Arginine kinase evolved twice: evidence that echinoderm arginine kinase originated from creatine kinase

Tomohiko SUZUKI^{*1}, Mika KAMIDOCHI[†], Naho INOUE^{*}, Hozumi KAWAMICHI^{*}, Yoichi YAZAWA[‡], Takahiro FURUKOHRI^{*} and W. Ross ELLINGTON[‡]

^{*}Laboratory of Biochemistry, Faculty of Science, Kochi University, Kochi 780-8520, Japan, [†]Hokkaido University of Education at Asahikawa, Asahikawa, Hokkaido 070-0825, Japan, and [‡]Department of Biological Science and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-4370, U.S.A.

Arginine kinase (AK) was isolated from the longitudinal muscle of the sea cucumber *Stichopus japonicus*. Unlike the monomeric 40 kDa AKs from molluscs and arthropods, but like the cytoplasmic isoenzymes of vertebrate creatine kinase (CK), the *Stichopus* enzyme was dimeric. To explore the evolutionary origin of the dimeric AK, we determined its cDNA-derived amino acid sequence of 370 residues. A comparison of the sequence with those of other enzymes belonging to the phosphagen kinase family indicated that the entire amino acid sequence of *Stichopus* AK is apparently much more similar to vertebrate CKs than to all other AKs. A phylogenetic tree also strongly suggests that the *Stichopus* AK has evolved from CK. These results support the conclusion that AK evolved at least twice during the evolution of phosphagen kinases: first at an early stage of phosphagen kinase evolution (its descendants are molluscan and arthropod AKs) and secondly from CK later in metazoan evolution. A comparison of the amino acid sequence

around the guanidino specificity (GS) region (which is a possible candidate for the guanidine substrate recognition site in the phosphagen kinase family) of the *Stichopus* enzyme with those of other phosphagen kinases showed that the GS region of the *Stichopus* enzyme was of the AK type: five amino acid deletions in the flexible loop region that might help to accommodate larger guanidine substrates in the active site. The presence of the AK-type deletions in the *Stichopus* AK, even though it seems that the enzyme's most immediate ancestor was probably CK, strongly suggests that the GS region has a role in substrate specificity. *Stichopus* AK and presumably other echinoderm AKs seem to have evolved from the CK gene; the sequence of GS region might have been replaced by the AK type via exon shuffling. The presence of an intron near the GS region in the *Stichopus* AK gene supports this hypothesis.

Key words: arginine kinase, creatine kinase, evolution.

INTRODUCTION

Phosphagen (guanidino) kinases constitute a family of highly conserved enzymes that catalyse the reversible transfer of phosphate from a phosphagen such as creatine phosphate. Creatine kinase (CK), the most widely studied member of this family, has a central role in both temporal and spatial ATP buffering in cells that display high and variable rates of energy turnover [1,2]. The remaining major members of this family [arginine kinase (AK), glycocyamine kinase (GK), lombricine kinase (LK), taurocyamine kinase and hypotaurocyamine kinase] have not been investigated as extensively as CK but probably have a similar physiological role [2–4].

The molecular properties of CK have been the subject of intense study over the past 40 years, recently culminating in the publication of a crystal structure for the enzyme [5]. In birds and mammals, CK exists as three dimeric, cytoplasmic isoenzymes (MM-, MB- and BB-CK) [6] as well as two mitochondrial (Mi) isoforms (Mia, acidic; Mib, basic) that assemble *in vivo* into homo-octamers (Mia-CK; Mib-CK) and dimers [7]. Lower vertebrates contain a number of dimeric, cytoplasmic CKs [8]; echinoderms, which have close affinities with chordates, have a unique contiguous trimeric CK that is present in sperm flagella [9] and probably in somatic tissues [10]. Furthermore, Wyss et al. [11] have shown that echinoderm sperm possess an Mi-CK that exists primarily as octamers. Cladistic analyses of available

vertebrate and echinoderm CK sequences have shown that both cytoplasmic and Mi-CK isoforms arose before the point of divergence of echinoderms and chordates [8].

AK is widely distributed in the lower and higher invertebrate groups including echinoderms; it is present in many lower chordates but is absent from the vertebrates [3,12]. Conventional wisdom would suggest that AK is the most primitive member of the phosphagen kinase family and that the other members, including CK, arose from tandem gene duplications and subsequent divergence [3,13,14]. This perspective is based on its use of the amino acid arginine rather than some secondarily derived guanidine compound as a substrate, its presence in the bulk of the invertebrate phyla including the most primitive groups and, lastly, the fact that AK is generally found as 40 kDa monomers in contrast with other phosphagen kinases, which typically are multimers. A crystal structure for the transition-state analogue complex (TSAC) of the monomeric AK from the horseshoe crab has been published recently [15].

