The *SalGI* restriction endonuclease

Purification and properties

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The type II restriction endonuclease *SalGI* has been purified to near homogeneity. At least 80% of the protein remaining after the final stage of the preparation is *SalGI* restriction endonuclease; no contaminating nucleases remain detectable. The principal form of the protein under both native and denaturing conditions is a monomer of M_r about 29000. The optimal conditions for both enzyme stability and enzyme activity have been determined.

Type II restriction endonucleases are enzymes that specifically recognize short nucleotide sequences in double-stranded DNA and cleave the DNA at fixed locations relative to their recognition sites (Roberts, 1976). The first type II restriction endonuclease to be discovered was the *HindII* enzyme (Smith & Wilcox, 1970); since then, over 200 enzymes of this type have been reported (Roberts, 1981). These enzymes have found wide application in the analysis and restructuring of DNA molecules and also provide convenient systems for studies on the interactions between proteins and specific sequences in nucleic acids since they require only Mg^{2+} as a cofactor (Roberts, 1976). Moreover, the recognition sites of many type II restriction enzymes possess an axis of two-fold rotational symmetry through the DNA, a factor common to many DNA–protein interactions (Jovin, 1976). However, studies of DNA–protein interactions require purified enzymes and only a few type II restriction endonucleases such as *EcoRI* have been obtained in pure form (Modrich, 1979).

SalGI is a type II restriction endonuclease isolated from *Streptomyces albus* G (Chater & Wilde, 1976). This enzyme was originally designated *SalI*, but the discovery of a type II restriction endonuclease from *S. albus* P (Chater, 1977) has required that these enzymes be named *SalGI* and *SalPI* respectively (Chater & Wilde, 1980). The

Abbreviations used: λ TF DNA, the DNA from the bacteriophage λ b221 *rex::tn5*, after digestion with *EcoRI* restriction endonuclease as described in the Materials and methods section; SDS, sodium dodecyl sulphate.

SalGI restriction endonuclease (EC 3.1.23.37) recognizes double stranded DNA at the sequence:

and cuts at the sites indicated by the arrows (Arrand *et al.*, 1978). Genetic studies have demonstrated that this enzyme is part of a host-controlled restriction–modification system (Chater & Wilde, 1980). Halford *et al.* (1979) showed that the *SalGI* endonuclease may simultaneously cleave both strands of the DNA at its recognition site, in contrast with the *EcoRI* enzyme which cleaves each strand in a separate reaction. Hence these two enzymes appear to interact with DNA through different mechanisms. In this paper, we describe the purification of the *SalGI* restriction endonuclease and several properties of the enzyme. The following papers (Maxwell & Halford, 1982*a,b*) describe studies on the mechanism of DNA cleavage by this enzyme and its specificity.

Materials and methods

DNA preparations

Bacteriophage λ b221 *rex::tn5* (bacteriophage number λ DB2523; Berg, 1977) was obtained from D. E. Berg (Washington University, St. Louis, MO, U.S.A.). The purification of this bacteriophage after infection of *Escherichia coli* C₆₀₀ and the isolation of its DNA were as described previously (Halford *et al.*, 1979). For use in enzyme assays, this DNA was digested with *EcoRI* restriction endonuclease, ex-

tracted twice with redistilled phenol, twice with chloroform and dialysed against 10 mM-Tris/0.1 mM-EDTA, pH 7.5; all reaction mixtures with DNA also contained 5 mM-Tris/0.05 mM-EDTA in addition to the stated constituents. Supercoiled DNA from plasmid pMB9 was prepared as described previously (Halford & Johnson, 1981) and that from plasmid pMB8 was a gift from Dr. S. J. Ford (this laboratory). The concentration of all DNA solutions were determined by absorbance with $A_{260}^{0.1\%} = 20 \text{ cm}^{-1}$ and are given in $\mu\text{g} \cdot \text{ml}^{-1}$. All DNA solutions were stored over chloroform at 4°C.

