explore the contribution of residues 113, 114, 117, and 120 to the dynamics of pyruvate kinase, the enzyme was analyzed with the DynDom software (58). DynDom is a program that identifies domains, hinge axes, and hinge bending residues in proteins. The program may be applied when there are crystallographic data of a protein in two or more conformations. The DynDom data base of all the crystallographic structures of pyruvate kinase available in the Protein Data Bank was used for the analysis.

The enzyme is formed by four structural domains: the N-terminal and the A, B, and C domains. The active site is in a cleft formed by A and B domains, which are linked by two loops. Rotation of the B domain over the A domain closes the active site cleft. DynDom assigns domain B as the mobile domain, and the N-terminal A and C domains as the fixed domain. Two hinge bending regions were assigned: a short hinge formed by the sequence 114–118 (green in Fig. 4) and a long hinge formed by residues 209–224 (yellow in Fig. 4). Therefore residues 113 and 120 are, respectively, at the beginning and end of the short hinge, whereas residues 114 and 117 are part of it. Thr$^{113}$, Lys$^{117}$, and Thr$^{120}$ are illustrated as sticks, and K$^+$ is shown in yellow. The sequence from residues 109–123 is shown at the bottom of the figure with the same color code (fixed in blue, moving in red, and short hinge in green). The sequence from residues 209–224 is not shown. The positions of residues 113, 114, 117, and 120 with respect to the short hinge are illustrated.
ies (15–19) have shown that monovalent cations induce structural rearrangements in the active site of rabbit muscle pyruvate kinase. Similar findings have also been documented in yeast pyruvate kinase where the interaction of the monovalent cation with the wild type enzyme (59, 60) and Thr298 mutants (60) was investigated by 205Tl NMR. The main results indicate that monovalent cation induces conformational alterations at the active site of yeast pyruvate kinase (59, 60). Moreover, kinetic and intrinsic fluorescence anisotropy measurements suggest that K+ induces the closure of the active site and the acquisition of the active conformation of rabbit muscle pyruvate kinase (20). In K+-dependent enzymes, the high conservation of Glu117 and its flanking residues Thr113/Lys114/Thr120, which are in a strategic structural location, indicates that these residues may be involved in the transduction of K+ binding to the movement of domain B. In the K+-independent enzymes, Lys117, along with Leu113/Gln114/(Val-Leu-Ile)120 may induce similar conformational changes but in the absence of K+.

Why Do Enzymes Use Monovalent Cations?—There are a large number of enzymes for which activity is dependent on either Na+ or K+. A salient feature of these enzymes is that extracellular enzymes are Na+-dependent, whereas K+-dependent enzymes are intracellular. Thus it may be asked, what is the evolutionary advantage of having Na+ or K+ as essential activators of these enzymes? Di Cera (4) has put forth that Na+ and K+ cannot function as regulators of enzyme activity because of their tightly controlled concentrations. Instead, he considers that monovalent cations provide a driving force for substrate binding and catalysis by lowering energy barriers (4). Thus monovalent cation-activated enzymes evolved to optimize their catalytic function by taking advantage of the large concentrations of Na+ and K+ in their surrounding media.

An alternative or additional explanation for the existence of the numerous proteins that require monovalent cations could involve the chemical rescue of an inactive enzyme. That is, we hypothesize that the biological activity of a mutated protein can be restored by the introduction of a chemical group that compensates for or overcomes the detrimental effect of a mutation. Indeed, it has been reported that imidazole compensates the loss of histidines that are essential for catalysis (61) and that guanidium ions restore the substitution of arginine residues (62). Accordingly, it is possible that the binding of monovalent cations compensate for the loss of essential positive charged amino acids followed by additional mutations as the result of functional adaptations to the presence of the new ligand. In pyruvate kinase, the substitution of Lys for Glu117 is compensated by the binding of K+. Mutations in residues 113, 114, and 120 could be the evolutional adaptations required to convert the enzyme into an efficient K+-dependent catalyst. Concerning cation selectivity, cation-dependent pyruvate kinases emerged to select K+ over Na+ because of evolutionary pressure that led to the optimization of the interactions of the protein with the K+ that abounds in the intracellular milieu.