Echinoderms, as deuterostomes, stand at the base of the chordate line [16]. Therefore early observations that both CK and AK (or their corresponding phosphagens) were present in this group attracted considerable attention [17–19] and ultimately led to the conclusion that echinoderms are in transition from the AK to the CK system [10,12,20,21]. Echinoderm AKs are all dimeric (see, for example, [10]) and can form ephemeral heterodimers with vertebrate cytoplasmic CK subunits [3,21,22], in-

Abbreviations used: AK, arginine kinase; CK, creatine kinase; GK, glycocyamine kinase; GS, guanidino specificity; LK, lombricine kinase; Mi, mitochondrial; TSAC, transition-state analogue complex.

¹ To whom correspondence should be addressed (e-mail suzuki@sc.kochi-u.ac.jp).

The nucleotide sequence data reported will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number AB025275.

dicating structural similarity in subunit association sites. Thus echinoderm AKs and vertebrate cytoplasmic CKs have probably shared a common immediate ancestor, although the exact evolutionary relationships are not clear.

To elucidate these relationships we have purified enzyme protein and obtained the cDNA and deduced amino acid sequences for a dimeric AK from an echinoderm, the sea cucumber *Stichopus japonicus*. We report the first cDNA and deduced amino acid sequences for an AK from a deuterostome. Our results show that this AK is much more closely related to CKs (and other CK-related phosphagen kinases) than to other metazoan AKs. Thus echinoderm AKs seem to have evolved from a CK-like ancestral protein.

MATERIALS AND METHODS

S. japonicus was purchased from the seafood market in Kochi, Japan. AK was isolated from the longitudinal muscle of *S. japonicus*. All procedures were performed at 4–8 °C. The muscle (5.3 g) was homogenized with 100 ml of 20 mM Tris/acetate buffer, pH 8.1, and the tissue extract was fractionated with 40–70% satd. $(\text{NH}_4)_2\text{SO}_4$. The precipitate was dissolved in a minimum volume of the same buffer and applied to an Ultrogel AcA 34 column (3 cm × 90 cm) equilibrated with the same buffer. The fractions possessing AK activity were pooled and applied to a DEAE-5PW column (7.5 cm × 75 mm; Tosoh) equilibrated with 20 mM Tris/acetate buffer, pH 8.1, containing 0.2 mM dithiothreitol. The column was washed with the same buffer, then eluted with a linear gradient of 0–500 mM NaCl in 20 mM Tris/acetate buffer. The purified AK was stored on ice or at –80 °C until use. Enzyme activity was assayed spectrophotometrically [4].

The molecular mass of native AK was estimated on a gel-filtration column of Superdex 75 (1 cm × 30 cm; Pharmacia) equilibrated with 20 mM Tris/phosphate buffer, pH 7.2. The column was eluted with the same buffer at a flow rate of 0.5 ml/min and calibrated with rabbit CK (80 kDa) and squid and octopus AKs (40 kDa). The isolated protein was pyridyl-ethylated and digested with lysyl endopeptidase at an enzyme-to-substrate ratio of 1:100 in 50 mM Tris/HCl, pH 8.0, at 37 °C for 6 h. The digested products were isolated by reverse-phase chromatography. The column (Asahipak ODP-50, 4.6 mm × 150 mm) was equilibrated with 0.1% (v/v) trifluoroacetic acid and eluted with a linear gradient of 0–99.9% (v/v) acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. Some peptides were purified further by rechromatography. The amino acid sequences of lysyl endopeptidase peptides were determined by an automated protein sequencer (Applied Biosystems 476A).

mRNA was prepared from the longitudinal muscle of *S. japonicus* with a QuickPrep Micro mRNA Purification Kit (Pharmacia). The single-stranded cDNA was synthesized with Ready-To-Go You-Prime First-Strand Beads (Pharmacia), with the oligo(dT) adaptor as a primer. The 3' half of the cDNA of *Stichopus* AK was first amplified for 30 cycles, each consisting of 1 min at 94 °C for denaturation, 1.5 min at 55 °C for annealing and 2 min at 72 °C for primer extension, by PCR. AmpliTaq Gold (PE Applied Biosystems) or Extra Taq DNA polymerase (Takara) was used as the amplifying enzyme. The primers used were the oligo(dT) adaptor and a 512-fold 'universal' redundant oligomer, GT(ACGT)TGG(AG)T(ACGT)AA(TC)GA(AG)G-A(AG)GA(TC)CA, designed for the amplification of phosphagen kinases [23]. The 800 bp products were sequenced directly with a PRISM dye terminator cycle-sequencing FS ready reaction kit (Applied Biosystems) and a Model 373-18 DNA sequencer

(Applied Biosystems), with the universal redundant oligomer as a sequence primer.

