Tosi, M., Duponchel, C., Meo, T., & Julier, C. (1987) *Biochemistry 26*, 8516–8524.
Villiers, C. L., Arlaud, G. J., & Colomb, M. G. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4477–4481.
Warburg, O., & Christian, W. (1941) *Biochem. Z. 310*, 384–421.
Weiss, V., Fauser, C., & Engel, J. (1986) *J. Mol. Biol. 189*, 573–581.

Amino-Terminal Nucleotide-Binding Sequences of a *Lactobacillus* Deoxynucleoside Kinase Complex Isolated by Novel Affinity Chromatography†

Seiichiro Ikeda, Richard P. Swenson, and David H. Ives*
Department of Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210
Received May 10, 1988; Revised Manuscript Received July 27, 1988

ABSTRACT: A highly efficient new affinity medium for deoxycytidine kinase, deoxycytidine 5′-tetraphosphate–Sepharose (dCp_4–Sepharose), has been constructed. A dCp_4–Sepharose column effects a one-step, 19 000-fold, purification to homogeneity of dCyd kinase from the ammonium sulfate fraction of *Lactobacillus acidophilus* R-26 extract, with 60% recovery. dCTP, a potent end-product inhibitor, is used as an eluent, and it also stabilizes the extremely labile purified enzyme. A noncompeting deoxyadenosine kinase activity accompanies the deoxycytidine kinase activity eluted. Native polyacrylamide gel electrophoresis shows a single protein band, which coincides with both deoxycytidine kinase and deoxyadenosine kinase activities at several gel concentrations. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis reveals a single polypeptide band of 26 000 daltons. Since the native enzyme is known to have an M_r of 50 000, it appears that the enzyme is composed of two subunits of similar size. Sequence analysis of the intact protein from the N-terminus reveals but a single amino acid species per residue up to the 17th residue; at the 18th, 21st, 26th, and 27th residue positions of the sequence, however, there appear to be two different amino acids in almost equal amounts. This may indicate that the enzyme is composed of two nonidentical subunits having the same amino acid sequence near the N-terminus. Residues 6–13 contain the highly conserved Gly-X-X-Gly-X-Gly-Lys sequence found at the active sites of kinases and other nucleotide-binding proteins.

Lactobacillus acidophilus R-26 has two unique paired deoxynucleoside kinases, namely, dCyd/dAdo kinase and dGuo/dAdo kinase (Deibel & Ives, 1977a). Kinetic studies have revealed that these two paired activities do not share a common active site in either case but exhibit positive allosteric interactions (Deibel et al., 1977; Chakravarty et al., 1984). The dGuo/dAdo enzyme was purified to homogeneity in very small amounts, with a combination of broadly specific affinity media, and shown to consist of a monomeric polypeptide. Selective chemical inactivation of its dGuo phosphorylation site concurrently eliminated the ability of dGuo, mediated by that site, to stimulate dAdo phosphorylation (Chakravarty et al., 1984).

In sharp contrast with the *Lactobacillus* enzyme, competition and mutation experiments with human cytosol dCyd kinase have revealed that its multiple deoxynucleoside specificities are associated with a common active site, which phosphorylates dCyd, dAdo, and dGuo with varying efficiencies (Verhoef et al., 1981; Hershfield et al., 1982; Bohman & Eriksson, 1988). A human mitochondrial deoxypyrimidine kinase isoenzyme also appears to phosphorylate both dCyd and dThd at the same active site (Lee & Cheng, 1977). Only one other bacterial dCyd kinase has been characterized; a dCyd/dAdo kinase isolated from *Bacillus subtilis* exhibited weak mutual inhibition by these substrates, while dCTP or dATP was each most effective in inhibiting the phosphorylation of its own cognate deoxynucleoside (Møllgard, 1980). This behavior suggests the possibility of separate but noninteracting sites on the *B. subtilis* kinase.

