Functional Assignment by Chimera Construction of the Domain Affecting Heterotropic Activation of Deoxyadenosine Kinase from Lactobacillus acidophilus R-26*

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The heterodimeric subunits of deoxyadenosine kinase (dAK)-deoxyguanosine kinase (dGK) from Lactobacillus acidophilus R-26 exhibit contrasting conformations manifested in the nearly unidirectional heterotropic activation of dAK when dGK binds deoxyguanosine. This is mediated, in part, by the conserved Ras switch I-like sequence (residues 153–161) (Guo et al. (1997) J. Biol. Chem. 272, 6890–6897). In an attempt to identify domains differentiating the specificities of dAK and dGK, we constructed several chimeras splicing heterodimeric dAK within this region. In Chimera-III, dAK residues 120–170 were replaced by the homologous section of dGK. dAK activity was elevated 40%, but although it retained its original specificity and $K_m$ values, it could no longer be activated by deoxyguanosine. Moreover, both the activated dAK and the “dAK” of Chimera-III exhibited (i) an increased $K_m$ for the leading substrate ATP-Mg$^{2+}$, suggesting the formation of intermediate enzyme species along their respective kinetic pathways, and (ii) broadened and lower pH optima for the dAK activities. These observations further indicate the importance of dAK residues 120–170, including the Ras-like segment, in catalysis and heterotropic activation. The other conformational properties of dAK (e.g. self-inactivity and MgATP being the leading substrate) were unaltered by this substitution, thus localizing the responsible domains even further upstream.

Lactobacillus acidophilus R-26 lacks a functional ribonucleotide reductase and thus depends on its four salvage deoxynucleoside kinases, each of which is highly specific, for the synthesis of DNA precursors. Whereas the thymidine kinase (TK) of cell extracts is readily separable from the other three kinase activities (1), the deoxyadenosine kinase peptide must be associated with either deoxyguanosine kinase (dAK-dGK) or the deoxycytidine kinase (dAK-dCK) (2–5). When cloned and independently expressed in Escherichia coli, dGK or dCK is almost fully active, whereas dAK expressed alone is nearly inactive; dAK becomes normally active when the dAK-dGK or dAK-dCK heterodimer is reconstituted in vitro (6). Heterodimeric dAK exhibits only about one-seventh of the activity of its partner dGK or dCK (7); however, the dAK activity of either heterodimer can be heterotropically activated by dGuo or deoxycytidine, respectively, by a factor of 3– to 5-fold (2–4). In the opposite direction, dAdo activates dGK or dCK only 20% at most (7). Differing by only three amino-terminal residues that switch their deoxynucleoside substrate specificities (8), dGK and dCK can otherwise be considered conformationally equivalent, having identical physical and kinetic properties in all aspects tested thus far. Together with homotropic end product inhibition of each of the four deoxynucleoside kinases (9), the heterotropic activation of dAK most likely plays a primary role in regulating the balanced deoxynucleotide metabolism of this unique organism (4).

The deoxynucleoside kinases from L. acidophilus R-26 are sequence-related to human dCK (10), dGK (11, 12), and TK2 (13). Their subunits are of similar size, and they share sequence homology along the entire span of the polypeptides, especially at sequences most likely comprising the active site, which include the P-loop, the D(E)E(R)S motif, and the arginine-rich site (14).

Chemical cross-linking studies and the adverse effects of dAdo, dATP, dGTP, and ATP on the cross-linking efficiency (6) as well as studies employing limited proteolysis (7) reveal conformational changes occurring near the intersubunit contact during heterotropic activation. A directed mutation within the Ras switch I-like sequence of dAK changing the structurally important Pro-155 residue to alanine permanently cis-activates dAK halfway toward its maximal activity potential, with a corresponding reduction in the magnitude of the heterotropic trans-activation by dGuo (6). Studies of comparable Ras-Ras-GAP interactions have shown switch I to be at the protein-protein interface, being a part of the Ras GTPase active site (15). By analogy, the Ras-like sequence of dAK is probably part of the dAK active site, being clearly involved in dAK-dGK intersubunit communication, specifically in heterotropic activation. It should be noted that unlike the Lactobacillus kinases, those human kinases do not possess the Ras switch I-like sequence. Instead, just after the arginine-rich site, they have a leucine-rich sequence that is thought to be involved in subunit-subunit interaction (16), although there is as yet no direct evidence for this.