The 5' half of the cDNA was amplified as follows. The single-stranded cDNA was newly synthesized with the non-redundant primer ScAK.R1; the poly(A)⁺ tail was added to the 3' end with a terminal deoxynucleotidyltransferase. Then the 5' half of the cDNA was amplified by the method described above, with the oligo(dT) adaptor and the non-redundant primer ScAK.R2. The 800 bp products were sequenced directly.

The genomic DNA was purified from a single specimen of *S. japonicus* with a conventional phenol/chloroform method; 1 µg of genomic DNA and two non-redundant primers ScAK.F1 and ScAK.R3 (1 pmol of each, see Figure 1 for the sequence) were used to amplify a DNA fragment containing the guanidine substrate recognition region by PCR. The PCR products of 2000 bp were partly sequenced by direct sequencing.

RESULTS

AK from the longitudinal muscle of *S. japonicus* was purified to homogeneity by $(\text{NH}_4)_2\text{SO}_4$ fractionation, gel-filtration on an Ultrogel AcA 34 column and ion exchange on a DEAE-5PW column. SDS/PAGE in the presence of a reducing agent showed that the isolated AK was highly purified and that the molecular

Figure 1 Nucleotide and deduced amino acid sequences of cDNA of *S. japonicus* AK

Arrows indicate primers used for amplification. Amino acid sequences determined chemically are underlined.

Figure 2 Alignment of amino acid sequences of *S. japonicus* AK with those of rabbit muscle CK, chicken Mi-CK, *Limulus* AK and abalone (*Nordotis*) AK

This alignment was obtained with the method of Feng and Doolittle [29]. Dashes correspond to alignment gaps. Residues enclosed in boxes correspond to highly conserved regions. Several deletions/insertions characteristic of CKs and AKs are shown by bold bars.

mass of subunit was approx. 42 kDa (results not shown). *Stichopus* AK was eluted from a Superdex 75 column at the same retention time as that of rabbit CK. Thus the mass of the native form was estimated to be approx. 84 kDa. We emphasize here that the isolated enzyme was absolutely specific for arginine, and showed no significant enzymic activity towards other guanidine substrates such as creatine, glycyamine or taurocyamine. No N-terminal amino acid of the whole protein was detected by protein sequencing, indicating that the N-terminus is blocked. The isolated enzyme was pyridylethylated and digested with lysyl endopeptidase. The peptides were purified by reverse-phase chromatography; eight peptides, L1–L8, were sequenced (Figure 1).

The 3' half of the cDNA of *Stichopus* AK was amplified by PCR with the phosphagen consensus primer and the oligo(dT) adaptor. Two new oligomers, SCAK.R1 and SCAK.R2, were designed for amplification of the 5' half. These 3' and 5' products were sequenced directly and the complete nucleotide sequence of 1439 bp of cDNA of *Stichopus* AK was determined (Figure 1). The sequence contains 27 and 301 bp of 5' and 3' untranslated sequences respectively. The open reading frame is 1113 nt long and encodes a protein with 370 amino acid residues. The molecular mass was calculated to be 42044.4 Da. The cDNA-derived amino acid sequence of *Stichopus* AK was completely consistent with the chemical sequencing of eight lysyl endopeptidase peptides (underlined in the amino acid sequence of Figure 1). This is the first dimeric AK ever to be sequenced.

Sequencing of the amplified PCR product of genomic DNA revealed that there is an intron (2 kbp) that separates the codon GAT of Asp-60. The partial sequence of this intron was

determined to be as follows: Ggtacgtatataaagacatta-(2 kbp)-cttattttttttcttaagAT.

DISCUSSION

AK is most widely distributed phosphagen kinase and is thought to be closer to the ancestral enzyme in this enzyme family [3,8,13,14,24]. Most AKs have a monomeric structure; however, there is also clear-cut evidence for dimeric AKs in all echinoderms investigated so far [3,10,20,22], which is also true of AK from *S. japonicus*. This dimeric structure is shared by other phosphagen kinases such as GK [25], LK [26,27] and taurocyamine kinase [26]. Furthermore, it has recently been shown that cytoplasmic CK from the protostome polychaete *Chaetopterus variopedatus* is dimeric in structure [28]. Therefore it is clear that dimeric quaternary structure is widespread throughout the phosphagen kinases.

