Enzymes activated by monovalent cations are abundantly represented in plants and the animal world. They have evolved to exploit Na\(^+\) and K\(^+\), readily available in biological environments, as major driving forces for substrate binding and catalysis. Recent progress in the structural biology of such enzymes has answered long standing questions about the molecular mechanism of activation and the origin of monovalent cation selectivity. That enables a simple classification of these functionally diverse enzymes and reveals unanticipated connections with ion transporters.

Over 60 years ago, Boyer et al. (1) reported in the pages of this Journal that pyruvate kinase would express appreciable catalytic activity only in the presence of K\(^+\). A similar effect was soon discovered in other systems, and just a few decades later the field of enzymes requiring a monovalent cation (M\(^+\)) for optimal activity encompassed hundreds of examples from plants and the animal world (2, 3). Since the beginning, this rapidly expanding field had to address two basic questions, namely the molecular mechanism of M\(^+\) activation and the structural basis of M\(^+\) selectivity. Because kinetic investigation would only provide indirect answers, further progress in the field had to await high resolution crystal structures of M\(^+\)-activated enzymes, which have become available only over the last decade.

A classification of M\(^+\)-activated enzymes can be based on the selectivity of the effect, as allowed by kinetic studies, and the mechanism of activation, as shown from structural analysis. The effect has exquisite specificity, with Na\(^+\) or K\(^+\) being the preferred M\(^+\). In general, enzymes requiring K\(^+\) such as kinases and molecular chaperones are also activated by NH\(_4\)^+ and Rb\(^+\) but are not activated as well or at all by the larger cation Cs\(^+\) or the smaller cations Na\(^+\) and Li\(^+\). Enzymes requiring Na\(^+\) such as \(\beta\)-galactosidase and clotting proteases are not activated as well by Li\(^+\) or the larger cations K\(^+\), Rb\(^+\), and Cs\(^+\). Because the concentration of Na\(^+\) and K\(^+\) is tightly controlled in vivo, M\(^+\)'s do not function as regulators of enzyme activity. Rather, they provide a driving force for substrate binding and catalysis by lowering energy barriers in the ground and/or transition states. Enzymes activated by M\(^+\)'s evolved to take advantage of the large availability of Na\(^+\) outside the cell and K\(^+\) inside the cell to optimize their catalytic function. Indeed, a strong correlation exists between the preference for K\(^+\) or Na\(^+\) and the intracellular or extracellular localization of such enzymes. The mechanism of M\(^+\) activation can be established from crystal structures as cofactor-like or allosteric. In the former case, the M\(^+\) anchors the substrate to the active site of the enzyme, often acting in tandem with a divalent cation like Mg\(^{2+}\). In such a mechanism of activation, the M\(^+\) is absolutely required for catalysis. In the latter, the M\(^+\) enhances enzyme activity through conformational transitions triggered upon binding to a site where the M\(^+\) makes no direct contact with substrate. In this case, the M\(^+\) is not absolutely required for catalysis.

Crystallographic assignment of Na\(^+\) or K\(^+\) is non-trivial, even for high resolution structures. Na\(^+\) has a small ionic radius (0.97 Å) and the same number of electrons as a water molecule. K\(^+\) has a higher electron density but an ionic radius (1.33 Å) almost identical to that of a water molecule. Hence, correct assignment of Na\(^+\) and K\(^+\) must be based on several criteria: the presence of a spherical electron density peak at a \(\sigma\) level in the \(F_o - F_c\) map considerably above that of all other water molecules, the number of surrounding oxygens (typically five to seven), and a \(B\) value that is comparable with that of neighbor atoms. Additional criteria involve proper O–M\(^+\) distances, on the average 2.4 Å for Na\(^+\)–O and 2.8 Å for K\(^+\)–O pairs (4), and valence values close to unity (5). Anomalous scattering often provides unequivocal evidence of K\(^+\).

Table I presents a classification of M\(^+\)-activated enzymes based on the mechanism of activation identified from structural data and on the M\(^+\) requirement established from kinetic studies. In Type I enzymes the M\(^+\) functions as a cofactor and the requirement is absolute. In Type II enzymes the M\(^+\) functions as an allosteric effector and the requirement is not absolute.

**K\(^+\)-activated Type I Enzymes**

Diol and glycerol dehydratases provide the simplest example of Type I enzymes. K\(^+\) is coordinated by five ligands from the protein and acts as a “bait” for the two hydroxyl oxygens from substrate (6, 7). Enzymes involved in phosphoryl transfer reactions were long recognized to be the dominant group among M\(^+\)-activated enzymes (2, 3), and this early observation is confirmed by the entries in Table I. In addition to K\(^+\), these enzymes have an absolute requirement for a divalent cation, typically Mg\(^{2+}\) (1, 8–11). The mechanism of activation involves K\(^+\) and Mg\(^{2+}\) acting in tandem to provide optimal docking for the phosphate moiety of substrate into the protein active site to enable nucleophilic attack on the P\(^\gamma\)-O of the phosphate group.