To understand the structural bases for the contrasting properties of dAK and dGK (or dCK), we constructed chimeras in this study in attempts to convert dAK into dGK. Although a comparison of primary sequences can implicate important motifs, in the absence of x-ray resolution, it is difficult to predict the actual size of a domain precisely. We therefore chose three splicing sites 50 residues apart, dividing the polypeptide into four nearly equal segments. Chimera III (Chi-III or “dAK”-...
dGK), with a segment of peptide from residues 120–170 on dAK replaced by its counterpart from dGK, is of particular interest. Upon such a transplant, the dAK activity was elevated about 40%, whereas its heterotropic response toward dGuo was abolished. Both “dAK” of Chi-III and heterotropically activated dAK had their pH optima reduced from pH 9.3 to around pH 8. Moreover, the chimeric “dAK” began to behave more like dGK in terms of a lower affinity for MgATP. Despite this, “dAK” remained inactive when expressed without dGK.

**EXPERIMENTAL PROCEDURES**

**Construction of Chimera I**—The plasmid pHBlueScript (+) KS (Stratagene) containing a single dak gene was linearized with a single cut by StyI (Life Technologies, Inc.). The tandem dak-dgk genes cloned in pHBlueScript (+) KS were also restricted by StyI, and the smaller resulting StyI fragment was isolated from the agarose gel using the Qiagen gel extraction kit and ligated to the above-mentioned linearized dak construct to yield chi-I. This chi-i' construct, like dak-dgk (Fig. 1), also contains tandem open reading frames; an unmodified dak gene is followed by a chimeric dgk gene that encodes dGK spliced to dAK at residue 170.

**Construction of Chimera II**—The chi-ii gene (Fig. 1) encodes a chimeric polypeptide Chi-II in which the amino-terminal half of dAK is spliced to the carboxyl-terminal half of dGK at residue 120. It was constructed using recombinant PCR techniques as generally described (17). Pfu DNA polymerase purchased from Stratagene was used according to the procedures recommended by the manufacturer. For PCR-1, the independently cloned dak (6) served as the template, and primer 1 (5'-CCCCCCCCCCGGGTTACCCACATCCATTACA-3') and Primer 2 (5'-ATCAAGGATTCTTCAGTTTACAGGTCG-3') functioned as upstream and downstream primers, respectively. For PCR-2, the independently cloned dgk (8) served as the template, and primer 3 (5'-GACCCTGGTAATCCAAAGAAGAATCCTGAT-3') and primer 4 (5'-CCCCCCTGGGTATCCAAAACTGATTTACAG-3') served as upstream and downstream templates. PCR products from the two reactions described above were purified using the Qiagig Quick PCR purification kit and joined and amplified in a third PCR reaction using primer 1 and primer 4 as the upstream and downstream primers, respectively. The PCR product from this reaction was purified and digested with KpnI and finally cloned into pHBlueScript (+) KS, as confirmed by restriction mapping with XhoI and XbaI. The chi-ii gene was then sequenced in its entirety at the Ohio State University Biopolymer Facility.

**Construction of Chimera III**—The plasmid pHBlueScript (+) KS containing chi-II was linearized with a single cut by StyI and ligated with the smaller StyI fragment cut from the unmodified dak-dgk tandem genes (6). Therefore, chi-iii (Fig. 1) is also a tandem gene construct, containing a chimeric gene encoding a “dAK” subunit with residues 120–170 replaced by the counterparts from dGK, followed by an unmodified dgk gene. The ligation product was confirmed by restriction mapping with StyI and XbaI and by DNA sequencing.

**Construction of Chimera IV**—The gene for Chi-IV (Fig. 1) was constructed in the same way as chi-ii, except that the primers corresponding to primer 2 (5'-GGCTGACTTAAATCATACGTAAACGCGTTT-3') and primer 3 (5'-ACCACCGGTGGATTTAGTAAAGGCGGC-3') were specifically designed for this particular case. Upon subcloning into the polypeptide vector, the chi-ii gene was sequenced in its entirety.

**Protein Purification**—All kinase proteins used in this study were partially purified at least through ammonium sulfate precipitation (8). When the intersubunit affinity was estimated by titrating the appropriate subunit with dCK or dGK, the dCK or dGK polypeptides had been further purified to about 80% purity through Sephacryl S-200 HR (Amersham Pharmacia Biotech) gel filtration chromatography, as described previously (6).

