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Homologies in the primary structure of GTP-binding proteins: the nucleotide-binding site of EF-Tu and p21

Reuben Leberman* and Ursula Egner

EMBL Outstation, B.P. 156X, 38042 Grenoble Cedex, France, and Max-Planck-Institut für medizinische Forschung, Jahnstrasse 29, D-6900 Heidelberg, FRG

*To whom reprint requests should be sent

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An examination of the available amino acid sequences of GTP-binding proteins has revealed that each contains a polypeptide essentially homologous for all of them. These sequences for elongation factor-Tu (EF-Tu) and the human bladder protein p21 exhibit a singular degree of homology (50%). Chemical and structural evidence indicates that this sequence in EF-Tu constitutes part of the nucleotide-binding site. The homologous sequences may therefore contribute to the GTP-binding sites of the other proteins.

Key words: EF-Tu/p21/GTP-binding/sequence homology

Introduction

Proteins which exhibit a high specific affinity for the guanosine nucleotides GTP and GDP are a diverse group. They include polypeptide elongation factors, tubulin, transducin, and receptor protein of eucaryotic adenylate cyclase. A recent addition to this list is the human bladder protein, p21, and its oncogenic variants (Scolnick *et al.*, 1979; Shih *et al.*, 1980; Papageorge *et al.*, 1982). These oncogenic variants are characterised by a base change in the gene at the codon for glycine at position 12 resulting in the substitution of this residue by either valine, arginine or serine (Tabin *et al.*, 1982; Reddy *et al.*, 1982; Dhar *et al.*, 1982; Tsuchida *et al.*, 1982).

Two reports have suggested that the N-terminal sequence of normal p21, encompassing glycine 12, is homologous to sequences of the nucleotide-binding regions of a number of adenine dinucleotide-binding proteins (Wierenga and Hol, 1983) and to a sequence present in the beta-subunit of bovine ATPase (Gay and Walker, 1983) which is believed to contribute to nucleotide binding. Together, these observations might indicate that normal p21 may possess an, as yet undetected, adenine nucleotide binding ability.

Although sequences homologous to that of the beta-subunit of bovine ATPase were not detectable in the primary structures of the GTP-binding proteins elongation factor-Tu (EF-Tu) and tubulin (Gay and Walker, 1983), other sequences might contribute to a nucleotide-binding site in these and other GTP-binding proteins. We have therefore compared the known amino acid sequences of six GTP-binding proteins to see if there exist homologous regions which might be tentatively identified as participating in nucleotide binding. The protein sequences examined were of *Escherichia coli* EF-Tu (Arai *et al.*, 1980), *E. coli* EF-G (Ovchinnikov *et al.*, 1982), alpha-tubulin (Ponstingl *et al.*, 1981), beta-tubulin (Krauhs *et al.* 1981), p21 (Capon *et al.*, 1983; Reddy, 1983), p21 *has* (Dhar *et al.*, 1982), p21 *kis* (Tsuchida *et al.*, 1982), and the partial sequence of *Artemia salina* eEF-Tu (Amons *et al.*, 1983).

Results

Comparisons of amino acid sequences were made by visual inspection and evaluated numerically using programmes written by Dr. G.E. Schulz (Williams *et al.*, 1982).

For the six GTP-binding proteins only one region of their primary structures contained sequences of significant homology (Table I). The central feature of this region is the sequence Val.X.X.X.hydrophobic.hydrophobic.hydrophobic.hydrophobic.Asn/Asp. Other homologies are apparent which are not found in all six sequences, and those which span three or more sequences are also indicated in Table I. Examination of pairs of sequences also showed that in this region there is a remarkable degree of homology between EF-Tu and p21 (Table II). Furthermore, when this alignment is made, then a relationship between these homologous sequences and the conserved B regions of adenine nucleotide-binding proteins of Walker *et al.* (1982) is apparent (Table II).