**K\(^+\)-activated Type II Enzymes**

Some kinases belong to Type II because K\(^+\) does not contact ATP directly. In this case, K\(^+\) exerts its influence indirectly by perturbing the conformation of active site residues. Ribokinase (21) and adeninomimidazole riboside kinase (22) break the K\(^+\)-Mg\(^{2+}\) tandem by embracing the M\(^+\) in a \(\beta\)-turn adjacent to the active site. In MutL, the K\(^+\) coordination resembles that of branched chain \(\alpha\)-ketoacid dehydrogenase kinase (13) and pyruvate dehydrogenase kinase (14) use a similar K\(^+\)-Mg\(^{2+}\) tandem, whereas the molecular chaperone Hsc70 (15) and the Rad51 recombinase homolog from *Methanococcus voltae* (16) utilize two K\(^+\) in tandem with Mg\(^{2+}\). Other variations are the K\(^+\)-Zn\(^{2+}\) tandem in pyridoxal kinase (17) and K\(^+\) coupled with two Mg\(^{2+}\) in fructose-1,6-biphosphatase (18), S-adenosylmethionine synthase (19), and pyruvate kinase (20).

**Na\(^+\)-activated Type I Enzymes**

The strategy used by K\(^+\)-activated Type I kinases to anchor substrate to the active site is also exploited by fructose-1,6-biphosphate aldolase (31) and...
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| TABLE 1 Classification of $M^+$-activated enzymes |
|-----------------------------------------------|
| Enzyme                                      | Protein Data Bank entry | Ligands | Ref. |
| **K⁺-activated Type I**                      |                            |         |      |
| Branched chain α-ketoacid dehydrogenase      |                            |         |      |
| kinase                                       | 1GJV, 1GKZ                | 5       | 4-0-1| 13  |
| Diol dehydratase                            | 1DI0, 1EEX, 1EGM, EGV, 1IWB, 1UC4, 1UC5 | 7       | 5-0-2| 6   |
| Fructose-1,6-bisphosphatase                  | 1FPI                       | 4       | 3-0-1| 18  |
| Glycerol dehydratase                        | 1IWP, 1MMF                | 7       | 5-0-2| 7   |
| GroEL                                        | 1KP8, 1PCQ, 1SVT, 1XS3     | 7       | 2-4-1| 12  |
| Hsc70                                        | 1BUP, 1HPM, 1KAX, 1KAY, 1KAZ, 1QQM, 1QQN, 1QQO; Na⁺ bound: 1BA0, 1BA1, 1UD0, 3H5C | 7       | 5-1-1| 15, 43 |
| MalvadA                                      | 1XU4                       | 4       | 2-1-1| 16  |
| Pyridoxal kinase                            | 1LHR, 1RTF                | 6       | 4-1-1| 17  |
| Pyruvate dehydrogenase kinase                | 1YSN, 1YSO, 1YSP          | 5       | 4-0-1| 14  |
| Pyruvate kinase                             | 1A3W, 1A3X, 1A49, 1AQF, 1F3W, 1F3X, 1LIU, 1LIW, 1LIX, 1LJY, 1PKN, 1T5A; Na⁺ bound: 1ASU | 6       | 4-1-1| 20  |
| S-Adenosylmethionine synthase                | 1MXA, 1MXB, 1MXC, 1O9Q, 1O9Q2, 1O9T, 1P7L, 1Q4M, 1RG9, 1IXA, 1XRB, 1XRC | 4       | 3-0-1| 19  |
| **K⁺-activated Type II**                     |                            |         |      |
| Branched chain α-ketoacid dehydrogenase      | 1DTW, 1OLS, 1OLU, 1OLX, 1I5B, 1V1I, 1V16, 1V1M, 1V1R, 1X7W, 1X7X, 1X7Y, 1X7Z, 1X80 | 5       | 5-0-0| 25  |
| Diallylglycine decarboxylase                  |                            |         |      |
| MutL                                         | 1NHI                      | 5       | 4-1-0| 23  |
| Ribokinase                                   | 1GQT, 1TTY, 1TZ3, 1TZ6      | 6       | 6-0-0| 21, 22 |
| Ser dehydratase                              | 1PWH                      | 6       | 6-0-0| 28  |
| Tryptophanase                                | 1AX4                       | 7       | 4-3-0| 29  |
| Tyrosinase                                   | 1TPL, 2TPL                | 7       | 4-3-0| 30  |
| **Na⁺-activated Type I**                     |                            |         |      |
| β-Galactosidase                              | 1DP0, 1JYN, 1JYV, 1JYW, 1JXY, 1JYY, 1JYZ, 1JZ0, 1JZ1, 1JZ2, 1JZ3, 1JZ4, 1JZ5, 1JZ6, 1JZ7, 1JZ8, 1IPX3, 1IPX4, 1ITG7, 1XC6 | 5       | 3-1-1| 33, 35 |
| Fructose-1,6-bisphosphate aldolase            | 1B57, 1RV8, 1RVG          | 6       | 4-1-1| 31  |
| Tagatose-1,6-bisphosphate aldolase            | 1GVF                      | 6       | 5-0-1| 32  |
| **Na⁺-activated Type II**                    |                            |         |      |
| Factor Xa                                    | 1P05, 2B0K                | 6       | 4-2-0| 41  |
| Thrombin                                     | 1A2C, 1A46, 1A4W, 1A5G, 1A61, 1A68, 1B5G, 1B80, 1ICU, 1C1V, 1C1W, 1C4U, 1C4V, 1C5L, 1C5N, 1C5O, 1CA8, 1D3D, 1D3P, 1D3Q, 1D3T, 1D4P, 1D6W, 1D9H, 1D7E, 1D7J, 1DX5, 1GHV, 1GHW, 1GHX, 1GHY, 1G4, 1GI5, 1JM0, 1KU0, 1K21, 1K22, 1O2G, 1O5G, 1OYT, 1SB1, 1SFQ, 1SG8, 1TB2, 1V7Q, 1XMN, 1Z8I, 1Z8J, 2THF, 7KME, 8KME; K⁺ bound: 2AOQ | 6       | 2-4-0| 40, 42 |
| Trp synthase                                 | 1A5O, 1A5S, 1BKS, 1C29, 1C3V, 1C9D, 1C2W2, 1C94, 1FYU, 1K3U, 1K7E, 1K7X, 1K8S, 1K8Y, 1K8Z, 1KFC, 1KFE, 1KFI, 1KFK, 1QOP, 1UB5, 1V82, 2ITR, 2TSY, 2TYS, 2WSY; K⁺ bound: 1A5A, 1A5B, 1B8E1, 1ITQ | 4       | 3-1-0| 38, 39 |