**Enzyme Assays and Steady-State Kinetic Studies**—Whenever possible, heterodimer reconstitution was carried out by the procedures described previously (6). Kinase activity was defined and routinely assayed as described previously (6, 18). $K_M$ and $V_{max}$ values were obtained from the secondary plots of the slopes or y-axis intercepts of the primary double reciprocal plots, as described previously (19). The pH values from 6.7 to pH 10.0 were encompassed by TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] and CHES [2-(cyclohexylamino)ethanesulfonic acid] buffers (20 mM, at 25°C, 5% glycerol), with two values overlapping.

**RESULTS AND DISCUSSION**

The Carboxyl Terminus of dGK (Residues 170–224) Affects Neither Substrate Specificity nor the Catalytic Turnover Rate of dGK—In a previous report of one chimeric dAK (6), the carboxyl-terminal segment downstream of residue 170 of dAK was shown not to be the structural element responsible for either its dAdo specificity or differentiating its conformational properties from dGK. That chimeric polypeptide, now called Chi-I, is encoded by chi-i (Fig. 1) and consists of the amino terminus of dAK and the carboxyl terminus of dAK joined at residue 170; it behaves just like the independently expressed unmodified dAK. To recapitulate, Chi-I is inactive when expressed alone, but it gains its dAK activity upon heterodimerizing with dGK and can then be further activated heterotropically by the deoxynucleoside of its heterologous partner (6). More recently, to probe the function of this carboxyl-terminal segment in dGK, a somewhat reciprocal chimera, heterodimeric Chi-I' encoded by chi-i' (Fig. 1), was constructed. In this case, a chimeric dGK subunit (the amino terminus of dGK spliced to the carboxyl terminus of dAK at residue 170) is co-expressed and heterodimerized with unmodified dAK. It behaved essentially like native dAK-dGK in terms of the ratio of dGK/dAK basal activities before heterotropic activation and also in the ability of dGuo to activate dAK. Because dAK is inactive by itself, and dGK is only partially active when expressed alone (6), the nearly unaltered ratio of dGK/dAK basal activities suggests that the affinity between the two heterologous subunits within Chi-I' is about the same as the affinity between the unmodified dAK and dGK. We can therefore infer that the carboxyl-terminal polypeptide segment does not contribute to the conformational differences between dAK and dGK.

The Structural Elements Determining dAK's Substrate Specificity and Self-Activity Must Reside Amino-terminal to Residue 120—Chemical cross-linking studies revealed that conformational changes occur near the subunit interface during heterotropic activation, and mutagenesis of the Ras switch I-like sequence on dAK implicates this motif in the transmission of heterotrophic differences between dAK and dGK.
includes conserved motifs such as the Ras switch I-like sequence (residues 153–161, in which dAK and dGK differ somewhat) and the arginine-rich site (residues 140–146, in which dAK and dGK are identical). Due to the uncertain location of the domain boundary, several residues upstream of the arginine-rich region were included in this transplant.

Interestingly, Chi-II was still inactive when expressed alone, and it exhibited dAK activity only when reconstituted into a heterodimer with either dCK or dGK. This observation clearly indicates that the structural elements responsible for dAK's substrate specificity and for dGK's being self-active (i.e. being active when expressed without dAK) must reside further upstream of residue 120. Unlike dAK or Chi-I, whose reconstituted dAK activity can be activated heterotropically (6), Chi-II's reconstituted dAK activity was incapable of heterotropic activation (data not shown). Chi-II's self-inactivity impeded further kinetic studies; nevertheless, it was useful in estimating the affinity between Chi-II and dCK. Earlier, the apparent dissociation constant ($K_d$) of unmodified dAK-dCK was determined to be 40 nM, and the presence of deoxycytidine does not change the affinity between Chi-II and dCK. Earlier, the apparent dissociation constant ($K_d$) of unmodified dAK-dCK was determined to be 40 nM, and the presence of deoxycytidine does not change the affinity between Chi-II and dCK. Earlier, the apparent dissociation constant ($K_d$) of unmodified dAK-dCK was determined to be 40 nM, and the presence of deoxycytidine does not change the affinity between Chi-II and dCK.

