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Short Sequence-Paper

The ferredoxin : sulphite reductase gene from *Synechococcus* PCC7942

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The structural gene of the ferredoxin : sulphite reductase (EC 1.8.7.1) from the cyanobacterium *Synechococcus* PCC7942 (formerly '*Anacystis nidulans*') was cloned and sequenced. The gene termed '*sir*' was detected by heterologous Southern hybridisation with the structural gene *cys*I from *Escherichia coli* encoding the iron-sulphur haemoprotein of the NADPH : sulphite reductase. The open reading frame is comprised of 1875 bp encoding for a polypeptide of M_r 70.028. The deduced amino acid sequence is 35.6% identical with the enterobacterial iron-sulphur haemoprotein. This putative fd-dependent sulphite reductase is only distantly related to the fd-dependent nitrite reductase (binary matching coefficient S_{AB}: 0.23) or with the NADPH-sulphite reductase (S_{AB}: 0.32). Highly conserved residues are found within the two Cys clusters forming the reactive Fe_4S_4-sirohaem centre of the enzyme. Expression of the *sir* gene using a fusion vector gave a single gene product which is immunologically related with the fd-sulphite reductase from the wild-type bacterium.

In plants or cyanobacteria, the reduction of sulphite to sulphide is catalysed by a ferredoxin-dependent sulphite reductase (EC 1.8.7.1 [20]):

$$SO_3^{2-} + 6\,Fd_{red} + H^+ \rightarrow HS^- + 6\,Fd_{ox} + 3\,H_2O$$

Like NADPH-dependent sulphite reductases (EC 1.8.2.1) from non-photosynthetic organisms, the enzyme contains a sirohaem and a tetrameric (Fe_4S_4) iron-sulphur cluster as prosthetic groups [13,16]. The iron-sulphur haemoprotein already suffices for catalysis, yet the NADPH-dependent sulphite reductases from enterobacteria are hetero-polymeric proteins containing additional flavoproteins. The FAD and FMN groups of these flavoprotein subunits are required for the transfer of electrons from NADPH to the iron-sulphur haemoprotein. This heteropolymeric type of enzyme is not yet observed to occur in plants or cyanobacteria, but the fd-dependent sulphite reductase. Its structure appears less complicated, consisting of a monomeric iron-sulphur haemoprotein only – although homo-polymers may be formed, depending on the ionic strength [1].

Elucidation of the structural genes encoding sulphite reductase from enterobacteria has considerably increased our knowledge about the structure and function of this polypeptide [17]. Yet, sulphite reductases occur also in sulphate dissimilating and sulphide oxidising bacteria [4]. Their wide distribution raises the question as to whether these different enzymes all have the same phylogenetic origin.

If such a genetic link between the different sulphite reductases does exist because the proteins are indeed originated from a common ancestral polypeptide, the structural genes encoding these proteins might also be highly homologous. In view of the rather unique biochemistry of the sulphite reductases, one would expect a conservation of the functional domain(s). This speculation was supported most recently by the finding that genomic DNA from sulphur bacteria, blue-green algae or higher plants gave distinct and specific signals when the corresponding structural genes from *E. coli* were used as probes [7]. Since the DNA probe *cys*I is specific for the gene encoding the haemprotein of the sulphite reductase, these signals were interpreted as

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heterologous DNA-hybrids of gene structures possibly related to the corresponding sulphite reductase genes. As detailed in this short report, we isolated and cloned a putative ferredoxin sulphite reductase gene from the photoautotrophic cyanobacterium *Synechococcus* using the structural gene of the *E. coli* haemoprotein as DNA probe. The identity of the cloned gene as the structural gene of the ferredoxin sulphite reductase was deduced from the similarity of the primary structures (DNA and amino acid sequence), and from the immunological cross-reactivity of gene product with sulphite reductase from *Synechococcus*.