*a From a total of 1508 structures containing Na⁺ or K⁺, as of September 8, 2005. Many M⁺-activated enzymes have been studied extensively both structurally and kinetically (36). The enzyme is peculiar in that it binds Na⁺ with only slightly higher affinity than K⁺ (37). The crystal structures of Trp synthase bound to Na⁺ or K⁺ show that the M⁺ makes no contact with substrate or PLP and binds to the β subunit near the tunnel enabling the indole intermediate to be shuttled to the active site for complexation with i-Ser (38). The tunnel is partially blocked by residues Phe-280 and Tyr-279 in the Na⁺ form and is more open in the K⁺ form. Long range allosteric communication in Trp synthase is further demonstrated by the fact that binding to the active site in the β subunit can displace Na⁺ from its site in the α subunit (39).

*b The format is N (p-w-s), where N is the sum of ligands from the protein (p), water (w), and substrate (s). Only the most relevant references are listed.

atose-1,6-bisphosphate aldolase (32), where the tandem Na⁺-Zn²⁺ replaces K⁺-Mg²⁺. Tagatose-1,6-bisphosphate is unique in that it replaces a water molecule in the Na⁺ coordination shell with a cation–π interaction. β-Galactosidase from Escherichia coli deserves special mention. As the gene product of the lac2 operon, it occupies a prominent place in the history of molecular biology (33). The activating effect of Na⁺ and Mg²⁺ was discovered by Monod in 1951 (34). In this enzyme, the interplay between Na⁺ and Mg²⁺ is quite different from the partnership seen in kinases, with Mg²⁺ binding away from substrate and Na⁺ being in contact with the galactosyl 6-hydroxy (Fig. 1) (33, 35). Na⁺ is coordinated by three protein atoms and two water molecules in the free enzyme, and lactose binding replaces one of the waters in the coordination shell. This change in Na⁺ coordination triggers a rearrangement of Phe-601, one of the Na⁺ ligands, to promote substrate binding.

Na⁺-activated Type II Enzymes

Among the enzymes involved in PLP catalysis, Trp synthase has been studied extensively both structurally and kinetically (36). The enzyme is peculiar in that it binds Na⁺ with only slightly higher affinity than K⁺ (37). The crystal structures of Trp synthase bound to Na⁺ or K⁺ show that the M⁺ makes no contact with substrate or PLP and binds to the β subunit near the tunnel enabling the indole intermediate to be shuttled to the active site for complexation with i-Ser (38). The tunnel is partially blocked by residues Phe-280 and Tyr-279 in the Na⁺ form and is more open in the K⁺ form. Long range allosteric communication in Trp synthase is further demonstrated by the fact that binding to the active site in the α subunit can displace Na⁺ from its site in the β subunit (39).