In sum, the phylogenetic analysis of pyruvate kinase showed that the K+-independent activity is broadly present in nature; about half of the reported sequences possess Lys in position 117, whereas the rest have Glu in that position and are K+-dependent. The K+ dependence feature clearly explains the dichotomic topology of the phylogenetic tree of pyruvate kinase family; the K+-dependent and -independent enzymes belong to each of the branches of the tree. Because organisms that evolved at a later time have K+-dependent pyruvate kinases, it may be suggested that they emerged from K+-independent enzymes through single nucleotide substitutions. The high association found between the amino acid in position 117 and those in positions 113, 114, and 120, along with their central location in the structure of the enzyme, indicates that these residues might be involved in the activating effect of K+ or in the expression of the K+-independent activity. Therefore, the primary structure of pyruvate kinases, related to the K+ requirement, provides a precise criterion for their classification.

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REFERENCES

1. Suelter, C. H. (1970) Science 168, 789–795
2. Larsen, T. M., and Reed, G. H. (2001) in Handbook of Metalloproteins (Bertini, I., Siegel, A., and Siegel, H., eds) pp. 9–37, Dekker, New York
3. Di Cera, E. (2004) C. R. Biol. 327, 1065–1076
4. Di Cera, E. (2006) J. Biol. Chem. 281, 1305–1308
5. Larsen, T. M., Benning, M. M., Rayment, I., and Reed, G. H. (1998). Biochemistry 37, 6247–6255
6. Andersson, C. E., and Mowbray, S. L. (2002) J. Mol. Biol. 315, 409–419
7. Ahmad, A., Akhtar, M. S., and Bhakuni, V. (2001) Biochemistry 40, 1945–1955
8. Kachmar, J. F., and Boyer, P. D. (1953) J. Biol. Chem. 200, 669–682
9. Kayne, F. J. (1971) Arch. Biochem. Biophys. 143, 232–239
10. Ramírez-Silva, L., Oria, J., Gómez-Puyou, A., and Tuena de Gómez-Puyou, M. (1997) Eur. J. Biochem. 250, 583–589
11. Suelter, C. H., and Melander, W. (1963) J. Biol. Chem. 238, PC4108–PC4109
12. Kayne, F. J., and Suelter, C. H. (1965) J. Am. Chem. Soc. 87, 897–900
13. Suelter, C. H., Singleton, R. R., Kayne, F. J., Arrington, S., Glass, J., and Mildvan A. S. (1966) Biochemistry 5, 131–139
14. Sorger, G. J., Ford, R. E., and Evans, H. J. (1965) Proc. Natl. Acad. Sci. U. S. A. 54, 1614–1621
15. Mildvan, A. S., and Cohn, M. (1966) J. Biol. Chem. 241, 1178–1193
16. Kayne, F. J., and Reuben, J. (1970) J. Am. Chem. Soc. 92, 220–222
17. Nowak, T., and Mildvan, A. S. (1972) Biochemistry 11, 2819–2828
18. Rausch, F. M., and Villafranca, J. J. (1980) Biochemistry 19, 5481–5485
19. Reuben, J., and Kayne, F. J. (1971) J. Biol. Chem. 246, 6227–6234
20. Oria-Hernandez, J., Cabrera, N., Perez-Montfort, R., and Ramírez-Silva, L. (2005) J. Biol. Chem. 280, 37924–37929
21. Larsen, T. M., Laughlin, L. T., Holden, H. M., Rayment, I., and Reed, G. H. (1994) Biochemistry 33, 6301–6309
22. Larsen, T. M., Benning, M. M., Wesenberg, G. E., Rayment, I., and Reed, G. H. (1997) Arch. Biochem. Biophys. 345, 199–206
23. Baranowska, B., and Baranowski, T. (1975) Mol. Cell. Biochem. 6, 197–201
24. Muirhead, H., Clayden, D. A., Cuffe, S. P., and Davies, C. (1987) Biochem. Soc. Trans. 15, 996–999
25. Kahn, A., and Marie, J. (1982) Methods Enzymol. 90, 131–140
26. Meixner-Monori, B., Kubicek, C. P., and Rohr, M. (1984) Can. J. Microbiol. 30, 16–22
27. Tsao, M. U., and Madley, T. I. (1972) Microbios 12, 125–142
28. Rhodes, N., Morris, C. N., Ainsworth, S., and Kindlerer, J. (1986) Biochem. J. 234, 705–715