The heterologous probe from *E. coli* used for Southern [22] hybridisation was isolated as a 1092 bp *Eco*RI/*Mlu*I fragment from the plasmid pCH10 [12]. This probe contained 702 bp of the 3′ end of gene *cys*I (encoding for 233 amino acid residues of the C-terminus of the haemoprotein), 13 bp of the intergenic region between *cys*H and *cys*I, and 377 bp of the pBR322 cloning vector. The fragment was useful as probe to detect by Southern hybridisation a 1.7 kb *Bam*HI restriction fragment from *Synechococcus* genomic DNA under conditions of moderate stringency as outlined in Ref. 7. In order to isolate and identify this homologous DNA fragment from *Synechococcus*, a partial library in pBluescriptII KS$^+$ was constructed from DNA fractionated by size [7,15]. The 1.7 kb DNA fragment hybridising with the *cys*I probe was found in the plasmid pGGAN1. Its identity as a putative sulphite reductase gene was concluded from DNA sequence homology with *cys*I. A 0.5 kb *Bam*HI/*Sal*I subfragment obtained from pGGAN1 was then used to detect full length clones from a representative library. The plasmid pGGAN2 selected by this procedure was sequenced by the chain termination method [19] using 46 clones with unidirectional deletions according to Ref. 8 which were constructed to cover both strands of the putative open reading frame.

The pTac [23] plasmid (Boehringer, Mannheim) was used for expression of *sir* as a putative fd : sulphite-reductase. The correct orientation was obtained by the use of a PCR product (499 nt constructed as to enable the ligation of the first 24 nt (from the ATG to the *Hin*dIII restriction site at nt 324) into the *Sma*I site of

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1    ctgccattgcgcgatcgcagcggctacaaaagaagcagggagttcttcggttccgacttc
61   tagtagaaaggccggcatagagcagcaatagcgcgaacagacaacctcatcagtctaccg
121  cgcacccttttccccc acctctgactccatt tcctaaagacttttgggcgatcgcggggtg
181  tggatccatcgaaaagtgttacaataaacaccgaaatccacagagacttaccggtcttgg
241  atttgtctcactgcc aagccattgtctggggcagtgcttttcttttctggacagaggaaca
301  ATG TCGCCGACTGCTGCACCCCAAAAGC TTTCTAAAGTTGAAGACCTCAAGGCACGCAGT
     M  S  P  T  A  A  P  Q  K  L  S  K  V  E  D  L  K  A  R  S
361  CAGTACCTGCTGGAGCCGATTCTAAGCCAGCTCCAGGAAGAGTCGACTCACTTTAATGAA
     Q  Y  L  L  E  P  I  L  S  Q  L  Q  E  E  S  T  H  F  N  E
421  GACGGGATCCAGATCCTTAA ATTCCACGGTTCCTATCAGCAAGACAACCGCGACAACCGC
     D  G  I  Q  I  L  K  F  H  G  S  Y  Q  Q  D  N  R  D  N  R
481  GTCAAGGGGCAGGAAAAAGAC TTTCAGTTCATGCTGCGCCTGCGCAGTCCCGGCGGCTAC
     V  K  G  Q  E  K  D  F  Q  F  M  L  R  L  R  S  P  G  G  Y
541  ATTCCGCCACAGCTCTATCTCACCCTTGATCAACTGGCCGATGACTATGGCAACGGCACC
     I  P  P  Q  L  Y  L  T  L  D  Q  L  A  D  D  Y  G  N  G  T
601  CTGCGAGCAACGACGCGCCAAGCCTTTCAGTTGCACGGTATTCTCAAGCGCGACCTGAAA
     L  R  A  T  T  R  Q  A  F  Q  L  H  G  I  L  K  R  D  L  K
661  ACGGTGATCCGTCGGATTGTCGAAAACCTGGGCTCGACGATCTCCGCTTGCGGCGATGTC
     T  V  I  R  R  I  V  E  N  L  G  S  T  I  S  A  C  G  D  V
721  AATCGCAACGTCATGGCACCGCCAGCACCCTTCCGCGATCGCCCGGAATATGAGTGGGCG
     N  R  N  V  M  A  P  P  A  P  F  R  D  R  P  E  Y  E  W  A
781  CGCACCTACGCTAACAACATTGCTGACTTGCTGACGCCGGAGTCTGGCGCCTATTACGAA
     R  T  Y  A  N  N  I  A  D  L  L  T  P  E  S  G  A  Y  Y  E
841  CTCTGGCTGGATGGTGAAAAAGTCCTGAGTGGTGAACCGGATCCAGCGGTACTAGCAGCC
     L  W  L  D  G  E  K  V  L  S  G  E  P  D  P  A  V  L  A  A
901  CGCCGCAATCCTAAAGGCCGTGTAGCCGATTCTGTCGAACCGCTCTACAGCGATCGCTAT
     R  R  N  P  K  G  R  V  A  D  S  V  E  P  L  Y  S  D  R  Y
961  CTGCCGCGCAAATTCAAGATTGCGGTCACGGTTCCCGGCGACAACTCAATTGACCTGTTC
     L  P  R  K  F  K  I  A  V  T  V  P  G  D  N  S  I  D  L  F
1021 ACCCAGGACATTGGCTTAGTCGTGATTGGCAATGATCGCGGCGAACTAGAGGGTTTCAAC
     T  Q  D  I  G  L  V  V  I  G  N  D  R  G  E  L  E  G  F  N
1081 GTCTACGTCGGTGGCGGCATGGGTCGCACCCACAACAAAGAGGAAACCTTTGCGCGGCTA
     V  Y  V  G  G  G  M  G  R  T  H  N  K  E  E  T  F  A  R  L
1141 GCGGATCCCCTCGGCTTTGTGCCTGCAGCTGATATCTATGCTGCTGTGCAGGCGATCGTG
     A  D  P  L  G  F  V  P  A  A  D  I  Y  A  A  V  Q  A  I  V
1201 GCGACCCAGCGGGACTACGGCGATCGCAGCAACCGTCGCCATGCCCGCATGAAGTACCTG
     A  T  Q  R  D  Y  G  D  R  S  N  R  R  H  A  R  M  K  Y  L