In the clotting protease thrombin the allosteric effect of Na⁺ affects a basic mechanism of substrate recognition. Na⁺ binds close to the primary specificity pocket and orients Asp-189 for correct engagement of the Arg side chain of substrate at the P1 position (Fig. 1), enabling the enzyme to accomplish its procoagulant role in the blood (40). Long range effects induced by Na⁺ binding propagate through a network of buried water molecules up to the catalytic Ser-195 located 15 Å away (40). A similar architecture of Na⁺ recognition is observed in clotting factor Xa (41).

M⁺ Selectivity

Several M⁺-activated enzymes have been crystallized free or in the presence of Na⁺, K⁺, or other M⁺, and the resulting information has broadened our understanding of M⁺ selectivity. In the case of Trp synthase, the changes between the Na⁺-bound and K⁺-bound structures are significant (38) but are
not matched by differences in the kinetics of activation (36). In pyruvate kinase the replacement of K\(^+\)/H\(_{11001}\) with Na\(^+\)/H\(_{11001}\) results in no structural changes (20) although the enzyme is practically inactive without K\(^+\) (1). Future structural studies will hopefully clarify this lack of correlation with function. In thrombin, however, changes in coordination between Na\(^+\)/H\(_{11001}\) and K\(^+\)/H\(_{11001}\) propagate to the oxyanion hole and explain the differences in the kinetics of activation (40, 42). In the case of Dialkylglycine dehydrogenase (26, 27) and Hsc70 (15, 43), replacement of the essential K\(^+\)/H\(_{11001}\) with Na\(^+\)/H\(_{11001}\) changes drastically the geometry of coordination and perturbs residues that control binding of PLP or ATP. These enzymes have evolved K\(^+\) selectivity by imposing geometric constraints on the coordination shell that cannot be obeyed by the smaller ionic radius of Na\(^+\). The linkage with enzyme activation is ensured by the functional connection of these constraints with the optimal orientation of catalytic residues. Rigidity of the coordination shell guarantees selectivity by increasing the entropic cost of any reorganization meant to accommodate a M\(^+\) of different size. Interestingly, an analogous strategy has been exploited successfully in the synthesis of selective chelators (44, 45).

Other examples are provided by ion transporters for which M\(^+\) selectivity is absolute. The V-type Na\(^+\)-ATPase (46), the F-type Na\(^+\)-ATPase (47), and the bacterial Na\(^+\)/Cl\(^-\)-dependent neurotransmitter homologue (48) cag Na\(^+\) in rigid environments, practically inaccessible to K\(^+\). In the KcsA K\(^+\) channel, the backbone oxygens lining the channel maintain distances suitable only for K\(^+\) coordination and provide an exact replica of the coordination shell of K\(^+\) in solution (49). One striking feature of the channel is the signature sequence GYG (residues 77–79) whose backbone oxygens shape part of the selectivity filter (Fig. 2). Remarkably, the conformation of this sequence relative to the bound K\(^+\) in the channel is very similar to the GYG sequence (residues 325–327) near the K\(^+\) binding site of pyruvate dehydrogenase kinase (14), the GFG sequence (residues 337–339) near the K\(^+\) binding site of branched chain \(\alpha\)-ketoacid dehydrogenase kinase (13), and the KYG sequence (residues 224–226) near the Na\(^+\) binding site of thrombin (42). Furthermore, mutation of Tyr in this sequence has very similar functional consequences in the K\(^+\) channel (50) and thrombin (51). This unexpected connection is a testimony to the basic
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similarity in the mechanism of M⁺ recognition that evolution has bestowed on proteins with widely different functions.

Concluding Remarks

The information that emerged from crystal structures of M⁺-activated enzymes rationalizes decades of kinetic studies and unravels details on the molecular origin of M⁺ activation and selectivity. A simple classification of these enzymes is now made possible by merging structural and functional data bases. Further studies should explore the intriguing connections between M⁺-activated enzymes and ion transporters. The enormous amount of knowledge gathered from kinetic and structural studies should facilitate the redesign of M⁺ specificity and activation (23, 52). A most exciting task would be to introduce M⁺ activation into proteins devoid of M⁺ binding, which could benefit many areas of biotechnology and medicine.

Acknowledgments—I am grateful to Drs. David Davies, Thomas Hurley, Yu Luo, Mischa Machius, Barry Stoddard, Michael Toney, Jimin Wang, and Wei Yang for helpful exchanges of information.

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