Purification of SalGI restriction endonuclease

Growth of cells. *S. albus* G was grown on agar plates [Difco malt extract ($24 \text{ g} \cdot \text{l}^{-1}$)/Oxoid yeast extract ($5 \text{ g} \cdot \text{l}^{-1}$)/Difco bacto agar ($20 \text{ g} \cdot \text{l}^{-1}$), pH 7.8] at 30°C until sporulation occurred (about 4 days). Spores from one plate were resuspended in 10 ml of sterile water, added to 10 ml of sterile glycerol/water (1:1, v/v) and stored at -20°C for periods of up to 1 year. Each spore suspension was used to inoculate 1 litre of growth medium containing 8 g of Difco nutrient broth, 340 g of sucrose, 10 g of MgCl_2 and 5 g of glucose, and the culture was shaken at 30°C for 48 h. The enzyme preparation described below is from an 8 litre culture of *S. albus* G.

Harvesting and lysis. Cells were harvested by centrifugation (15 min at 23 000 g, 4°C) and cell pellets were washed with 0.2 M-NaCl in enzyme buffer (10 mM- K_2HPO_4 /10 mM- β -mercaptoethanol/1 mM-EDTA, pH 8.0). (Cell pellets at this stage could be stored for several weeks at -20°C.) Pellets were resuspended in 500 ml of 0.2 M-NaCl in enzyme buffer and 2.5 g of lysozyme was added. After 30 min at 37°C, the suspension was cooled to 4°C. (All further stages in the procedure were conducted at 4°C.) This suspension was then disrupted by sonication in small batches. Cell debris was removed by centrifugation (30 min at 100 000 g) and the supernatant was retained.

Phosphocellulose chromatography. The 100 000 g supernatant was applied to a 150 ml phosphocellulose column (Whatman P11, Whatman, Maidstone, Kent, U.K.) prepared as described by Greene *et al.* (1978) and equilibrated in 0.2 M-NaCl in enzyme buffer. The column was washed with the same buffer until the A_{280} of the eluant fell to zero; the eluant was monitored for A_{280} with an Isco UA-5 (MSE Scientific Instruments, Crawley, Sussex, U.K.). The column was developed with 0.75 M-NaCl in enzyme buffer and the eluant was collected across the single peak of A_{280} -absorbing material. The peak was immediately dialysed against 50 mM-NaCl in enzyme buffer. After dialysis, the resulting precipitate was removed by centrifugation (10 min at 38 000 g).

DEAE-Sephadex chromatography. The supernatant was applied to a 65 ml DEAE-Sephadex column (Sephadex A-50, Pharmacia Fine Chemicals, Uppsala, Sweden), equilibrated in 50 mM-NaCl in enzyme buffer. The column was washed with the same buffer and the eluant was monitored to zero A_{280} as before. The column was developed with a linear gradient (300 ml) of 0.05–0.6 M-NaCl in enzyme buffer. Fractions were collected and assayed for *SalGI* endonuclease. Fractions containing the peak of enzyme activity (0.2–0.3 M-NaCl) were pooled and dialysed against 0.1 M-NaCl in enzyme buffer.

Heparin-agarose chromatography. The DEAE-Sephadex pool was applied to a 4 ml heparin-agarose column (Heparin-Sepharose CL-6B, Pharmacia), equilibrated in 0.1 M-NaCl in enzyme buffer. The column was washed in the same buffer, developed with a linear gradient (40 ml) of 0.1–1.0 M-NaCl in enzyme buffer and fractions were collected. After assaying, peak fractions (0.45–0.55 M-NaCl) were pooled and dialysed against 0.1 M-NaCl in enzyme buffer.

Hydroxyapatite chromatography. The heparin-agarose pool was applied to a 0.5 ml hydroxyapatite column (Bio-Gel HTP, from Bio-Rad Laboratories, Richmond, CA, U.S.A.) equilibrated in 0.1 M-NaCl in enzyme buffer. The column was washed in the same buffer and the eluant was monitored as before. The column was developed with a linear gradient (6 ml) of 0.01–0.3 M- K_2HPO_4 in 10 mM- β -mercaptoethanol/1 mM-EDTA/0.1 M-NaCl, pH 8.0. After assaying, peak fractions (0.12–0.17 M- K_2HPO_4) were dialysed against 0.2 M-NaCl/50% (v/v) glycerol in enzyme buffer. Enzyme preparations were stored at -20°C in this buffer.