The Heterotropic Activation of “dAK” in Chimera III Was Abolished—To simplify the kinetic studies on Chi-II, Chi-III was constructed. The chi-iii gene is a tandem construct like dak-dgk (Fig. 1), transcribed into a polycistronic messenger RNA followed by separate but presumably simultaneous translation of the two subunits. Subunit heterodimerization occurs either during or immediately after translation. The “dAK” subunit of Chi-III has its amino acid residues 120–170 substituted by the counterpart sequence from dGK, whereas the dGK subunit was unmodified. The Chi-III and native dAK-dGK were expressed in E. coli, and ammonium sulfate fractions were prepared. Because both Chi-I and Chi-II are inactive when expressed alone, the chimeric “dAK” subunit of Chi-III would most likely be inactive if not associated with dGK.

Whereas the dAK activity of dAK-dGK is normally heterotropically activated 4-fold by dGuo (at 400 μM), the dAK activity of Chi-III in the presence of dGuo was instead reduced by about 30% (Fig. 3). There are several possible causes for this heterotropic deactivation: (i) dGuo binding to dGK might induce an adverse conformational change transmitted to “dAK” of Chi-III across the interface, allosterically reducing the dAK activity; (ii) the “dAK” subunit of Chi-III could have a broadened deoxynucleoside substrate specificity, allowing dGuo to compete with dAdo for the active site on “dAK”, thus inhibiting the dAK activity isosterically; and (iii) dGuo binding to dGK might induce partial dissociation of “dAK” and dGK, reducing the dAK activity, because free “dAK” would be inactive.

The third possibility is readily discounted because, as shown above in a comparable case, Chi-II and dAK subunits had nearly equal affinities for dCK in the presence of deoxyctydine; thus, all of the Chi-II subunits would be heterodimeric. Therefore, the lack of heterotropic activation of Chi-II’s reconstituted dAK activity, as well as that of dAK bearing an even smaller substitution, cannot be due to heterodimer dissociation.

The second possibility can also be excluded. In Fig. 3, the half-maximal reduction of Chi-III dAK activity occurs at the $K_m$ (dGuo) concentration (5–10 μM). If the “dAK” subunit in Chi-III had a broader specificity accommodating dGuo to any significant extent, its dAK activity (assayed with 20 μM dAdo) would have been completely blocked by 400 μM dGuo. Therefore, the apparent inhibition of the dAK activity must be due to dGuo binding to the dGK subunit.

Although highly specific for dGuo, the dGK subunit has a secondary dAK activity with a much larger $K_m$ (dAdo) (8). Based on the secondary $K_m$ (dAdo) and $V_{max}$ of the dAMP formation attributed to the dGK subunit, heterodimeric dGK should contribute about 30% of the measured dAK activity under the standard dAK assay conditions. Therefore, the 30% inhibition of the measured dAK activity of Chi-III by dGuo is mostly due to blocking the secondary dAK activity of the dGK subunit. Consequently, it can be concluded that the “dAK” subunit of Chi-III has little, if any, direct response to the binding of dGuo by dGK. The heterotropic response of the “dAK” subunit is therefore largely abolished by replacing residues 120–170 with the counterpart segment from dGK.

Chi-III Has an Elevated dAK Activity—The dAK activity of Chi-III was elevated by about 40% relative to that of dAK-dGK, and like heterotropically activated dAK, this increased activity of “dAK” is mainly a $V_{max}$ effect because the $K_m$ values for the two substrates were not significantly changed. Its $K_m$ (dAdo) was 5–10 μM, and a comparison of $K_m$ (MgATP) values is shown
In Table I. In view of the abolished heterotropic response of “dAK”, one might ideally expect this chimeric dAK to possess the maximal catalytic potential attainable by heterotropically activated native dAK. However, the 40% increase in the activity of chimeric dAK is considerably less than the normal 4-fold activation, and one might suppose that this is due to weakened affinity between “dAK” and dGK. Chi-II has about half the affinity for dCK shown by native dAK, and the “dAK” of Chi-III, with its smaller replaced segment, should bind the dCK peptide at least as strongly, but a slightly reduced affinity between “dAK” and dGK could at least partially explain the less than optimal dAK activity of Chi-III.

The 40% increase in $V_{\text{max}}$ and the lack of significant changes in $K_m$ values together testify to the strong likelihood that the “dAK” polypeptide has not been detrimentally misfolded. In fact, we will show that the “dAK” subunit, with residues 120–170 transplanted from dGK, has already started to behave like a dGK subunit in certain respects.