Studies on the structure–function relationship of the interesting multifunctional-type deoxynucleoside kinases of *L. acidophilus* have been impeded by the extremely small amounts of purified proteins attainable by conventional purification procedures (Chakravarty et al., 1984). We have developed several affinity media using deoxynucleosides linked to Sepharose through the 3′-hydroxyls or through various positions on the purine or pyrimidine bases. Although deoxynucleoside kinases from other sources were purified successfully on some of these media, none of them retained the bacterial kinases effectively (Ikeda et al., 1984). Greater success was attained with multisubstrate-type affinity media (dNp_4A–Sepharose) directed specifically toward the dCyd, dAdo, or dGuo sites (Ikeda & Ives, 1985). Copurification of two activities (dCyd/dAdo or dGuo/dAdo kinase) on these media, in conjunction with competition experiments, has provided further proof of the existence of two distinct active sites on each protein. However, even these highly specific media had relatively limited capacity for the *Lactobacillus* kinases. Recently, we have found that natural triphosphate end products (dNTP)[1] bind still more tightly (K_i = 0.4–3 μM)

† This work was supported in part by National Science Foundation Grant DMB-8416134 and Ohio Cancer Research Associates Grant OCRA-88-02-05.
* To whom correspondence should be addressed.

[1] Abbreviations: dNp_4A, deoxynucleoside 5′-adenosine 5‴-P^1,P^4-tetraphosphate; dNTP, deoxynucleoside triphosphate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; dNp_3A, deoxynucleoside 5′-adenosine 5‴-P^1,P^3-triphosphate; dCp_4–Sepharose, deoxycytidine 5′-tetraphosphate–Sepharose.

0006-2960/88/0427-8648$01.50/0

to the active sites of the corresponding bacterial kinase than do the dNp_4A bisubstrate analogues (K_i = 1.4–9.2 μM) (Ikeda et al., 1986). The deoxynucleoside moiety of dNTP fits optimally at the deoxynucleoside binding site and provides the basis for its inhibition specificity, whereas the triphosphate group interacts with the ATP binding site. These multiple binding determinants reinforce the affinity of the molecule, making it a potent end-product inhibitor. It seems that the bulky adenosine portion of dNp_4A does not fit optimally at the ATP binding site, perhaps even interfering with proper alignment of phosphate groups within the ATP site. On the basis of these observations, we have decided to construct another affinity medium in which the elements of dCTP are linked to Sepharose through its terminal phosphate, inserting an extra phosphate group between the dCTP and a hexyl group to replace the fourth negative charge lost in the covalent attachment of dCTP. The resulting ligand appears to provide the basis for the ideal affinity medium for dCyd/dAdo kinase, yielding enough pure enzyme to begin structural analysis of the protein.

Experimental Procedures

Materials. 1,1′-Carbonyldiimidazole, 6-aminohexanol 1-phosphate, and cyanogen bromide were purchased from Aldrich Chemical Co. *S*-Ethyl trifluorothioacetate was obtained from Pierce Chemical Co. The disodium salt of dCTP was from Sigma Chemical Co. *N*,*N*-Dimethylformamide (Fisher) and methanol (Mallinckrodt) were distilled over CaH_2 before use. Tributylamine (Aldrich Chemical Co.) was distilled over ninhydrin. Sepharose CL-6B and Sephadex G-10 were from Pharmacia. Trisacryl M DEAE was obtained from LKB. Tritiated nucleosides and nucleotides were from ICN. Reagents for gel electrophoresis were supplied by Bio-Rad.