1261 ATCCACGATTGGGGCATCGCCAAATTTAAAGAAGCTGTCGAATCCGTTTTCGGTAAAGCG
     I  H  D  W  G  I  A  K  F  K  E  A  V  E  S  V  F  G  K  A
1321 ATCGCCCCTGTGCGCGAACTGCCGCCCTTCCGCTATCGCGACTATCTCGGCTGGCATGAG
     I  A  P  V  R  E  L  P  P  F  R  Y  R  D  Y  L  G  W  H  E
1381 CAGGGCGATGGCAAGTGGTTCTTGGGTCTACCGATCACCAGTGGCCGGATTAAAGATGAC
     Q  G  D  G  K  W  F  L  G  L  P  I  T  S  G  R  I  K  D  D
1441 GGTAATTGGCAACTGCGATCGGCTTTGCGGGAGATCGTCAGCCGTTGGCAACTACCGCTG
     G  N  W  Q  L  R  S  A  L  R  E  I  V  S  R  W  Q  L  P  L
1501 TTGCTGACCGGCAGCCAAGACGTCTTGATCTACGATGTTCAGCCTGGCGATCGCGCTGCG
     L  L  T  G  S  Q  D  V  L  I  Y  D  V  Q  P  G  D  R  A  A
1561 ATTGATAAACTCCTCCGCGATCGCGGAGTTCATACGGTTGAGGCGATCGACTCCCTGCAG
     I  D  K  L  L  R  D  R  G  V  H  T  V  E  A  I  D  S  L  Q
1621 CGCTACGCGATGGCCTGCCCTGCCTTGCCAACCTGCGGTCTGGCGATTACGGAATCGGAG
     R  Y  A  M  A  C  P  A  L  P  T  C  G  L  A  I  T  E  S  E
1681 CGAGCCTTGCCGGGACTATTGGTGCGGATCCGTCGCCTCCTGGAAGAGCAAGGGTTGCCC
     R  A  L  P  G  L  L  V  R  I  R  R  L  L  E  E  Q  G  L  P
1741 GATGAGCATTTCGTGGTGCGGATGACTGGTTGTCCCAATGGTTGTGCTCGTCCCTACATG
     D  E  H  F  V  V  R  M  T  G  C  P  N  G  C  A  R  P  Y  M
1801 GCGGAACTGGCCTTTGTGGGTAGTGCACCGAATACCTACCAGCTCTGGCTAGGGGGGTCG
     A  E  L  A  F  V  G  S  A  P  N  T  Y  Q  L  W  L  G  G  S
1861 CCAGATCAAACCCGCTTGGCTCGACCCTTCATCGATCGCTTAGCGGATGGCGATGTAGAA
     P  D  Q  T  R  L  A  R  P  F  I  D  R  L  A  D  G  D  V  E
1921 ACCCAACTACGACCGCTGTTTGTCTTCTTCAAACAGAGTCGGCAAGCGGGCGAAAGTTTT
     T  Q  L  R  P  L  F  V  F  F  K  Q  S  R  Q  A  G  E  S  F
1981 GGTGATTTCTGCGATCGCGTCGGCTTCGACGCCCTGCGTCAGTTTAGTGAAAGCTACCAG
     G  D  F  C  D  R  V  G  F  D  A  L  R  Q  F  S  E  S  Y  Q
2041 CACGAAGCAGCCAAACCCGGCTATCGGGTAGGTCTACGGGCGGATGTGCACGGTCGTCTG
     H  E  A  A  K  P  G  Y  R  V  G  L  R  A  D  V  H  G  R  L
2101 AAAGCCGAAGCCGACAAACGTGGCGTCTCGCTGACTGACCTGGCCTGTGAGGCGATCGCC
     K  A  E  A  D  K  R  G  V  S  L  T  D  L  A  C  E  A  I  A
2161 GCCTACCTGCGCTAAgtgcggctcaaccgccgctggcgctatggcctagggctgtcgact
     A  Y  L  R  ***
2221 ctgttcatcgttcttggtgtacagggttggcagcaatggcagcaacgtcgctgggcagac
2281 actctggggattaccgccgcaccttt gccgagcgatcgcctagtgacgttggccgactgg
2341 cagcgccgccttgatcccttgcggttgacgccgcgccagcagcaacaattggagcctttg
2401 ctgatcagcttgcagcggctgcgggtctca gtggaactcgaagccgaccccccacgatcgc
2461 tatgctgggctgtggttaccaagtcagcggcagattcgcctcaatcctcgcttgctgcga
2521 tcgccttccgctttactgcaaagcctcagtcacgagagtgttcatgtagctcaaagctgt
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Fig. 1. Structure of the ferredoxin : sulphite reductase gene-nucleotide sequence and amino acid sequence deduced from the DNA of the gene encoding the fd-dependent sulphite reductase. Coding sequence is numbered from nt 301 to 2175, the putative ribosome binding site is underlined at nt 294 to 297.