Assay of SalGI restriction endonuclease

Wild type bacteriophage λ DNA possesses two *SalGI* recognition sites, both near the centre of the molecule (Chater & Wilde, 1976), and the products obtained by cleavage at these sites cannot readily be resolved from intact substrate by electrophoresis. DNA from the bacteriophage λ b221 *rex::tn5* carries an extra *SalGI* site within the *tn5* element and its cleavage by the *SalGI* enzyme yields a DNA band of higher electrophoretic mobility, which can easily be resolved from intact substrate. By using *EcoRI* fragments of this DNA (λ TF) as a substrate for the *SalGI* restriction endonuclease, partial products formed after the cleavage of the DNA at some but not all *SalGI* sites can be observed. Hence an estimate of the *SalGI* activity can be made from the time taken to reach the reaction end-point with the complete disappearance of partial products (Fig. 1). For qualitative assays, 1–5 μl of enzyme were incubated with 0.5 μg of λ TF DNA in assay buffer (50 mM-Tris/10 mM- MgCl_2 /125 mM-NaCl/10 mM- β -

mercaptoethanol/0.5 mg·ml⁻¹ bovine serum albumin) in a total volume of 20 µl, at 37°C. Digestion was stopped by the addition of 10 µl of stop mix [40% (w/v) sucrose/0.1 M-Tris/0.1 M-EDTA/0.5 mg·ml⁻¹ Bromophenol Blue, pH 8.0]. After incubation at 67°C for 10 min and quenching on ice, samples were analysed by electrophoresis through 0.67% agarose slab gels (Sharp *et al.*, 1973) at 9 V·cm⁻¹ for 1 h. Quantitative assays were performed by withdrawing samples (15–20 µl) at timed intervals from 100 µl reaction mixtures containing 2.5 µg of λTF DNA and *SalGI* enzyme in assay buffer at 37°C. Each sample was immediately added to 10 µl of stop mix and analysed as before (Fig. 1). One unit of *SalGI* restriction endonuclease was taken as the amount of enzyme required to completely digest 0.5 µg of λTF DNA in 20 µl of assay buffer in 1 h at 37°C.

Other materials and methods

Restriction endonuclease *EcoRI* and *E. coli* alkaline phosphatase were prepared as described previously (Halford, 1971; Halford *et al.*, 1979). DNA ligase of phage T₄ was a gift from Dr. A. G.

Hepburn (John Innes Institute, Norwich, U.K.). Hen ovalbumin and human type C transferrin were gifts from Dr. J. Williams (this department). Horse heart myoglobin, bovine erythrocyte carbonic anhydrase and bovine serum albumin were obtained from Sigma. Protein concentrations were determined by the method of Bradford (1976) using *EcoRI* restriction endonuclease as the standard. Solutions containing bovine serum albumin were heated at 67°C for 2 h before use in assays in order to inactivate contaminating nucleases.

Results and discussion

Purification of restriction endonuclease *SalGI*

Previous preparations of the *SalGI* restriction endonuclease have only separated the enzyme from contaminating nucleases (Chater & Wilde, 1976; Arrand *et al.*, 1978; Greene *et al.*, 1978). The procedure described in the Materials and methods section yields *SalGI* restriction enzyme of greater than 80% homogeneity (as shown below), with higher yields of enzyme per litre of bacterial culture

Fig. 1. Assay of *SalGI* restriction endonuclease. Samples (15 µl) were withdrawn, at timed intervals after the addition of enzyme, from 100 µl containing 2.5 µg of λTF DNA and 10 units of *SalGI* enzyme in 50 mM-Tris/10 mM-MgCl₂/125 mM-NaCl/10 mM-β-mercaptoethanol/0.5 mg·ml⁻¹ bovine serum albumin, pH 8.0, at 37°C. Samples were immediately added to 10 µl of stop mix and analysed by gel electrophoresis; the gel is shown above. The time of withdrawal (in min) of each sample is noted by each track on the gel; the zero-time point was taken before the addition of enzyme. The polarity of the gel is marked on the left-hand side and the positions of partial digestion products are marked by arrows on the right hand side.