Preparation of P^3-[(6-Aminohex-1-yl)phosphoryl]-dCTP (IV). An affinity ligand (IV) for dCyd kinase was newly synthesized by the imidazolide method (Hoard & Ott, 1965) which uses carbonyldiimidazole as phosphate group activating reagent. Hoffmann and Blakley (1975) employed this method to prepare P^3-(6-aminohex-1-yl)-dGTP from dGMP and 6-aminohexanol 1-phosphate (I) as starting materials. In their synthetic scheme, compound I was first converted to the *N*-(trifluoroacetyl) derivative (II). Then, orthophosphate was coupled to compound II with carbonyldiimidazole to give *N*-(trifluoroacetyl)-6-aminohexanol 1-pyrophosphate, which was then reacted with the second component, dGMP, previously activated with carbonyldiimidazole. The final product was obtained by removing the *N*-(trifluoroacetyl) group upon hydrolysis. To synthesize compound IV, we have followed the basic reaction conditions of Hoffmann and Blakley (1975), with the following modifications. First, dCTP and compound II were chosen as coupling components. dCTP was activated with carbonyldiimidazole and then coupled to compound II to give the tetraphosphate derivative. In another preparation, compound II was first activated by carbonyldiimidazole and then coupled to dCTP. However, the yield of the coupling reaction was found to be significantly lower than that in the first case. This is probably because of the disproportionation reaction of dCTP in organic solvent. In our first procedure, however, dCTP is considered not to be subject to this disproportionation since it is converted to the imidazolide in a short time by the activation reaction.

The sodium salt of dCTP (0.786 mmol) was converted to the tributylammonium salt, through the pyridinium salt, by a cation-exchange column. *N*-(Trifluoroacetyl)-6-aminohexanol 1-phosphate (II) was prepared from 6-aminohexanol 1-phosphate (I) according to Barker et al. (1972). On the basis of phosphate analysis, 1.42 mmol of II was obtained from 1.52 mmol of I. The free acid form of II was converted to the tributylammonium salt by treatment with 1 molar equiv of tributylamine. Both starting materials were dried by repeated addition and evaporation of anhydrous DMF and were stored in a vacuum desiccator over P_2O_5.

1,1′-Carbonyldiimidazole (3.93 mmol) was added to a solution of the tributylammonium salt of dCTP (0.786 mmol) in 5 mL of dry DMF. After the solution was stirred at room temperature for 4 h in a desiccator over P_2O_5, 0.159 mL of dry methanol (3.93 mmol) was added, and the solution was stirred for another 30 min. To this solution, 1.42 mmol of compound II in 5 mL of dry DMF was added. The solution was stirred at room temperature in a desiccator over P_2O_5. The formation of new product (III) was detected, along with several byproducts by high-performance liquid anion-exchange chromatography on a Pharmacia FPLC system equipped with a Mono-Q column, using a 0–0.5 M gradient of NaCl in 0.01 N HCl. Compared to the 24-h reaction period reported by Hoffman and Blakley (1975), the present coupling reaction was found to be fairly slow. The stirring was continued for 7 days before the reaction approached completion. After the solvent was evaporated, the residue was dissolved in 10 mL of methanol–H_2O (1:1).

Five milliliters of the solution containing the products was applied to a column of Trisacryl M DEAE (370 mL, formate form) at 4 °C. The column was washed with 100 mL of 50% methanol and then with 500 mL of H_2O. The products were eluted with a gradient generated by 0.2 M ammonium formate (pH 2.0, 2 L) and 0.6 M ammonium formate (pH 2.0, 2 L). Fractions at the major UV-absorbing peak containing the desired product (III) were combined and lyophilized. The residue was dissolved in H_2O and incubated at pH 11.5 at room temperature overnight. The resulting free amine (IV) was desalted through Sephadex G-10, and the aqueous solution was evaporated to dryness (yield 0.129 mmol, or 16.3%, based on the amount of dCTP used). On the basis of the UV absorption of the base, compound IV gave mole ratios of 3.6 and 0.8 per mole of compound for total phosphate and amine, respectively. This compound was found to inhibit *L. acidophilus* dCyd kinase activity competitively versus dCyd [K_i(app) = 61 μM, at 1 mM ATP].

