The glycine-rich loop of adenylate kinase forms a giant anion hole

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The conformation of the glycine-rich loop of adenylate kinase is described in detail. It forms a giant anion hole for a sulfate ion, which presumably mimicks a nucleotide phosphoryl group. This loop had been called flexible, because at pH values of 6 or below it is displaced in the crystal. In the region of this loop the adenylate kinases are probably homologous to the p21 proteins. Is is known that a mutation in this loop at residue 12 of p21 causes cell transformation and therefore cancer. Other potentially homologous proteins are indicated.

Adenylate kinase *X-ray diffraction* *Structural homology* *Nucleotide-binding protein* *Oncogene*

1. INTRODUCTION

Adenylate kinases (myokinase, ATP:AMP phosphotransferase, EC 2.7.4.3, AK) are small monomeric enzymes with M_r values in the range 22000–26000 [1,2] catalyzing the reaction MgATP + AMP $\rightleftharpoons$ MgADP + ADP. The spatial structures of 2 species, porcine cytosolic (AK1) and yeast cytosolic (AKy), are known [3–6]. X-ray diffraction studies using substrate analogues revealed the approximate positions of the ligands [5]. Recently, at least one of the sites could be firmly established [6]. The glycine-rich loop of these enzymes has attracted widespread interest, because it is likely to play a special role in phosphoryl binding and it appears to be a homologous chain segment in various nucleotide-binding proteins, among them the p21 proteins [7–17]. Although well conserved and deeply buried in the catalytic cleft, this loop has been called flexible because it is displaced in the crystal at pH values of 6.0 or below [4]. Moreover, its modification in p21 proteins is known to lead to cell transformation and thus to cancer [9,10].

The glycine-rich loop is located in the deep cleft of AK1 [3] and at an equivalent position in AKy [6]. It seems clear that it binds one of the phosphoryl groups of the substrates [3,5,6], but the correct one among the 4 candidates cannot be identified yet. Here, we report the detailed conformation of this loop in AK1.

2. MATERIALS AND METHODS

AK1 was crystallized according to Schirmer et al. [18]. The crystals were stored and mounted in a solution of 3 M $(NH_4)_2SO_4$, 0.15 M Tris-HCl at pH 7.7. X-ray data were collected from crystal form A [4] with space group $P3_121$ and cell parameters $a = b = 48.5$ Å, $c = 141$ Å as described earlier [19]. The structure had been solved at 3 Å resolution by multiple isomorphous replacement (MIR) [3]. X-ray data collection from native protein crystals was then extended to 2.1 Å resolution yielding altogether 11399 independent reflections, which is 96% of the complete set. The quality of the data set was checked by comparing additionally measured symmetry-related reflections in the $l = 0$ plane. The internal difference amounts to $R_F = 7.7\%$.

Starting from the 3 Å MIR model the structure was refined using the procedure of Tronrud et al.

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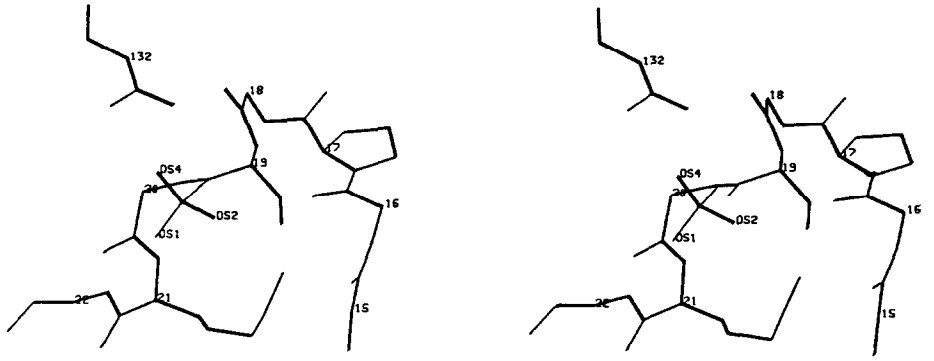

Fig.1. Stereo view of the segment -Gly-Gly-Pro-Gly-Ser-Gly-Lys-Gly- (residues 15–22) and Arg-132 of AK1 in crystal form A together with a bound sulfate ion occupying a phosphoryl binding site. The view is into the deep cleft of the enzyme [3] towards the center of the molecule. The loop is not much exposed to solvent. The model is based on a refinement at 2.1 Å resolution.