Fig. 2. Growth of *Streptomyces albus* G. The cell mass (○) and yield of *SalGI* restriction endonuclease (●) were determined from 100 ml samples taken at timed intervals from a 1 litre culture of *S. albus* G inoculated at time zero and shaken at 30°C. Left-hand ordinate: cell mass was determined by weighing the washed pellet after centrifugation at 23 000 g. Right-hand ordinate: enzyme yields were determined by following the preparative procedure, as described in the Materials and methods section, to the 100 000 g supernatant stage for each sample and assaying for *SalGI* restriction endonuclease.

than had previously been described. We have found that the time of harvesting *S. albus* cultures affects the yield of enzyme; the peak of production of *SalGI* enzyme occurs during the exponential phase of growth (Fig. 2). Growth of the bacteria at 30°C produced a high yield of enzyme whereas at 37°C very little enzyme was obtained. As discussed below, restriction endonuclease *SalGI* is more unstable under certain conditions of pH and ionic strength than under other conditions, so we employ a pH of 8.0 and minimize the exposure of the enzyme to high salt concentrations (>0.35 M) in order to favour enzyme stability. Moreover, as enzyme activity is lost with time even under optimal conditions, we minimize the time taken for each stage; the procedure is routinely completed within 4 days.

The use of phosphocellulose in the preparation of the *SalGI* restriction enzyme has been described previously (Greene *et al.*, 1978). This step gives a large purification because the vast majority of the material applied to this column (as judged by A_{280}) does not bind to the resin. We have replaced elution by a NaCl gradient (Greene *et al.*, 1978) by a single step elution with 0.75 M-NaCl followed immediately by dialysis against 50 mM-NaCl. This eliminates lengthy exposure to high salt without reducing the purification. In our preparation, we have replaced DEAE-cellulose (Arrand *et al.*, 1978) with DEAE-Sephadex as this permits faster flow rates. Again the vast majority of the material applied to this column does not bind to the resin and an NaCl gradient resolves the *SalGI* enzyme from many other proteins. SDS/polyacrylamide electrophoresis of the pooled fractions from the DEAE-Sephadex column revealed about 10 bands of separate proteins, in contrast with more than 50 bands remaining after the phosphocellulose column. However, we experience large losses in enzyme activity at this stage (Table 1), probably due to the time taken to run this column and to assay the column fractions. Heparin-agarose has been widely used in restriction enzyme preparations, including the partial purification of *SalGI* (Chater & Wilde, 1980). Although a proportion of the proteins in the DEAE-Sephadex pool bind to heparin-agarose, an NaCl gradient elutes most of these in advance of the *SalGI* enzyme. SDS/

polyacrylamide-gel electrophoresis showed the heparin-agarose pool to contain four bands, of which *SalGI* enzyme constituted the major component. The minor contaminants from the heparin-agarose pool were removed on hydroxyapatite.

Our procedure yields a single major band of protein upon SDS/polyacrylamide-gel electrophoresis by the method of Laemmli (1970). This band was shown by densitometry to account for at least 80% of the total protein (Fig. 3). Analysis by polyacrylamide-gel electrophoresis under native conditions (Davis, 1964) again showed only one major band after staining for protein (results not shown). An identical sample on this gel was cut into 3 mm slices and the protein was eluted from each slice by incubating in 0.2 M-NaCl in enzyme buffer. The eluant from each slice was assayed for *SalGI* restriction enzyme; the peak of activity was comigrant with the major band in the stained sample.

Fig. 3. *Purity of SalGI restriction endonuclease*
A sample of purified *SalGI* restriction endonuclease (16 000 units) was denatured in 2% SDS by boiling and subjected to electrophoresis through a 15% polyacrylamide slab gel containing 0.1% SDS, as described by Laemmli (1970). After staining for protein, the gel track containing the *SalGI* enzyme was analysed by densitometry; the densitometric trace is shown above. Electrophoretic mobilities are expressed relative to the mobility of Bromophenol Blue; zero denotes the gel boundary.