Preparation of Affinity Adsorbent, dCp_4-Bound Sepharose. Compound IV was coupled to Sepharose CL-6B according to the simplified CNBr activation method of March et al. (1974). The amount of bound ligand was calculated to be 1.5 μmol/mL of gel, on the basis of the decrease in the absorbance of the supernatant of the coupling reaction mixture. As an alternative coupling procedure, we attempted to use carbonyldiimidazole-activated Sepharose (Bethell et al., 1979), which has been successfully used for coupling a high concentration of spacer arms in our previous work (Ikeda et al., 1984). However, the substitution was found to be very low (less than 0.1 μmol/mL of gel). This is probably because the concentration of compound IV used in the coupling reaction was somewhat limited (<3 μmol/mL). Under such conditions, it seems that CNBr-activated Sepharose couples this ligand more efficiently.

Enzyme Preparation and Assays. An ammonium sulfate fraction was prepared from the extract of *Lactobacillus acidophilus* R-26 cells (ATCC 11506) as described previously (Chakravarty et al., 1984; Deibel & Ives, 1977b), except that 0.1 mM phenylmethanesulfonyl fluoride was added to the extraction buffer. dCyd kinase and dAdo kinase assays were carried out radiometrically as described by Deibel and Ives

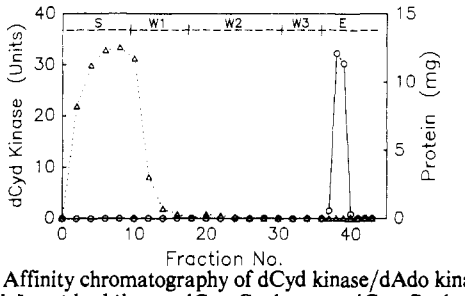

FIGURE 1: Affinity chromatography of dCyd kinase/dAdo kinase from dCK/dAK *L. acidophilus* on dCp_4–Sepharose. dCp_4–Sepharose (18 mL) was packed in a column (1.6-cm diameter) and equilibrated at 4 °C with 15 mM potassium phosphate buffer, pH 8.0, containing 20% glycerol. Three milliliters of an ammonium sulfate fraction of *L. acidophilus* extract, containing 102 units of dCyd kinase (776 mg of protein), was diluted to 60 mL with the equilibration buffer (EB) and applied to the column (step S). Fractions of 6.6 mL were collected. The column was washed with 50 mL of EB, 100 mL of 0.1 M potassium chloride in EB, and 50 mL of EB, successively (steps W1, W2, and W3). Then, dCyd kinase/dAdo kinase was eluted with 30 mL of 0.3 mM dCTP in EB (step E). (○) dCyd kinase activity; (△) protein.

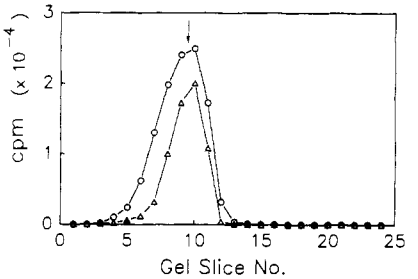

FIGURE 2: Nondenaturing PAGE of purified dCyd kinase/dAdo kinase. Electrophoresis in 10% polyacrylamide and enzyme assays with gel slices were carried out as described under Experimental Procedures. One lane was stained for protein, and a parallel lane was cut into 2-mm slices for enzyme assays. (○) dCyd kinase; (△) dAdo kinase. The arrow denotes the position of the stained band.

(1977b). One unit of activity is defined as the amount producing 1 nmol of deoxynucleoside monophosphate per minute at 20 °C. Protein determinations employed the Bradford (1978) method.

Gel Electrophoresis. The discontinuous Tris–glycine/Tris-HCl buffer system of Laemmli (1970) was used for both denaturing and nondenaturing PAGE. Protein bands were visualized by staining with Coomassie Blue R-250. The enzyme activities were localized by cutting an unstained parallel channel of the nondenaturing gel into 2-mm slices and assaying as described previously (Chakravarty et al., 1984).

Protein Sequence Analysis. Amino acid sequence analyses were performed by automated gas-phase Edman degradation chemistry on an Applied Biosystems Model 470A sequencer at the Ohio State University Biochemical Instrument Center. The phenylthiohydantoin (PTH) amino acid derivatives were identified and quantified by high-performance liquid chromatography (HPLC) on an Applied Biosystem Model 120A system interfaced to the sequencer. The analysis was carried out twice on 25-μg batches of purified dCyd/dAdo kinase.