		1o							17						
AK1	[2]	I	I	F	V	V	G	G	P	G	S	G	K	G	T
		2													
AKe	[2]	R	I	I	L	L	G	A	P	G	A	G	K	G	T
		6													
AKy	[2]	R	M	V	L	I	G	P	P	G	A	G	K	G	T
		18													
AK2	[2]	R	A	V	L	L	G	P	P	G	A	G	K	G	T
		7													
AK3	[2]	L	R	A	I	M	G	A	P	G	S	G	K	G	T
		5							12						
p21 c-has/bas	[9,1o]	K	L	V	V	V	G	A	G	G	V	G	K	S	A
		151													
F1-ATPase beta-subunit	[8]	K	I	G	L	F	G	G	A	G	V	G	K	T	V
		172													
myosin nematode	[23]	S	M	L	I	T	G	E	S	G	A	G	K	T	E
		3o													
G-protein	[24]	K	L	L	L	L	G	A	G	E	S	G	K	S	T

Fig.2. Sequence alignment at the glycine-rich loop in the adenylate kinase family together with the normal version of p21 protein and other mononucleotide-binding proteins [2].

[20]. All calculations were done on a Vax 11-730 computer, which needed 5–6 h per cycle. At 8 intermediate stages we inspected $(2 \cdot F_{obs} - F_{calc})$-maps and at 4 stages also $(F_{obs} - F_{calc})$-difference maps on an interactive vector display and readjusted the model. After a new fitting to the 3 Å MIR map the refinement started with an R value of 44%. It finished with a residual error of $R = 19\%$ after a total of 340 cycles. The remaining root mean square (rms) deviation of the model from standard geometry was 0.02 Å in bond lengths and 3° in bond angles. The final model contains 2 sulfate ions and 156 enzyme-bound water molecules. The rms-difference between the 3 Å MIR and the refined model is 1.6 Å in the backbone and 2.6 Å in the side chains.

Table 1

Conformation of the glycine-rich loop of AK1 in crystal form A

Residue	ϕ (°)	ψ (°)	Atom-1	Atom-2	Distance (Å)
Gly-15	−171	+163	O	21-NZ	2.8
Gly-16	−62	+157	O	21-NZ	3.3
			O	19-OG	2.4
Pro-17	−64	+126			
Gly-18	+92	−1	N	OS3	3.4
			N	OS2	3.7
Ser-19	−73	−14	N	OS2	3.5
			OG	16-O	2.4
Gly-20	+88	+34	N	OS4	3.0
			N	OS2	3.1
Lys-21	−75	−30	N	OS2	3.0
			N	OS1	3.4
			N	OS4	3.5
			NZ	OS2	3.1
			NZ	15-O	2.8
			NZ	16-O	3.3
Gly-22	−67	−47	N	OS1	2.8
Arg-132			NH1	OS4	3.0
			NH2	OS3	2.8

The backbone dihedral angles ϕ, ψ and atom names are defined as usual. OS1 to OS4 are the 4 oxygen atoms of the sulfate ion

3. RESULTS

The glycine-rich loop in the refined structure is depicted in fig.1. In the previous 3 Å MIR analysis the exact conformation of this loop could not be established. In the course of the refinement the loop adopted a new conformation in which the 5 peptide hydrogens of residues 18–22 point to the encircled sulfate as shown in fig.1. The final $(2 \cdot F_{obs} - F_{calc})$-electron density map confirms this interpretation. The orientation of the sulfate ion refined unambiguously. The resulting distances between peptide nitrogens and sulfate oxygens are given in table 1 together with other distances within the loop and the backbone dihedral angles. The 2 negative charges of the sulfate ion can be compensated by the contacting positively charged amino and guanidinium groups of Lys-21 and Arg-132 as well as by the dipole field of helix 21–30 [21]. As shown in fig.1 and confirmed by table 1 the loop forms an anion hole for the sulfate ion reminiscent of the anion hole of the serine proteases [22]. In contrast to the 2 involved peptide nitrogens in the serine proteases, however, AK1 uses 5 peptide nitrogens prompting us to call this feature a 'giant' anion hole. The other sulfate binds at a less conspicuous site near Arg-149.

4. DISCUSSION

Several sequences have been aligned to the glycine-rich loop and the preceding β-strand of porcine cytosolic adenylate kinase in fig.2. This loop is well conserved in the family of adenylate kinases, being one of about 6 regions of high homology [2]. Furthermore, it appears to be conserved in the other proteins given in fig.2. But with none of these proteins can one detect other regions that are clearly homologous to any one of the known adenylate kinases. Lacking sequence homology does not exclude, however, that the chain folds are closely related. An extraordinary conservation of chain fold together with the binding mode of the dinucleotide FAD has been reported for p-hydroxybenzoate hydroxylase and glutathione reductase [25], where sequence homology can only be found in the first β-strand and in the following glycine-rich loop connecting to an α-helix. The same applies for a number of other dinucleotide-binding proteins.

It should be mentioned that the conformation of the short glycine-rich loop of dinucleotide-binding proteins is quite different from the long glycine-rich loop of the mononucleotide-binding adenylate kinases. Contrary to an earlier assignment [12] we think that the glycine-rich segment of the mononucleotide-binding cancerogenic p21 proteins can be better fitted to the latter group of proteins.