EF-Tu

The soluble protein factors participating in the biosynthesis of polypeptides in both prokaryotes and eukaryotes have a functional requirement for GTP. Of these the best documented is the bacterial elongation factor EF-Tu from *E. coli*. The three-dimensional structure analysis of proteolysed EF-Tu.GDP is being pursued in three laboratories (Kabsch *et al.*, 1977; Morikawa *et al.*, 1978; Jurnak *et al.*, 1980) and an essentially complete tracing of the polypeptide chain is now available (T. la Cour and J. Nyborg, personal communication). This protein has three cysteine residues and modification studies of their thiol functions has led to the proposition that Cys-137 is at or near the nucleotide-binding site (Miller *et al.*, 1971; Arai *et al.*, 1974; Wade *et al.*, 1975) although it may play no active role in this process (Wittinghofer and Leberman, 1979). These data would support the argument that sequences shown in Table I constitute part of the nucleotide-binding site. More convincingly the data of la Cour and Nyborg show that in the EF-Tu.GDP complex, Asn-135 H-bonds to the oxygen at the 6-position of the guanine [in agreement with the solution studies of Wittinghofer *et al.* (1977)] but there is no direct interaction of Cys-137 with the GDP.

eEF-Tu

The partial sequence of this elongation factor from *A. salina* shows in general a high homology with EF-Tu (Amons *et al.*, 1983). Thus, the region shown in Table I is 66% homologous with EF-Tu if we consider identical or conservative amino acid residues.

EF-G

The high degree of homology between the N-terminal sequences of this protein and EF-Tu (Jones *et al.*, 1980) would

Table I. Homologous sequences among GTP-binding proteins

Protein	Sequence	Ref.
eEF-Tu:	120 ala- leu- leu- ala- tyr- thr- leu- gly- val- lys- X- 130 leu- ile- val- gly- val- asn- lys-	a
EF-Tu:	120 ile- leu- leu- gly- arg- gln- val- gly- val- pro- tyr- 130 ile- ile- val- phe- leu- asn- lys- cys- asp- - met- 140 val- asp- asp- glu- glu- leu- leu- glu- 150 leu- val- glu- met-	b
EF-G:	trp- arg- gln- ala- 130 asn- lys- tyr- lys- val- pro- arg - ile- ala- phe- val- 140 asn- lys- met- asp- arg- met- gly- ala- asn- 150 phe- leu- lys- val- val- asn- gln- ile- lys-	c
α-Tubulin:	glu- thr- gly- ala- 60 gly- lys- his- - val- pro- arg - ala- val- phe- val- asp- 70 leu- glu- pro- thr- val- ile- asp- glu- val- arg- 80 thr- gly- thr- tyr- arg- gln- leu-	d
β-Tubulin:	glu- ala- ala ser gly ser asn his lys- tyr- - 60 val- pro- arg - ala- ile- leu- val- asp- 70 leu- glu- pro- gly- thr- met- asp- ser- val- arg- ser- 80 gly- pro- phe- gly- gln- ile-	e
p21:	lys- arg- val- lys- asp- ser- asp- asp- 110 val- pro- met - val- leu- val- gly- asn- lys- cys- asp- 120 leu- ala- ala- arg- thr- val- glu- ser- 130 arg- gln- ala- gln- asp- leu-	f

a, Amons *et al.* (1983); b, Arai *et al.* (1980); c, Ovchinnikov *et al.* (1982); d, Ponstingl *et al.* (1981); e, Krauhs *et al.* (1981); f, Capon *et al.* (1983) and Reddy (1983) for c-*has/bas* gene product. p21-*has* (Dhar *et al.* 1982) has glycine at position 122 and p21-*kis* (Suchida *et al.*, 1982) has, in this region, differences at residues 107 (glu), 121-122 (pro-ser), 126-128 (asp-thr-lys) and 132 (glu).

Table II. Homologies between EF-Tu, p21 and some adenine nucleotide-binding proteins

Protein	Sequence	Ref.
EF-Tu:	110 thr- asp- gly- pro- met- pro- gln- thr- arg- glu- his- ile- 120 leu- leu- gly- arg- gln - val- gly- val- pro- tyr- 130 ile- ile- val- phe- leu- 140 asn- lys- cys- asp- met- val- asp-	a
p21:	90 ser- phe- glu- asp- ile- his- gln- tyr- arg- glu- gln- ile- 100 lys- arg- val- lys- asp- ser- asp- asp- val- pro- met- 110 val- leu- val- gly - 120 asn- lys- cys- asp- leu- ala- ala-	b
Bovine ATPase β:	-phe- arg- asp- gln- glu- 250 gly- gln- asp- val- leu- leu- phe- ile- asp- gln-	c
E. coli ATPase β:	-phe- arg- asp - glu- gly- arg- asp- val- 240 leu- leu- phe- val- asp- gln-	c
E. coli ATPase α:	270 -phe- arg- asp - arg- gly- glu- asp- ala- leu- ile- ile- 280 tyr- asp- asp-	c
ADP/ATP translocase:	280 - arg- gly- met- gly- gly- ala- phe- val- leu- val- leu- 290 tyr- asp- glu-	c
Adenylate kinase:	-glu- arg- lys - ile- 110 gly- gln- pro- thr- leu- leu- leu- tyr- val- asp	c
Phospho-fructokinase:	90 -leu- lys- lys - his- gly- ile- gln- gly- leu- val- val- 100 ile- gly- gly- asp-	c

a and b are references b and f, respectively, of Table I. With the addition that p21-*kis* has histidine at position 95. c, data from Walker *et al.* (1982).