the vector. The missing part of the gene was then excised by *Hin*dIII from pMal-Sir (for the 1.8 kb fragment) or from pGGAN3 (for the 8.5 kb fragment).

pB*cys*G was constructed from a 1.6 kb *Pvu*I/*Dra*I fragment of the gene *cys*G from *S. typhimurium* cloned in pJYW609. The fragment was subcloned in the *Sca*I site of pBR322 to give pB*cys*G, which could be propagated in *E. coli* TG1.

For expression and purification of a *sir* gene product a fusion gene vector was used (pMal-c2, New England Biolabs). The putative '*sir*'-gene (nt 301–2214) was ligated into the *Xmn*I/*Sal*I restriction site and expressed in *E. coli* TG1. The fusion protein consists of the *mal*E gene product fused to the *sir* gene product. This fusion protein was isolated by chromatography on DEAE-Sepharose, Superose 12 and affinity chromatography on a maltose matrix from IPTG-induced (1 mM) bacteria. The Sir polypeptide then is recovered from the fusion protein after treatment with factor Xa proteinase which specifically cleaves the fusion protein at the IEGR amino acid sequence separating MalE from the Sir gene product. Electrophoretically pure Sir polypeptide was used for the production of polyclonal antibodies [5] and Western immunoblotting was done by established procedures [25].