The first observed p21 mutant causing cell transformation had a valine instead of Gly-12 [9,10], which corresponds to the conserved Pro-17 (AK1) of the adenylate kinases (fig.2). Interestingly enough, a survey of mutations of Gly-12 of p21 showed that all exchanges except to proline caused cell transformation [26], corroborating the similarity to the adenylate kinases.

The most prominent features of the alignment of fig.2 are (AK1-numbering) the high β-strand forming potential of residues 10–14, the glycines at positions 15, 18, 20, and Lys-21. From table 1 we recognize that the backbone dihedral angles of Gly-18 and Gly-20 are forbidden for residues with side chains, which explains the conservation of glycines. Lys-21 forms contacts to the anion and to residues 15–16 (table 1). A closer inspection of Gly-15 shows that a side chain at this position would collide with the bound anion.

The described glycine-rich loop seems to be a very well conserved feature of mononucleotide-binding proteins. We expect that it is intimately involved in the transfer of a phosphoryl group. Conspicuously, the interactions in the giant anion hole are mostly with the backbone, i.e. the basic structure of a polypeptide. Thus, this hole may reveal an archaic way of anion-protein interactions: adenylate kinase may allow us to visualize a primordial encounter between nucleotides and peptides.

REFERENCES

[1] Noda, L.H. (1973) in: The Enzymes (Boyer, P.D. ed.) vol.8, pp.279–305, Academic Press, New York.
[2] Schulz, G.E., Schiltz, E., Tomasselli, A.G., Frank, R., Brune, M., Wittinghofer, A. and Schirmer, R.H. (1986) Eur. J. Biochem., in press.
[3] Schulz, G.E., Elzinga, M., Marx, F. and Schirmer, R.H. (1974) Nature 250, 120–123.
[4] Sachsenheimer, W. and Schulz, G.E. (1977) J. Mol. Biol. 114, 23–36.
[5] Pai, E.F., Sachsenheimer, W., Schirmer, R.H. and Schulz, G.E. (1977) J. Mol. Biol. 114, 37–45.
[6] Egner, U., Tomasselli, A.G. and Schulz, G.E., personal communication, manuscript in preparation.
[7] Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R., Ade, W. and Ponstingl, H. (1981) Proc. Natl. Acad. Sci. USA 78, 4156–4160.
[8] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 945–951.
[9] Tabin, C.J., Bradley, S.M., Bargmann, C.I., Weinberg, R.A., Papageorge, A.G., Scolnick, E.M., Dhar, R., Lowy, D.R. and Chang, E.H. (1982) Nature 300, 143–149.
[10] Reddy, E.P., Reynolds, R.K., Santos, E. and Barbacid, M. (1982) Nature 300, 149–152.
[11] Gay, N.J. and Walker, J.E. (1983) Nature 301, 262–264.
[12] Wierenga, R.K. and Hol, W.G.J. (1983) Nature 302, 842–844.
[13] Leberman, R. and Egner, U. (1984) EMBO J. 3, 339–341.
[14] Möller, W. and Amons, R. (1985) FEBS Lett. 186, 1–7.
[15] Argos, P. and Leberman, R. (1985) Eur. J. Biochem. 152, 651–656.
[16] Medynski, D.C., Sullivan, K., Smith, D., Van Dop, C., Chang, F.H., Fung, B.K.-K., Seeburg, P.H. and Bourne, H.R. (1985) Proc. Natl. Acad. Sci. USA 82, 4311–4315.
[17] Fry, D.C., Kuby, S.A. and Mildvan, A.S. (1986) Proc. Natl. Acad. Sci. USA 83, 907–911.
[18] Schirmer, I., Schirmer, R.H., Schulz, G.E. and Thuma, E. (1970) FEBS Lett. 10, 333–338.
[19] Schulz, G.E., Biedermann, K., Kabsch, W. and Schirmer, R.H. (1973) J. Mol. Biol. 80, 857–864.
[20] Tronrud, D.E., TenEyck, L.F. and Matthews, B.W. (1986) Acta Crystallogr., in press.
[21] Hol, W.G.J., Van Duijnen, P.T. and Berendsen, H.J.C. (1978) Nature 273, 443–446.
[22] Blow, D.M. (1976) Acc. Chem. Res. 9, 145–152.
[23] Karn, J., Brenner, S. and Barnett, L. (1983) Proc. Natl. Acad. Sci. USA 80, 4253–4257.
[24] Hurley, J.B., Simon, M.I., Teplow, D.B., Robishaw, J.D. and Gilman, A.G. (1984) Science 226, 860–862.
[25] Wierenga, R.K., Drenth, J. and Schulz, G.E. (1983) J. Mol. Biol. 167, 725–739.
[26] Seeburg, P.H., Colby, W.W., Capon, D.J., Goeddel, D.V. and Levinson, A.D. (1984) Nature 312, 71–75.