The cDNA-derived amino acid sequence of 370 residues for the dimeric AK from *S. japonicus* was aligned with those of 12 CKs, a GK, an LK and the 15 AKs sequenced so far, by using the alignment program of Feng and Doolittle [29]. The alignment showed that the *Stichopus* dimeric AK has 58–62% sequence identity with vertebrate CKs, 54–56% with invertebrate CKs (sea urchin sperm flagellar CKs), 51–53% with GK and LK, and 33–47% with all AKs. Thus it is evident that the entire sequence of *Stichopus* dimeric AK is more similar to CKs than to AKs.

Of special interest in terms of the structure of phosphagen kinases is the question of which residues are associated with the recognition of the different substrates, creatine, glycyamine, arginine and lombricine. An alignment of representative

	1	5	#	10	15	20
GK- <i>Neanthes</i>	TGVDNPGNK	FY	G	K	K	TGCVFGD
LK- <i>Eisenia</i>	PSVDNT				G	RIIGLVAGD
CK-Human.M	TGVDNPGHPF					IMTVGCVAGD
CK-Rabbit.M	TGVDNPGHPF					IMTVGCVAGD
CK-Chicken.M	TGVDNPGHPF					IMTVGCVAGD
CK-Torpedo	TGVDNPGHPF					IMTVGCVAGD
CK-Human.B	TGVDNPGHPY					IMTVGCVAGD
CK-Rabbit.B	TGVDNPGHPF					IMTVGCVAGD
CK-Chicken.B	TGVDNPGHPF					IMTVGCVAGD
CK-Chicken.Mi	TGVDNPGHPF					IKTVGMVAGD
CK-Human.Mi	TGVDNPGHPF					IKTVGMVAGD
CK-SeaUrchin.D1	TGVDNPGHPF					IKTVGLVAGD
CK-SeaUrchin.D2	TGVMNPGHPH					IMTVGMVAGD
CK-SeaUrchin.D3	TGIDNPGHPY					IMTVGLVAGD
↓ Intron						
AK-SeaCucumber	NGVDNPD					FHLGLLAGD
AK-Lobster	SGVENLD					SGVGIYAPD
AK-Shrimp	SGVENLD					SGVGIYAPD
AK-GrassHopper	SGLENHD					SGVGIYAPD
AK-Limulus	SGVENLD					SGVGIYAPD
AK-Drosophila	SGLENHD					SASAIYAPD
AK-C.elegance	SGVANLD					SGVGVIYAPD
AK-SeaAnemone.D1	SGVVNLD					SGVGVIYAGD
AK-SeaAnemone.D2	SGVENLD					SSCGVIYAGD
AK-Battilus	SGCLNLD					SGVGIYACD
AK-Abalone	SGCLNLD					SGVGIYACD
AK-Chiton	SGAKNLD					SGVGVIYASD
AK-Pseudocardium.D1	SGALHPG					SKVGIYACD
AK-Pseudocardium.D2	SGSLHLD					SKVGIYACD
PK-Schistosoma.D1	TNAYNPG					ALLPR SCD
PK-Schistosoma.D2	NVH					TIIVQYVHE

Figure 3 Alignment of the amino acid sequences of the GS region

The seventh residue in the GS region is indicated by #. Abbreviations: B, brain type; M, muscle type; D1, domain 1 of two- or three-domain phosphagen kinase; D2, domain 2; D3, domain 3; PK, phosphagen kinase.

sequences (rabbit muscle CK [30], chicken Mi-CK [31], abalone (*Nordotis*) AK [28], *Limulus* AK [32] and *Stichopus* AK) is shown in Figure 2. In the alignment there are several deletions/insertions, shown by bold lines, characteristic of CKs and AKs: deletions at residues 67–70 for all AKs, deletions at 127–128 for all CKs (also in GK and LK), a deletion at 200 for all AKs, and deletions at residues 267–270 for all AKs. From an earlier amino acid sequence alignment of CKs, GK, LK and AKs, we proposed a region displaying remarkable amino acid deletions (referred to as the GS region) as a possible candidate for the guanidine recognition site [27]. This region is represented by deletions 67–70 in Figure 2. We again emphasize here that the amino acid sequence of *Stichopus* AK shares the same deletions/insertions as those for CKs, except for the GS region.