Results and Discussion

Purification of dCyd Kinase/dAdo Kinase by Affinity Chromatography. Figure 1 shows a typical affinity chromatography elution profile of *L. acidophilus* dCyd kinase on dCp_4–Sepharose. The column proved both to be very specific for dCyd kinase and to have a high capacity for that enzyme. About 100 units of dCyd kinase from an ammonium sulfate fraction was retained on an 18-mL column, with the bulk of other proteins running through. After washing with 0.1 M KCl, about 63 units of dCyd kinase was eluted with the end-product inhibitor, dCTP (0.3 mM), a yield of 62% of the activity applied. dAdo kinase activity accompanied the peak of dCyd kinase activity in the eluate (not shown). After the combined eluate was concentrated to a small volume (50 μL) with a Centricon Microconcentrator (Amicon), the total protein recovered was estimated to be 25 μg, indicating a one-step purification of 19 000-fold relative to the ammonium sulfate fraction. The specific activity of the purified dCyd kinase (2540 units/mg of protein) appears to be about seven times higher than the best preparation obtained so far by repeated Blue Sepharose chromatography (Deibel & Ives, 1977b). We have recently developed another group of affinity media for deoxynucleoside kinases on the basis of the multisubstrate-type inhibitor dNp_4A (Ikeda & Ives, 1985). Although these affinity columns were very useful in distinguishing different kinase species in bacterial extract, their capacity as practical affinity media was found to be limited (about 0.2 unit/mL of gel). Comparative kinetic studies have indicated that dNp_4A does not fit optimally within the active site of a kinase, despite the fact that its dissociation constant is smaller than that of either substrate, indicating that it is indeed a multisubstrate inhibitor. On the other hand, the natural end-product inhibitor, dNTP, has exhibited the tighter binding to the kinase, while functioning also as a multisubstrate analogue (Ikeda et al., 1986). The desirability of using dCTP as affinity ligand has been demonstrated by the above results. In order to compensate for the fourth negative charge lost from the γ-phosphate upon covalent attachment of dCTP, an extra phosphate was inserted between the γ-phosphate and the hexyl linker group. We do not know whether a triphosphate—rather than a tetraphosphate—group should have been sufficient for the efficient binding of bacterial dCyd kinase, but an analogy drawn from the behavior of the dNp_4A vs dNp_3A compounds (Ikeda & Ives, 1985) suggests that the extra phosphate should strengthen considerably the interaction of a derivatized deoxynucleotide with the *Lactobacillus* deoxynucleoside kinases. Hoffmann and Blakley (1975) constructed a similar affinity medium in which dGTP was linked to Sepharose through the γ-phosphate, via a six-carbon chain. Directed toward a regulatory site, that adsorbent was used successfully for the large-scale purification of ribonucleotide reductase from *Lactobacillus leichmannii.* Derivatization of the elements of dNTP through its γ-phosphate joined to a linear hydrocarbon or phosphoryl–hydrocarbon linker arm seems to be the most promising way of constructing affinity media for various proteins having dNTP binding sites.

Association of dCyd Kinase and dAdo Kinase. The copurification of dCyd kinase and dAdo kinase on dCp_4–Sepharose, which is directed specifically to dCyd kinase, confirms the association of these two kinase activities previously suggested by their stimulatory kinetic interaction (Deibel et al., 1977) and by their joint retention on bisubstrate affinity columns directed toward either dCyd or dAdo kinase (Ikeda & Ives, 1985). Figure 2 shows the congruence of dCyd kinase and dAdo kinase activities and a stained protein band, developed on a 10% polyacrylamide gel. Similar results were obtained with 8% and 12% gels (not shown), again attesting the homogeneity of the enzyme and also the fact that dCyd kinase and dAdo kinase activities reside on a single protein.