29. Nairn, J., Duncan, D., Gray, L. M., Urequhart, G., Binnie, M., Byron, O., Fothergill-Gilmore, L. A., and Price, N. C. (1998) Protein Expression Purif. 14, 247–253
30. Cullen, M., Kuntz, D. A., and Opperdoes, F. R. (1991) Mol. Biochem.
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Parasitol. 47, 19–29
31. Ernest, I., Callens, M., Opperdoes, F. R., and Michels, P. A. (1994) Mol. Biochem. Parasitol. 64, 43–54
32. Maeda, T., Saito, T., Oguchi, Y., Nakazawa, M., Takeuchi, T., and Asai, T. (2003) Parasitol. Res. 89, 259–265
33. Valentini, G., Bartolucci, S., and Malcovati, M. (1979) Ital. J. Biochem. (Engl. Ed.) 28, 345–361
34. Asanuma, N., and Hino, T. (2001) Microbiology 147, 681–690
35. Du, W., Wallis, N. G., Mazzulla, M. J., Chalker, A. F., Zhang, L., Liu, W. S., Kallender, H., and Payne, D. J. (2000) Eur. J. Biochem. 267, 222–227
36. Yamada, T., and Carlsson, J. (1975) J. Bacteriol. 124, 562–563
37. Sakai, H., Suzuki, K., and Imahori, K. (1986) J. Biochem. (Tokyo) 99, 1157–1167
38. Iliffe-Lee, E. R., and McClarty, G. (2002) Mol. Microbiol. 44, 819–828
39. Malcovati, M., and Valentini, G. (1982) Methods Enzymol. 90, 170–179
40. Busto, F., Del Valle, P., and Soler, J. (1988) Biochem. Cell Biol. 66, 148–157
41. Jetten, M. S. M., Gubler., M. E., Lee, S. H., and Sinskey, A. J. (1994) Appl. Environ. Microbiol. 60, 2501–2507
42. Steiner, P., Fussenegger, M., Bailey, J. E., and Sauer, U. (1998) Gene 220, 31–38
43. Schramm, A., Siebers, B., Tjaden, B., Brinkmann, H., and Hensel, R. (2000) J. Bacteriol. 182, 2001–2009
44. Knowles, V. L., Smith, C. S., Smith, C. R., and Plaxton, W. C. (2001) J. Biol. Chem. 276, 20966–20972
45. Johnsen, U., Hansen, T., and Schonheit, P. (2003) J. Biol. Chem. 278, 25417–25427
46. Laughlin, L. T., and Reed, G. H. (1997) Arch. Biochem. Biophys. 348, 262–267
47. Wilbanks, S. M., and McKay, D. B. (1998) Biochemistry 37, 7456–7462
48. Belogurov, G. A., and Lahti, R. (2002) J. Biol. Chem. 277, 49651–49654
49. Muñoz, M. E., and Ponce, E. (2003) Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135, 197–218
50. Doolittle, W. F., and Brown, J. R. Proc. (1994) Natl. Acad. Sci. U. S. A. 91, 6721–6728
51. Walsh, D. A., and Doolittle, W. F. (2005) Curr. Biol. 15, R237–R240
52. Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Rapp, B. A., and Wheeler, D. L. (2000) Nucleic Acids Res. 28, 15–18
53. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
54. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876–4882
55. Chrusciel, T., Anderson, J. B., DeWeese-Scott, C., Fedorova, N. D., Geer, L. Y., He, S., Hurwitz, D. I., Jackson, J. D., Jacobs, A. R., Lanzczewski, C. J., Liebert, C. A., Liu, C., Madej, T., Marchler-Bauer, A., Marchler, G. H., Mazumder, R., Nikolskaya, A. N., Rao, B. S., Panchenko, A. R., Shoemaker, B. A., Simonov, Y., Song, J. S., Thiessen, P. A., Vasudevan, S., Wang, Y., Yamashita, R. A., Yin, J. I., and Bryant, S. H. (2003) Nucleic Acids Res. 31, 474–477
56. Kumar, S., Tamura, K., Jakobsen, I. B., and Nei, M. (2001) Bioinformatics 17, 1244–1245
57. Schmidt, H. A., Strimmer, K., Vingron, M., and von Haeseler, A. (2002) Bioinformatics 18, 502–504
58. Qi, G., Lee, R., and Hayward, S. (2005) Bioinformatics 21, 2832–2838
59. Loria, J. P., and Nowak, T. (1999) Biochemistry 37, 6967–6974
60. Susan-Resiga, D., and Nowak, T. (2003) J. Biol. Chem. 278, 40943–40952
61. McCartney, S. A., Brignole, E. J., Kolegraff, K. N., Loveland, A. N., Ussin, L. M., and Gibson, W. (2005) J. Biol. Chem. 280, 33206–33212
62. Williams, D. M., Wang, D., and Cole, P. A. (2000) J. Biol. Chem. 275, 38127–38130