Table 1. *Purification of SalGI restriction endonuclease*

Fraction	Total protein (mg)	$10^{-6} \times$ Total enzyme activity (units)	$10^{-4} \times$ Specific activity (units $\cdot$ mg $^{-1}$)
100 000 g supernatant	1800	50	2.8
Phosphocellulose pool	450	30	6.7
DEAE-Sephadex pool	4.1	3	73
Heparin-agarose pool	0.4	1	250
Hydroxyapatite pool	0.15	0.4	300

Fig. 4. Molecular weight of *SalGI* restriction endonuclease

(a) Electrophoresis of purified *SalGI* restriction endonuclease and proteins of known molecular weight on an SDS/polyacrylamide-gel was as described in the legend to Fig. 3. Molecular weight standards were; human transferrin (78 000), bovine serum albumin (65 000), carbonic anhydrase (29 500) and myoglobin (17 200): (○) indicates the relative mobility of molecular weight standards. The vertical arrow indicates the relative mobility of *SalGI* restriction endonuclease. Relative mobilities were calculated as the mobility of a protein band compared with the mobility of Bromophenol Blue.

(b) Gel filtration of purified *SalGI* restriction endonuclease and proteins of known molecular weights was carried out using a Sephacryl S-200 column (90 cm $\times$ 1.4 cm²) with elution by 0.2 M-NaCl in enzyme buffer. Elution volumes for standard proteins were determined by A_{280} and the elution volume for the *SalGI* enzyme was determined by assaying column fractions. The K_{av} for standard

Hence the major protein in this preparation is *SalGI* restriction endonuclease.

Although the enzyme has been 12 000-fold purified in terms of protein (Table 1), a large loss in enzyme activity is experienced during the procedure due to the extreme instability of the enzyme under certain conditions. It is therefore possible that the purified protein contains both active and denatured forms of the enzyme. However, the final specific activity (3×10^6 units $\cdot$ mg⁻¹) is similar to those reported for other restriction endonucleases, such as *Bst*II, 6×10^5 units $\cdot$ mg⁻¹ (Clarke & Hartley, 1979) and *Eco*RI, 1×10^7 units $\cdot$ mg⁻¹ (Halford *et al.*, 1979); for each of these enzymes, slightly different definitions for units of enzyme activity were used. Because of their utility in genetic engineering, it is desirable that high yields should be achieved in the purification of restriction enzymes. One strategy to enhance yields has been the cloning of the genes coding for restriction enzymes (Smith, 1979). Our studies on the preparation of *SalGI* have shown that the yield of one restriction endonuclease can be improved by manipulating existing procedures without the necessity for cloning. It is probable that improvements in the yields of other restriction endonucleases could be made in a similar way.

Molecular weight studies

SDS/polyacrylamide electrophoresis of purified *SalGI* restriction endonuclease gave an apparent subunit M_r of 29 000 for the enzyme (Fig. 4a). To determine the M_r under native conditions, gel-filtration studies were performed under two sets of conditions by the method of Andrews (1965). Elution with enzyme buffer containing 0.2 M-NaCl gave a single peak of enzyme activity corresponding to an M_r of about 30 000 (Fig. 4b). However, elution with assay buffer gave both a predominant peak of activity corresponding to an M_r of 30 000 and also a broad peak of low activity corresponding to a range of M_r values from 50 000 to 120 000. It is therefore possible that, under assay conditions, the enzyme exists in an equilibrium between monomer and aggregated states. Maxwell & Halford (1982a) suggest that the active form of the enzyme is a dimer. Several other restriction endonucleases are active in the dimeric form; for example, *Eco*RI and *Bam*HI (Modrich, 1979). Clarke & Hartley (1979) have shown that the *Bst*II restriction enzyme behaves

proteins are shown by (●) and the vertical arrow indicates the K_{av} for the *SalGI* enzyme. Molecular weight standards were: alkaline phosphatase (94 000), bovine serum albumin (65 000), ovalbumin (45 000), carbonic anhydrase (29 500) and myoglobin (17 200). K_{av} is defined by $K_{av} = (V_e - V_o) / (V_t - V_o)$, where V_e is the elution volume of the sample, V_o the void volume of the column and V_t the total column volume.

as a monomer during gel filtration under one set of conditions but as a dimer under other conditions. They suggest that the active form of the enzyme is a dimer but that the association between subunits is weak. It is possible that a similar phenomenon occurs with the *SalGI* restriction endonuclease.