The purified, unconcentrated enzyme eluate (in 15 mM potassium phosphate, 20% glycerol, and 0.3 mM dCTP) loses only a few percent of the dCyd/dAdo kinase activities when stored for a week at 4 °C. After concentration by Centricon ultrafiltration, the enzyme in the same buffer has been stored

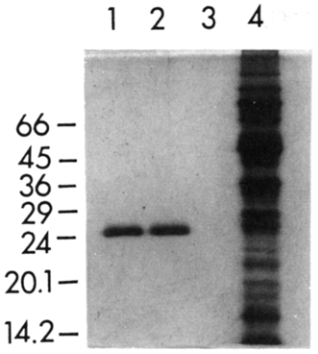

FIGURE 3: SDS–polyacrylamide gel electrophoresis of purified dCyd kinase/dAdo kinase. Electrophoresis was carried out in 12% polyacrylamide gel as described under Experimental Procedures. Lanes 1 and 2, 5 μg of purified dCyd/dAdo kinase; lane 3, blank; lane 4, ammonium sulfate fraction (260 μg of protein). Dithioerythritol was omitted during denaturation of the sample in lane 1 only.

```
                  5                   10
Met-Ile-Val-Leu-Ser-Gly-Pro-Ile-Gly-Ala-

                  15                  20
Gly-Lys-Ser-Ser-Leu-Thr-Gly-Leu/Ile-Leu-Ala

                  25          28
Glu/Lys-Tyr-Leu-Gly-Thr-Asn/Gln-Pro/Ala-Phe
```

FIGURE 4: Partial amino acid sequence of dCyd kinase/dAdo kinase from *L. acidophilus*.

in a freezer (−20 °C) over several months without any significant loss of activity. This contrasts with previous methods of preparation, where the enzyme became extremely labile upon purification, with only limited protection by glycerol and ATP (Deibel & Ives, 1977a). The dCTP used as an eluent appears to be very effective at stabilizing dCyd/dAdo kinase activities since, when it is removed by dialysis or gel filtration, both kinase activities are lost upon overnight storage at 4 °C. It was shown in our previous work that dCTP potently inhibits dCyd kinase activity competitively with respect to both ATP and dCyd by binding to the active site of dCyd kinase as a multisubstrate inhibitor (Ikeda et al., 1986), whereas the same dCTP appears to stimulate the dAdo kinase activity associated with dCyd kinase (Deibel et al., 1977). Although a multisubstrate mechanism provides a very plausible explanation for the effect of dCTP on dCyd kinase, it is yet to be clarified how dAdo kinase activity is activated and also stabilized by the binding of dCTP at the dCyd kinase active site.

Partial Amino Acid Sequence and Subunit Composition. Figure 3 shows the stained protein band resulting from electrophoresis of the purified enzyme run on an SDS–polyacrylamide gel. The enzyme samples on lanes 1 and 2 were denatured with SDS in the absence and presence of dithioerythritol, respectively. Only a single band is seen at the same position of either lane, indicating both the homogeneity of the enzyme preparation and the absence of disulfide bonds in the quaternary structure. The molecular weight of the denatured polypeptide(s) is estimated to be 26 000. The molecular weight of native dCyd/dAdo kinase had been determined by two substantially different procedures in a previous study in this laboratory (Chakravarty et al., 1984). Gel permeation chromatography on Bio-Gel P-150 yielded a molecular weight of 50 000 ± 4000. Sedimentation equilibrium on a Beckman Airfuge gave a value of 60 000. On the basis of these results, it appears that the enzyme is composed of two subunits of the same, or very similar, molecular size.