The “dAK” of Chi-III and Heterotropically Activated dAK of dAK-dGK Both Have Weakened Affinities for MgATP and More Acidic pH Optima—Earlier work from this laboratory has established the utility of the deoxynucleotide triphosphate end products as convenient probes of the steady-state kinetic mechanism by behaving as a bisubstrate analogs (20). For example,

![Fig. 4. Representative double reciprocal plots for kinase activities of different heterodimeric subunits.](image)

- **A**, for dAK of dAK-dGK, the $K_m$(MgATP) value equals the $K_s$(MgATP) value.
- **B**, for dAK of Chi-III, the $K_s$(MgATP) value is larger than the $K_m$(MgATP) value.
- **C**, for the heterotropically activated dAK of dAK-dGK, the $K_s$(MgATP) value is larger than the $K_m$(MgATP) value.
- **D**, for dGK of dAK-dGK, the $K_s$(MgATP) value is larger than the $K_m$(MgATP) value as well.
dATP is competitive with the leading substrate of normal dAK, MgATP, but is noncompetitive with dAdo, the second substrate to bind. Similar experiments with Chi-III reveal that MgATP is still the first substrate to bind to the “dAK” subunit (data not shown); thus, in this respect, the “dAK” subunit remains essentially a dAK subunit.

However, for unmodified dAK, $K_m(MgATP) = K_s$, the dissociation constant of the enzyme-MgATP complex. This was revealed by the primary steady-state kinetics of unmodified dAK without heterotropic activation, in which plot lines converge on the abscissa of double reciprocal graphs (Fig. 4A). By contrast, the plot lines obtained with Chi-III converged on a point above the x-axis, due to the $K_s$ for the enzyme-MgATP complex being substantially larger than the $K_m(MgATP)$ (Fig. 4B). In other words, whereas for native dAK, the $K_s(MgATP)$ value is numerically equal to the $K_m(MgATP)$ value, for the “dAK” subunit of Chi-III, the $K_s(MgATP)$ value has increased, but its $K_m(MgATP)$ value has hardly changed relative to that of native dAK (Table I). It was surprising to discover that heterotropically activated native dAK also has an increased $K_s(MgATP)$ value, whereas its $K_m(MgATP)$ value remains unaltered (Fig. 4C and Table I). Furthermore, dGK of dAK-dGK also has a $K_s(MgATP)$ value larger than its $K_m(MgATP)$ value (Fig. 4D and Table I). Therefore, both the unmodified but heterotropically activated dAK and the “dAK” of Chi-III have started to behave more like dGK in terms of their weakened affinities for the substrate MgATP. Both cases suggest that heterotropic activation may involve a conformational change that shifts the binding preference away from the ground state with the formation of intermediate enzyme species along the kinetic pathway.

The acidic shift in the pH dependence of the dAK activities of the “dAK” of Chi-III and heterotropically activated dAK (Fig. 5) is a further indication that the chimera emulates the activated state. Heterodimeric native dAK has a prominent activity optimum around pH 9.5 (Fig. 5, A and C), but, when heterotropically activated, the dAK pH activity profile becomes broader, with a maximum around pH 8 (Fig. 5B). The “dAK” of Chi-III

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**Fig. 5. pH dependence of the kinase activities.** □, CHES buffer; ■, TES buffer.
also has the more acidic pH optimum (Fig. 5D), again like the activated native dAK. dCK exhibits an optimum velocity around pH 7.5 (9, 21). The pH dependence pattern shared by different subunits (i.e. “dAK,” heterorotopically activated dAK and dCK) suggests that the several active sites may also share a common configuration that is optimal for catalysis, but the rate-limiting kinetic step related to such a pattern remains to be determined.

The segment between residues 120 and 170 is responsible for the conformational difference between dAK and dCK (or dGK). We propose that this segment approximates an important independently folded domain whose conformation is modulated through subunit-subunit interaction during heterotropic activation.

G Proteins, HSV-1 Thymidine Kinase, and Lactobacillus Deoxyribooside Kinases: a Case of a Rosetta Stone—The three-dimensional structures of G proteins (22, 23) and HSV-1 thymidine kinase (24–26) have all been resolved and correlated with their catalytic functions, but a better understanding of the structure-function relationship of the Lactobacillus deoxyribooside kinases still awaits a crystallographic solution. Meanwhile, sequence conservations shared among those proteins provide some clues as to which portions of the Lactobacillus kinases are of functional importance. Part of the information that can be inferred is quite consistent with the results reported here and previously for the Lactobacillus kinases, whereas other inferences suggest intriguing future experimental explorations.