suggest an evolutionary relationship between the two proteins (Laursen *et al.*, 1981). This is particularly true for the regions listed in Table I although Girshovich *et al.* (1978) found, by photo-affinity labelling, that the GTP-binding site of EF-G is associated with the tryptic peptide containing Cys-113 which is situated towards the N terminus.

Tubulin

The heterodimer of alpha-beta tubulin binds two molecules of GTP; one bound to alpha-tubulin which is non-exchangeable, and one to beta-tubulin which is exchangeable. The total primary structure of both subunits of porcine brain tubulin has been determined and are 41% homologous (Krauhs *et al.*, 1981). Glycine-rich sequences have been identified which are homologous with similar sequences of adenylate kinase and alcohol dehydrogenase (Krauhs *et al.*, 1981) but do not correspond to the glycine-rich regions (A sequences) of adenine nucleotide-binding proteins described by Walker *et al.* (1982). The alignment of sequences from the tubulins with those of polypeptide elongation factors in Table I is pleasing, with seven indentities between alpha-tubulin and EF-G and between beta-tubulin and EF-G.

p21

The physiological function of this protein is still undefined but the affinity of the normal protein and its oncogenic variants for guanine nucleotides has been established (Papageorge *et al.*, 1982). These proteins bind GDP, GTP and dGTP but exhibit no affinity for adenine nucleotides (Scolnick *et al.*, 1979; Shih *et al.*, 1980). The primary structures of both normal and oncogenic variants contain a sequence which can be generally aligned with sequences from the other GTP-binding proteins (Table I) and specifically with EF-Tu (Table II). Furthermore, the combined homologous sequences of p21 and EF-Tu show a homology with the B sequences of adenine nucleotide proteins described by Walker *et al.* (1982).

The 34 residue sections of EF-Tu and p21 compared in Table II required only one deletion in each polypeptide to align 11 identities. If we consider the other six conservative

replacements then the homology is 50%. In addition there are a further seven positions where the amino acids can be related by a single base change.

Discussion

In their study of adenine nucleotide-binding protein homologies, Walker *et al.* (1982) emphasised the value of aligning the sequences of several proteins with the same substrate requirements. We have done this for a group of GTP-binding proteins and find a region of the primary structure which is conserved in all of them. Furthermore, for this region, we find a marked homology between bacterial elongation factor EF-Tu and human bladder protein p21, which can be related to homologies within a group of adenine nucleotide-binding proteins. The homology between EF-Tu and adenylate kinase is rather striking, with 10 of the 14 relevant amino acids being either identical or having conservative differences. These results, together with the observations of la Cour and Nyborg (personal communication) that Asn-135 of EF-Tu interacts with the guanine moiety of the GDP and that the primary structure homology between EF-Tu and adenylate kinase (Table II) corresponds to a tertiary structure homology between the two proteins, strongly suggests that the homologous sequences presented in Table I constitute part of the nucleotide-binding sites for the respective proteins.

It has been suggested by Allende (1982), using the examples of polypeptide biosynthesis factors, tubulin, transducin, G/F protein of adenylate cyclase, that the control function of GTP-binding proteins are to regulate the reversible association of macromolecules. Although the function of p21 has not yet been determined, attention has been focussed on the sequence homology between the N terminus of normal protein and some adenine nucleotide-binding proteins (Gay and Walker, 1983; Wierenga and Hol, 1983), and it has been postulated that p21 may act as a kinase or be part of an electrogenic ATPase or GTPase (Gay and Walker, 1983). The established GTP-binding property of p21 can now be related to a sequence homology present in a set of five other GTP-binding proteins, of which the homology with EF-Tu is the most striking. This would support the idea of p21 being part of a GTPase and also poses the question as to whether p21 has a regulatory function similar to other GTP-binding proteins.

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