This GS region is overlapped partly by the so-called flexible loop in the crystal structure of chicken Mi-CK that has been speculated to move nearer to the active site so as to exclude water during catalysis [5]. This movement has been confirmed in the recent TSAC AK crystal structure [15]. We compare the amino acid sequence around the GS region of *Stichopus* enzyme with those of other phosphagen kinases in Figure 3. There is a proportional relationship between the size of the deletion in GS region and the mass of guanidine substrate used [27]: namely, LK and AK, which use relatively large guanidine substrates, lombricine and arginine, have a five-residue deletion in this region; CK has one-amino acid deletion; and GK has no deletion. It should be noted that the size of the deletion in the GS

Figure 4 Phylogenetic tree constructed from 30 sequences of phosphagen kinases

The tree was constructed with the maximum-likelihood method [33]. The numbers at each branching point show the quartet puzzling support value (%) [33], which can be considered as comparable with the bootstrap value. The bar in the lower left corner represents 0.1 amino acid substitution per amino acid site for the branch length. Abbreviations: B, brain type; M, muscle type; D1, domain 1 of two- or three-domain phosphagen kinase; D2, domain 2; D3, domain 3; PK, phosphagen kinase.

region of *Stichopus* enzyme is of the AK type, even though the enzyme has a high similarity to CKs in its entire amino acid sequence.

The crystal structure of the TSAC AK, which contains the substrates bound in the active site, is consistent with this hypothesized role for the length of the flexible loop in determining some aspects of guanidine specificity [15]. If our hypothesis that echinoderm AK evolved from an ancestral CK is correct, then the deletion must have occurred during the process of acquisition of arginine specificity. The partial sequence of the gene for *Stichopus* AK is suggestive of a mechanism for such a deletion. The codon for Asp-60 (the seventh residue in Figure 3) is split by a very large 2 kb intron. Therefore it is possible that an exon-shuffling type mechanism might have been responsible for the observed change in *Stichopus* AK.

The phylogenetic tree for all sequences, constructed with the maximum-likelihood method [33], is shown in Figure 4. The phylogenetic tree separates the sequences into two major clusters, a cluster containing protostome AKs and the trematode *Schistosoma* phosphagen kinase [34], and a cluster containing all CKs, GK, LK and the *Stichopus* AK. All AK sequences, except that

of *Stichopus*, are apparently distant from the sequences of CK, GK and LK.

In the latter cluster, the LK and GK sequences form a subcluster with a high probability (87%) (Figure 3). Vertebrate and sea-urchin flagellar CKs have high sequence identity (60–68%) and form the CK subcluster. *Stichopus* AK is placed just outside the CK subcluster. Thus it seems that CK, GK, LK and *Stichopus* AK probably evolved from a common immediate ancestral protein. Because the distribution of GK, LK and dimeric AK is restricted to annelids, allied protostome worm groups and echinoderms, the direct ancestor for these enzymes might well be CK. Although the amino acid sequences of taurocyamine kinase and hypotaurocyamine kinase have not yet been determined, we assume that these enzymes will also be included in the subcluster for LK and GK. This is supported by the observation that LKs use both lombricine and taurocyamine as substrates [27]. Therefore it seems probable that members of the phosphagen kinase family are derived from two genes, for AK and for CK (Figure 4), which originally resulted from the duplication of an ancestral gene (probably the gene for AK) at an early stage of phosphagen kinase evolution.

Our phylogenetic analyses of *Stichopus* dimeric AK show clearly that AKs have appeared independently twice in the evolution of phosphagen kinase: first at an early stage of phosphagen kinase evolution (its descendants are molluscan and arthropod monomeric AKs) and secondly from CK later in metazoan evolution. It should be noted that, although *Stichopus* AK is dimeric and has more a similar amino acid sequence to that of CKs, it is absolutely specific for arginine as in the molluscan and arthropod AKs. The two independent origins of AKs constitute a rather unusual occurrence in protein evolution. This fact also underscores the possibility that substrate recognition is controlled by a very restricted region in phosphagen kinases. This is an area that is currently under intense investigation by our groups.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and U.S. National Science Foundation grant IBN-9631907 to W.R.E.

