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Antiproliferative plant and synthetic polyphenolics are specific inhibitors of vertebrate inositol-1,4,5-trisphosphate 3-kinases and inositol polyphosphate multikinase.

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In this paper we analyzed putative amino acids involved in inhibitor binding and identified Lys^{268} and Lys^{272} as inhibitor binding sites. However, verification of these results showed that not these amino acids but Lys^{336} is critical for inhibitor binding. The failure was corrected (as shown in bold) in the updated sections that follow.

PAGE 13229:

In the Abstract, the sentence in lines 25–28 should read: "Mutagenesis studies revealed that both the calmodulin binding and the **ATP** binding domains in IP3K are involved in inhibitor binding."

PAGE 13235:

In the right column, under the subheading "Search for Inhibitor Binding Sites," lines 31–47 should read: "The point mutant **K336Q** revealed a drastically reduced inhibition by THF (Table VII). Substitution of **Lys^{336}** led to a 260-fold increase in the IC_{50}. On the other hand, kinetic parameters of the enzyme with respect to both substrates were nearly unchanged (Table VII). Thus, by substituting **lysine 336, which is involved in ATP binding** and located in the **C-lobe** (37, 38), we have created inhibitor-resistant IP3K-A with full enzymatic activity and normal substrate affinity. As shown in the sequence alignment of IP3K isoforms and IPMK in **Fig. 4*B*, this region shows high homology between IP3K isoforms but not between IP3K and IPMK.** Both the absence of the CaM binding domain (above) **and the differences in the ATP binding site** (55) may thus contribute to the observed differences in inhibitor selectivity of IPMK as compared with IP3K (see Fig. 1*C*).

PAGE 13237:

Table VII has been updated.

Fig. 4*B* has been corrected as shown, and its legend should read: **"Identification of sites of GgIP3K-A involved in inhibitor binding.** Partial sequences from the catalytic domains of mammalian IP3K isoforms and HsIPMK comprising the **"GFR"** motif (*underlined*) and parts of the ATP binding domain downstream of this motif (55) are aligned. **The amino acid** in GgIP3K-A (*bold*) shown by mutagenesis to be involved in inhibitor binding (**Lys^{336}**) **is** *boxed*."

PAGE 13238:

In the right column, under the subheading "Structural Basis of Inhibitor Selectivity," lines 1–16 should read: "The markedly differing inhibitor selectivity of IP3K and IPMK reflects different inhibitor binding sites within these two homologous enzymes (see the structural sketch in Fig. 4*A*). One important position in the IP3K catalytic domain for inhibitor binding seems to be located within the ATP binding segment, since substitution of **Lys^{336}** downstream of the **GFR** motif (Ref. 56, see Fig. 4) caused a 260-fold decrease of the inhibitory effect. No corresponding **Lys residue** is present in IPMK (see Fig. 4*B*). The location of **Lys^{336}** in the three-dimensional structure of IP3K-A (37, 38) indicates that **it is** located in the **C-lobe and is involved in ATP** binding. An interaction of inhibitor with **this** side chain thus may induce a major conformational change modifying the nucleotide binding site."

TABLE VII

Kinetic constants and IC_{50} values for THF inhibition of mutated or truncated IP3K isoforms or IPMK

Enzyme	K_m $InsP_3$	K_m ATP	Specific activity	Inhibitor	IC_{50}
	μM	μM	*units/mg*		μM
RnIP3K-$C_{CaM/cat}$ [a]	0.2	33	3	THF	0.19
RnIP3K-C_{cat} [b]	1.7	42	16	THF	**0.61**
GgIP3K-A [a]	0.40	74	15	THF	0.29
GgIP3K-A_{K336Q} [c]	0.54	105	16	THF	**84.3**
HsIPMK wt	0.35	17	2.5	THF	>100
				Others	See Table V
HsIPMKΔ [d]	0.40	39	5.5	THF	**0.52**
				Others	See Table V

[a] Fragment comprising CaM binding and catalytic domain (assay without Ca^{2+}-CaM).
[b] Fragment comprising only catalytic domain.
[c] Fragment comprising CaM binding and catalytic domain. **Lys^{336}** is substituted by Gln (assay without Ca^{2+}-CaM).
[d] **Deletion of amino acids 266–371.**

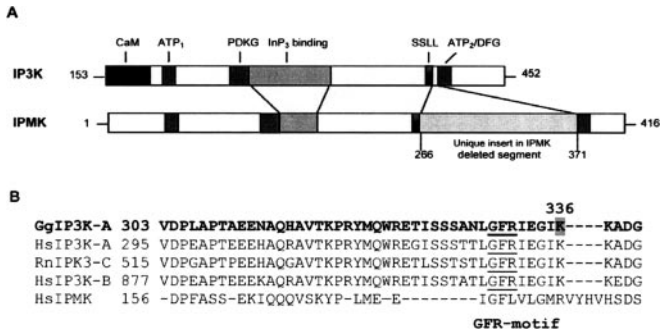

FIGURE 4.

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