The amino acid sequence of the enzyme was determined, by an automated Edman degradation, up to the 28th amino acid residue from the N-terminus (Figure 4). Only a single amino acid (800–80 pmol) was identified at each cycle of analysis up to the 17th amino acid, attesting to the homogeneity of the enzyme preparation. However, two different amino acids were detected at the 18th, 21st, 26th, and 27th cycles. The amounts of two amino acids detected at each of these cycles seem to be roughly equal, considering the possibly variable extent of recovery of each amino acid derivative (45 pmol of Leu and 49 pmol of Ile at the 18th cycle; 15 pmol of Glu and 19 pmol of Lys at the 21st cycle; 16 pmol of Asn and 9 pmol of Gln at the 26th cycle; 12 pmol of Pro and 8 pmol of Ala at the 27th cycle). Moreover, these divergencies seem, overall, to be fairly conservative, particulary the Leu/Ile and Asn/Gln pairs, and possibly the charged-polar Glu/Lys pair. One reasonable interpretation is that these diverging sequences indicate that the two subunits of the enzyme are different polypeptides, despite their similarity in terms of molecular size and amino acid sequence nearer the N-terminus.

Recently, homology in the sequence Gly-X-X-Gly-X-Gly-Lys has been recognized among several ATP-requiring enzymes (Walker et al., 1982) and many other purine nucleotide binding proteins (Gay & Walker, 1983; Dever et al., 1987), including thymidine kinases (Otsuka & Kit, 1984) and ade-

Protein	Amino Acid Sequence																
LBA dCK/dAK:			**M**	I	V	L	S	G	P	I	G	A	G	K	S	S	L	**T**
MarHV TK:	10 I	L	R	V	Y	L	D	G	P	H	G	V	G	K	S	T	T	27 A
HSV-1 TK:	49 L	L	R	V	Y	I	D	G	P	H	G	M	G	K	T	T	T	66 T
Rabbit AMP kinase:	8 A	K	I	I	F	V	V	G	G	P	G	S	G	K	G	T	Q	25 C
Bovine GTP-AMP PT:	5 R	L	L	R	A	I	M	G	A	P	G	S	G	K	G	T	V	22 S
Human oncogene p21:	3 E	Y	K	L	V	V	V	G	A	V	G	V	G	K	S	A	L	20 T
Bovine ATPase:	149 G	G	K	I	G	L	F	G	G	A	G	V	G	K	T	V	F	166 I
Rabbit Myosin:	172 N	Q	S	I	L	I	T	G	E	S	G	A	G	K	T	V	N	189 T

FIGURE 5: Sequence homologies of NTP-binding proteins: LBA dCK/dAK, *L. acidophilus* dCyd kinase/dAdo kinase; MarHV TK, marmoset herpes virus thymidine kinase (Otsuka & Kit, 1984); HSV-1 TK, herpes simplex virus type 1 thymidine kinase (Otsuka & Kit, 1984); rabbit AMP kinase, rabbit muscle adenylate kinase (Fry et al., 1986); bovine GTP-AMP PT, bovine heart mitochondrial GTP-AMP phosphotransferase (Tomasselli et al., 1986); human oncogene p21, human bladder carcinoma oncogene p21 protein (Gay & Walker, 1983); bovine ATPase, bovine mitochondrial ATP synthase β-subunit (Walker et al., 1982).

nylate kinase (Fry et al., 1986). There is accumulating evidence that this region of the polypeptide forms a flexible nucleotide binding loop (Pai et al., 1977; Fry et al., 1986). Affinity labeling experiments with adenylate kinase have provided direct proof that the ϵ-amino group of the Lys in this sequence contributes to the binding of ATP, possibly by interacting with the γ-phosphate of the nucleotide (Tagaya et al., 1987). Moreover, recent experiments using site-directed mutagenesis to modify the same sequence on adenylate kinase (Reinstein et al., 1988) or F_1-ATPase (Parsonage et al., 1988) have revealed that this nucleotide binding loop is important, not only for the binding of substrates but also as a flexible element in the conformational changes necessary for enzymic catalysis. It is, therefore, of particular interest to recognize the same sequence homology in the N-terminal region (residues 6–13) of bacterial dCyd kinase/dAdo kinase, shown in Figure 5, together with data quoted by Otsuka and Kit (1984).

It seems likely that dCyd kinase and dAdo kinase are situated on separate subunits but have a common structure at their ATP-binding sites which, in both cases, are located at the N-termini. If so, this protein would represent the very unusual case of a dimeric structure in which the two subunits catalyze the first committed steps of parallel biosynthetic pathways, each with end-product regulation and with positive regulatory interaction between the subunits (stimulation of dAdo kinase activity by saturation of the dCyd kinase site).