A large open reading frame (Fig. 1) extending over 1875 nt was found in the *Synechococcus* DNA fragment cloned in pGGAN2. The acronym '*sir*' was given to the open reading frame tentatively identified as the structural gene of the ferredoxin sulphite reductase. The DNA with the *sir* gene was not contiguous to the *par* gene [15] from the same strain of *Synechococcus*; hence, sulphite reductase and PAPS-reductase genes do not form a *cys*JIH-like operon as the enterobacteria [17]. When compared on the basis of nucleotides, it is 49.5% identical with the sulphite reductase structural gene *cys*I from *E. coli* encoding the iron-sulphur haemoprotein. The deduced amino acid sequence of the polypeptide from *Synechococcus* still is homologous to the iron-sulphur haemoprotein of the NADPH-sulphite reductase by 35% when compared over the complete sequence of 583 amino acid residues of the *cys*I gene described by Ostrowski et al. [17]. Most striking are the similarities in the domains which the authors suggested form the Fe_4S_4-sirohaem catalytic centre of sulphite and nitrite reductases. These two Cys clusters are selectively compiled from sulphite and nitrite reductases (EMBL Data Base, release 32, Table 1):

The C-terminal Cys clusters are invariably spaced by the highly conserved sequence 'PNG'. With the exception of the low molecular mass sulphite reductases from *Desulfovibrio vulgaris*, the sulphite reductases differ from the nitrite reductases only in that the glycine is replaced by serine and that the position of this glycine changed from inner left side of cysteine-495

sulphite reductases ("biosynthetic")							
sir	*Synechococcus*	444	M A C P A L P T C G L	454	489	T G C P N G C A	496
*cys*I	*E. coli* B	432	M A C V S F P T C P L	442	477	T G C P N G C G	484
MET	*S. cerevisiae*		S S C V P L P T C G L			T G C P N G C S	
cys	*T. roseopersicina*		M S C V A L P T C P L			T G C P N G C A	
sir	*A. thaliana*		M A C P A F P L C P L			T G C P N G C S	
nitrite reductases ("biosynthetic")							
nir A	*Synechococcus*	394	V S C T G S Q Y C N F	404	435	T G C P N S C G	442
nir	*Spinacea oleracea*	471	V A C T G S Q F C G Q	481	512	T G C P N S C G	519
	B.pendula		V A C T G N Q F C G Q			T G C P N S C G	
	Z. mays		V A C T G N Q F C G Q			T G C P N S C G	
sulphite reductases ("respiratory")							
asr C	*S. typhimurium*	113	V A C Q G N R I C Q K	123	151	V G C P N D C A	158
*dsr*A	*Archaeoglobus f.*	174	S A C M G P A L C E F	184	218	A G C P N D C V	225
	Desulfovibrio v.	89	Q A C P T G A V C P F	99	129	S G C P F C C G	126
nitrite reductase ("respiratory")							
*nii*A	*Aspergillus nidul.*	718	K S C V G T T W C R F	728	758	S G C V R E C A	765
nir B	*E. coli* K12	639	K T C V G S T W C R Y	649	679	S G C T R E C S	686

Fig. 2. Sulphite and nitrite reductase Cys clusters – alignment of the amino acids involved in the binding of the Fe_4S_4-sirohaem cluster in the six electron transferring homologous enzymes, numerals refer to the position of the residue when known: *sir* from *Synechococcus* – this paper, *cys*I from *E. coli* [16], *met* from yeast – Gisselmann, unpublished data, *sir* from *Arabiopsis thaliana* – Gisselmann, unpublished data, *cys*I from *Thiocapsa roseopersicina* – Haverkamp and Schwenn, unpublished data, nir A from *Synechococcus* PCC7942 – Omata, unpublished data, *nir* from spinach [3], from *B. pendula* [5], from *Z. mays* [13], *asr*C from *S. typhimurium* – [8], *dsr*A – alpha subunit of dissimilatory sulphite reductase from *Archaeoglobus fulgidus* – Dahl et al., unpublished data, low molecular weight form of 'assimilatory' sulphite-reductase from *Desulfovibrio vulgaris* [23], inducable nitrite reductases *nii*A from *Aspergillus nidulans* [9], *nir*B from *E. coli* [17].