REFERENCES

- Meyer, R. A., Sweeney, H. L. and Kushmerick, M. J. (1984) *Am. J. Physiol.* **246**, C365–C377
- Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K. and Eppenberger, H. M. (1992) *Biochem. J.* **281**, 21–40
- Watts, D. C. (1971) in *Biochemical Evolution and the Origin of Life* (Schoffeniels, E., ed.), pp. 150–173, North-Holland, Amsterdam
- Ellington, W. R. (1989) *J. Exp. Biol.* **143**, 177–194
- Fritz-Wolf, K., Schnyder, T., Wallimann, T. and Kabsch, W. (1996) *Nature (London)* **381**, 341–345
- Eppenberger, H. M., Dawson, D. M. and Kaplan, N. O. (1967) *J. Biol. Chem.* **242**, 204–209
- Wyss, M., Smeitink, J., Wevers, R. A. and Wallimann, T. (1992) *Biochim. Biophys. Acta* **1102**, 119–166
- Muehlebach, S. M., Gross, M., Wirz, T., Wallimann, T., Perriard, J.-C. and Wyss, M. (1994) *Mol. Cell. Biochem.* **133/134**, 245–262
- Wotho, D. D., Charbonneau, H. and Shapiro, B. M. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5203–5207
- Ratto, A., Shapiro, B. M. and Christen, R. (1989) *Eur. J. Biochem.* **186**, 195–203
- Wyss, M., Maughan, D. and Wallimann, T. (1995) *Biochem. J.* **309**, 255–261
- Watts, D. C. (1975) *Symp. Zool. Soc. Lond.* **36**, 105–127
- Van Thoai, N. (1968) in *Homologous Enzymes and Biochemical Evolution* (Van Thoai, N. and Roche, J., eds.), pp. 199–229, Gordon & Breach, New York
- Suzuki, T., Kawasaki, Y. and Furukohri, T. (1997) *Biochem. J.* **328**, 301–306
- Zhou, G., Somasundaram, T., Blanc, E., Parthasarathy, G., Ellington, W. R. and Chapman, M. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8449–8454
- Conway Morris, S. (1993) *Nature (London)* **361**, 219–225
- Needham, D. M., Needham, J., Baldwin, E. and Yudkin, W. H. (1932) *Proc. R. Soc. Lond.* **110**, 260–294
- Baldwin, E. and Yudkin, W. H. (1950) *Proc. R. Soc. Lond.* **B 136**, 614–631
- Griffiths, D. E., Morrison, J. F. and Ennor, A. H. (1957) *Biochem. J.* **65**, 612–617
- Moreland, B., Watts, D. C. and Virden, R. (1967) *Nature (London)* **214**, 458–462
- Reddy, S. R. and Watts, D. C. (1994) *Comp. Biochem. Physiol.* **108B**, 73–78
- Seals, J. D. and Grossman, S. H. (1988) *Comp. Biochem. Physiol.* **89B**, 701–707
- Suzuki, T. and Furukohri, T. (1994) *J. Mol. Biol.* **237**, 353–357
- Morrison, J. F. (1973) in *The Enzymes* (Boyer, P. C., ed.), pp. 457–486, New York, Academic Press
- Suzuki, T., Nishimura, Y., Umekawa, U., Yamamoto, Y., Kawamichi, H. and Furukohri, T. (1999) *J. Protein Chem.* **18**, 13–19
- Oriol, C., Landon, M. F. and van Thoai, N. (1970) *Biochim. Biophys. Acta* **207**, 514–521
- Suzuki, T., Kawasaki, Y., Furukohri, T. and Ellington, W. R. (1997) *Biochim. Biophys. Acta* **1348**, 152–159
- Ellington, W. R., Roux, K. and Pineda, G. (1998) *FEBS Lett.* **425**, 75–78
- Feng, D. F. and Doolittle, R. F. (1987) *J. Mol. Evol.* **25**, 351–360
- Putney, S., Herlihy, W., Royal, N., Pang, P., Aposhian, H. V., Pickering, L., Belagaje, R., Biemann, K., Page, D., Kuby, S. and Schimmel, P. (1984) *J. Biol. Chem.* **259**, 14317–14320
- Ordahl, C. P., Evans, G. L., Cooper, T. A., Kunz, G. and Perriard, J.-C. (1984) *J. Biol. Chem.* **259**, 15224–15227
- Strong, S. J. and Ellington, W. R. (1995) *Biochim. Biophys. Acta* **1246**, 197–200
- Strimmer, K. and von Haeseler, A. (1996) *Mol. Biol. Evol.* **13**, 964–969
- Stein, R. K., Harn, D. A. and David, J. R. (1990) *J. Biol. Chem.* **265**, 6582–6588

Received 12 October 1998/1 March 1999; accepted 30 March 1999