Registry No. II, 40248-29-1; II (tributylammonium salt), 57680-98-5; III, 116669-41-1; IV, 116669-42-2; ATP, 56-65-5; dCyd/dAdo kinase, 75302-35-1; dCTP sodium salt, 18423-41-1; dCTP tributylammonium salt, 90290-79-2; Sepharose CL-6B, 62610-50-8.

REFERENCES

Barker, R., Olsen, K. W., Shapa, J. H., & Hill, R. L. (1972) *J. Biol. Chem. 247*, 7135–7147.

Bethell, G. S., Ayers, J. S., Hancock, W. S., & Hearn, M. T. W. (1979) *J. Biol. Chem. 254*, 2572–2574.

Bohman, C., & Eriksson, S. (1988) *Biochemistry 27*, 4258–4265.

Bradford, M. (1978) *Anal. Biochem. 72*, 248–254.

Chakravarty, R., Ikeda, S., & Ives, D. H. (1984) *Biochemistry 23*, 6235–6240.

Deibel, M. R., & Ives, D. H. (1977a) *J. Biol. Chem. 252*, 8235–8239.

Deibel, M. R., & Ives, D. H. (1977b) *Methods Enzymol. 63*, 467–486.

Deibel, M. R., Reznik, R. B., & Ives, D. H. (1977) *J. Biol. Chem. 252*, 8240–8244.

Dever, T., E., Glynias, M. J., & Merrick, W. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 1814–1818.

Fry, D. C., Kuby, S. T., & Mildvan, A. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 907–911.

Gay, N. J., & Walker, J. E. (1983) *Nature (London) 301*, 262–264.

Hershfield, M. S., Fetter, J. E., Small, W. C., Bagnara, A. S., Williams, S. R., Ullman, B., Martin, D. W., Jr., Wasson, D. B., & Carson, D. A. (1982) *J. Biol. Chem. 257*, 6380–6386.

Hoard, D. E., & Ott, D. G. (1965) *J. Am. Chem. Soc. 87*, 1785–1788.

Hoffmann, P. J., & Blakley, R. L. (1975) *Biochemistry 14*, 4804–4812.

Ikeda, S., & Ives, D. H. (1985) *J. Biol. Chem. 260*, 12659–12664.

Ikeda, S., Park, I., Gardner, P., & Ives, D. H. (1984) *Biochemistry 23*, 1914–1921.

Ikeda, S., Chakravarty, R., & Ives, D. H. (1986) *J. Biol. Chem. 261*, 15836–15843.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lee, L.-S., & Cheng, Y. (1977) *Biochemistry 15*, 3686–3690.

March, S. C., Parikh, I., & Cuatrecasas, P. (1974) *Anal. Biochem. 60*, 149–152.

Møllgaard, H. (1980) *J. Biol. Chem. 255*, 8216–8220.

Otsuka, H., & Kit, S. (1984) *Virology 135*, 316–330.

Pai, E. G., Sachsenheimer, W., Schirmer, R. H., & Schultz, G. E. (1977) *J. Mol. Biol. 114*, 37–45.

Parsonage, D. Al-Shawi, M. K., & Senior, A. E. (1988) *J. Biol. Chem. 263*, 4740–4744.

Reinstein, J., Brune, M., & Wittinghofer, A. (1988) *Biochemistry 27*, 4712–4720.

Tagaya, M., Yagami, T., & Fukui, T. (1987) *J. Biol. Chem. 262*, 8257–8261.

Tomasselli, A. G., Frank, R., & Schiltz, E. (1986) *FEBS Lett. 202*, 303–308.

Verhoef, V., Sarup, J., & Fridland, A. (1981) *Cancer Res. 41*, 4478–4483.

Walker, J. E., Saraste, M., Runswick, M. G., & Gay, N. J. (1982) *EMBO J. 1*, 945–951.