to a position on the right side. This generalization seems to cease with the respiratory enzymes – sulphite and nitrite reductases alike. Of the two Cys clusters thought to be involved as bridging ligands of the haem-Fe_4S_4 cluster, the amino acids of the C-terminal Cys cluster (position 491) appears to be conserved rather strictly when compared with the second Cys cluster at position cys_{446}. This second Cys cluster is spaced by five amino acids of which only the proline seems to be conserved among sulphite reductases. This may be coincidental though, and, when compared with the other six electron-transferring reductases like nitrite reductase, including the respiratory enzymes, the homology only remains for the number and spacing of the cysteine residues in the clusters. It should be noted that the whole stretch of amino acids is hydrophobic in the sulphite reductases with a significantly higher content of leucine and proline favouring a bend or turn in the primary structure. In addition to homologies in the Cys clusters, the polypeptides were compared by their binary matching coefficients which consider identities in the position of the amino acids in combination with the size of the gene product. The coefficient between *Synechococcus* sulphite reductase and *E. coli* iron-

sulphur haemoprotein is 0.32 and 0.23 for the ferredoxin-dependent nitrite reductase from spinach. These rather low values may be seen as indication of a considerable phylogenetic distance between the enzymes, but straight diagonals were obtained by dot matrix comparison of the deduced amino acids (data not shown), supporting the view of homologous proteins.

As the overall homology with the NADPH : sulphite reductase haemoprotein together with the conservation of the Cys clusters of the putative Fe_4S_4-sirohaem binding site at positions 446/491 of the polypeptide led us to the suggestion that the cloned DNA is identical with the structural gene for ferredoxin : sulphite reductase, we wanted to confirm its identity. Protein sequence data are not yet available, so that the identification is based on a correlation of (a) enzymatic activity of the gene product, and (b) the immunological relationship between the recombinant gene product and the fd : sulphite reductase purified from a wild-type *Synechococcus*.

In order to express a *sir* gene product that could be used as enzymatically active sulphite reductase, the pBTac1 expression vector system [23] was used where the *sir* gene was cloned into the *Sma*I site. The *sir* gene was inserted in two steps: first, a 499 bp PCR product (from nt 301 to 798) of the gene *sir* including the ATG and a *Hin*dIII restriction site (corresponding to nucleotide position 324 of the gene in Table I) was ligated blunt-end into the *Sma*I site of pBTac. Second: the resultant plasmid was restricted with *Hin*dIII thus enabling the insertion of the large 1.8 kb *Hin*dIII fragment of *sir* (derived from pMal-Sir) including the 3′-end of the gene-yielding plasmid pTacSir1. pTacSir2 was considerably larger because it was constructed using an 8 kb *Hin*dIII fragment for insertion. Both plasmids were cloned in *E. coli* TG1. As Wu et al. [26] reported that the activity of the recombinant sulphite reductase from *E. coli cys*I was limited by an unsufficient supply of the sirohaem due to a lack of *cys*G gene product, we additionally complemented two of the transformants with pB*cys*G. Of the two types of construct monitored for sulphite reductase in vitro by its MVH-dependent formation of H_2S (Ref. 2, modified in Ref. 21), only pTac-Sir1 and pTac-Sir1 + pB*cys*G exhibited activity (103 and 171 nmol H_2S mg^{-1}) which corroborated with the amount of sulphite reductase detected by ELISA using the antibodies against the *sir* gene product (Fig. 3). The pTac-Sir2 constructs, harbouring additional DNA downstream of *sir*, both contained little activity (14 and 35 nmol H_2S mg^{-1}) while some activity was also found in the extract from the clone harbouring pMal-Sir (123 nmol H_2S mg^{-1}). The enzyme activity of the pMal-Sir clone, however, is expected to result from free unfused enzyme protein rather than the fusion protein.