The subunits of the heterodimeric Lactobacillus deoxyribooside kinases have identical arginine-rich motifs followed immediately by (slightly divergent) Ras switch I-like sequences (Fig. 6). This arginine-rich motif is also conserved in HSV-1 TK, along with its family members human dCK, dGK, and TK2, and has been shown to interact with the ATP molecule (24). Ras protein does not possess this counterpart arginine residue in its own switch I (Fig. 6), but it is provided in trans by interacting GAP. This Arg-789 inserted by GAP almost identically mimics the position of Arg-178 of dGK (15). In G\(\alpha_i\), one of the types of \(\alpha\) subunits of the heterotrimetric G proteins, transition state stabilization is carried out by Arg-178 within G\(\alpha_i\)'s own switch I (G-2) sequence (27). Like Ga subunits, Lactobacillus kinases possess in cis arginine residues that presumably are also involved in transition state stabilization. In this regard, and with respect to their Ras-like motifs, these Lactobacillus kinases are hybrid molecules with structural characters resembling both Ras proteins and Ga subunits.

In G proteins, a highly conserved aspartate residue from G-3 participates in coordinating Mg\(^{2+}\) through a water molecule (Ref. 22; Fig. 6). A similar function is carried out in HSV-1 TK by Asp-162 of motif 3 (161FDHP; Ref. 24; Fig. 6). It has been pointed out (26) that this aspartate is at the same relative position as Asp-93 of adenylate kinase (28) and Asp-80 (from G-3) of E. coli elongation factor Tu (one of the Ga proteins; Ref. 29). At first glance, Lactobacillus deoxyribooside kinases do not appear to have G proteins' G-3-like sequences; however, in common with HSV-1 TK (Fig. 6) and human dCK, dGK, and TK2, they share the D(E)RS(H) motif (11–13, 16). Indeed, upon mutating the aspartate residue of the \(70^\text{DRS}\) motif to alanine, glutamate, or asparagine, Lactobacillus deoxyribooside kinase activity was virtually eliminated (30). In HSV-1 TK, 10 residues downstream of Asp-162, Tyr-172 is shown to stack against the thymine base of the substrate, and this tyrosine residue can be functionally replaced by phenylalanine (31). In Lactobacillus deoxyribooside kinases as well as in the human kinases, 10 residues downstream of the aspartate (glutamate) residue of the D(E)RS motif also lies the residue Phe-88. However, the functional importance of this phenylalanine residue remains to be assessed.

According to the data available from mutagenesis (6, 30) and from the chimera constructions presented above, as well as the preservation of conserved motifs, heterodimeric Lactobacillus deoxyribooside kinases can be considered to possess at least three of the five nucleotide binding motifs (or their functional equivalents) commonly identified in G proteins. Those three motifs in Lactobacillus kinases, listed in order from amino to carboxyl terminus, are the P-loop, the G-3 equivalent DRS motif, and the Ras G-2 (switch I)-like sequence. During the Ras-GAP interaction and GAP-induced GTPase action, the conformational changes in switch I and switch II (which is just downstream of G-3) are coupled, because both regions are involved in the binding of ATP and Mg\(^{2+}\) (23). In the Lactobacillus deoxyribooside kinases, a roughly 50-residue segment of dAK including the Ras switch I-like sequence is involved in the intersubunit communication, affecting dAK's activity in cis. An additional chimera (Chi-IV, encoded by chi-iv; Fig. 1) was constructed such that dAK was spliced to dGK at residue 65 just amino-terminal to the \(78^\text{DRS}\) motif. Unfortunately, this new chimera was found to be inactive, even when reconstituted into heterodimers with dGK or dCK. Clearly, splicing at this position created a distortion in the dAK tertiary structure rendering it inactive. Additional site-directed mutagenesis studies are needed to individually dissect the roles of amino acid residues upstream of 120, possibly leading to the conversion of dAK to dGK (or dCK) in terms of the deoxyribooside substrate specificity.

The question also remains as to which segment on dGK is the \textit{in trans} structural element for dAK, which transmits the conformational change to dAK during heterotropic activation. It is an intriguing observation that a R79K substitution on dGK had a profound stimulatory effect on dAK's catalytic turnover rate (9, 30). Is dGK's \(70^\text{DRS}\) motif, upon modification, undergoing a conformational change that is in turn propagated to dAK, or is dGK's \(70^\text{DRS}\) directly participating in dAK's catalysis \textit{in trans}? X-ray crystallographic data are indispensable to answer this question.

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