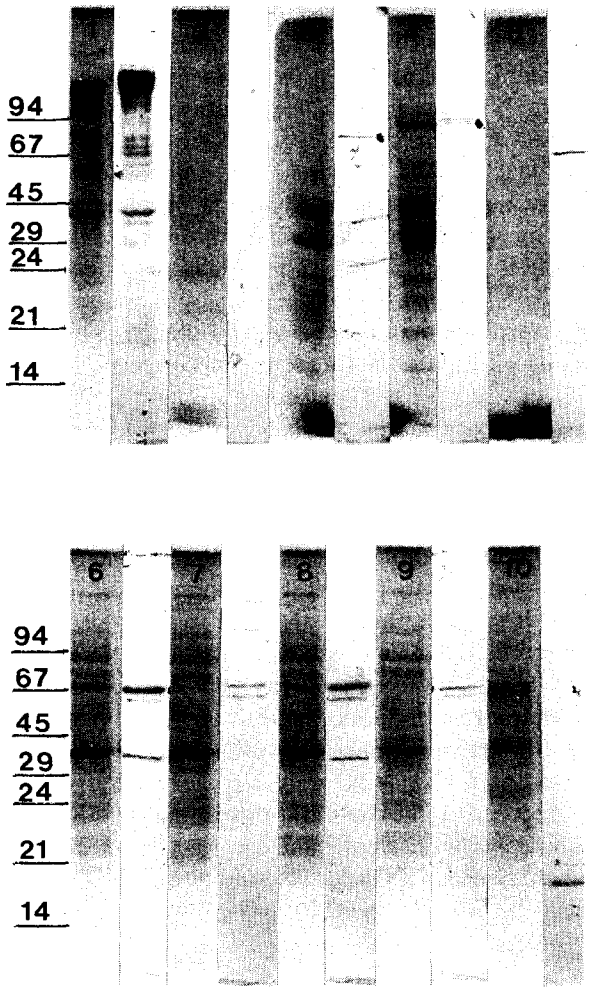

Fig. 3. Expression and immunological identification of the *sir* gene product – SDS-polyacrylamide gel electrophoresis (left) and Western blot (right lanes) of cell extracts containing recombinant fd : sulphite reductase and fd : sulphite reductase partially enriched from *Synechococcus*. Lane 1: cell extract from host *E. coli* TB1 harbouring the fusion vector pMal-Sir, and Western blot with anti : sulphite reductase antibodies. 2: total protein extract from *Synechococcus* wild-type, no detectable sulphite reductase; 3: sulphite reductase enriched (from DEAE-Sepharose, low Cl-eluting fractions); 4: sulphite reductase enriched (from DEAE-Sepharose, high Cl-eluting fractions); 5: sulphite reductase enriched (Phenyl-Sepharose, Brij 35 step); 6: expression of pSir1, 7: of pSir2, 8: pSir1 plus pB*cys*G, 9: pSir2 plus pB*cys*G,10: pJYW609 as control. Protein content in all lanes 15 μg except markers. For the Western immunoblots, anti-Sir-antibodies were raised against electrophoretically pure recombinant sulphite reductase purified from the fusion protein shown in lane 1. Detection of antigen-antibody complexes by the use of peroxidase-conjugated goat anti-rabbit immunoglobulins.

Expression of the cloned *sir* should yield a polypeptide of 70 028 Da – including the MAL-binding protein, a fusion-protein of 114 kDa has to be obtained (Fig. 3a). A pure Sir gene product was isolated from the fusion by maltose affinity chromatography and factor Xa proteinase treatment. The molecular mass of this gene product estimated by SDS-polyacrylamide gel electrophoresis was 69–70 kDa. The cyanobacterial

sulphite reductase is larger than the enterobacterial *cysI* gene product because the C-terminus is extended by 42 amino acid residues. In its size it strongly resembles the 71 kDa fd:sulphite reductase monomer purified from spinach [1]. Krueger and Siegel [13] observed that spinach leaves contained two immunologically related forms of the enzyme: a 69 and a 63 kDa monomer, assuming that the smaller polypeptide was formed by proteolytic degradation. The fd:sulphite reductase from *Spirulina platensis* purified by Tamura's goup using ferredoxin-affinity chromatography showed a mass of 63 kDa with the tendency to form a homodimer of 120 kDa at higher ionic strength [1,11].

The identity of the *sir* gene product as the structural gene of the fd:sulphite reductase was concluded from its cross-reactivity towards the fd:sulphite reductase which was purified from the wild-type *Synechococcus*. Polyclonal antibodies raised against the *sir* gene product were used for Western immunoblotting (Fig. 3b). In view of the highly disputed role of this enzyme in the process of assimilatory sulphate reduction by plant, the study of gene activity and expression will be most helpful to re-define its position in the pathway.

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