Specificity of DNA cleavage

Many applications of restriction endonucleases do not require that the enzymes be pure. In most cases, it is sufficient that the activities of contaminating nucleases are minimal so that DNA cleavage occurs exclusively at the recognition site for the restriction enzyme. We have used sensitive assays to detect contamination by either non-specific endonucleases or exonucleases at each stage of the enzyme preparation.

Endonucleases can be readily observed by the conversion of supercoiled DNA into either its open-circle form, following a single strand cleavage at any site on the DNA or into its linear form, where both strands of the DNA have been cleaved at the same site. Supercoiled, open-circle and linear forms of many DNA molecules may be separated by electrophoresis through agarose (Johnson & Grossman, 1977). The DNA from the related plasmids, pMB8 and pMB9 (Rodriguez *et al.*, 1976), can both be prepared in the supercoiled form; pMB9 has a single *SalGI* recognition site but pMB8 has no *SalGI* site. Thus, comparisons of the cleavage of

these two DNA molecules by preparations of the *SalGI* enzyme give a measure of the level of the contamination by non-specific endonucleases at each stage of the enzyme purification (Table 2a). For experiments where the presence of non-specific endonucleases is undesirable, such as the mapping of DNA molecules, *SalGI* restriction endonuclease prepared to the DEAE-Sephadex stage would be of sufficient purity.

Restriction endonuclease *SalGI* cleaves DNA at its recognition site to yield complementary 5' single stranded extensions of four bases (Arrand *et al.*, 1978) which can be re-annealed to generate novel DNA molecules. Contaminating exonucleases would remove these single stranded extensions and prevent reannealing. Hence the efficiency with which DNA molecules, cleaved by the *SalGI* enzyme, may be re-ligated by DNA ligase gives an indication of the activity of contaminating exonucleases (Table 2b). However, if the exonuclease removes one or more bases of the single strand extensions, it is still possible that the DNA molecules may be re-ligated. But such molecules would no longer be substrates for the *SalGI* endonuclease as they lack the correct recognition sequence. Thus the complete cleavage of re-ligated DNA molecules by the *SalGI* enzyme demonstrates that the single strand extensions have remained intact (Table 2c). For experiments involving the re-annealing of DNA fragments that were obtained by cleavage with the *SalGI* endonuclease

Table 2. *Specificity of DNA cleavage by restriction endonuclease SalGI*

(a) Values refer to the ratio of the minimum amount of *SalGI* enzyme required to cleave 1 μ g of plasmid pMB8 DNA over the minimum amount required to cleave 1 μ g of plasmid pMB9 DNA in 20 μ l of 50 mM-Tris/10 mM-MgCl₂/125 mM-NaCl/10 mM- β -mercaptoethanol/0.5 mg·ml⁻¹ bovine serum albumin, pH 8.0, in 1 h at 37°C. Values for the last three stages of the preparation are lower limits as no digestion of plasmid pMB8 could be observed at the maximum enzyme concentration. (b) Plasmid pMB9 DNA (³H-labelled, 1 μ g) in 40 μ l of 50 mM-Tris/10 mM-MgCl₂/125 mM-NaCl/10 mM- β -mercaptoethanol/0.5 mg·ml⁻¹ bovine serum albumin, pH 8.0, was digested for 1 h at 37°C with 10-fold excesses of *SalGI* enzyme from each stage of the preparation. After cooling on ice, the same amount of T4 DNA ligase was added to each sample and each was made 0.1 mM in ATP. Samples, at 16°C, were then allowed to cool to 0°C over several hours. A 20 μ l aliquot was removed from each, added to 10 μ l of stop mix and subjected to electrophoresis on a 1% agarose gel at 5 V·cm⁻¹. Linear DNA was separated from re-ligated forms and the proportions of each was estimated by scintillation counting (Halford *et al.*, 1979). Values represent the proportions of re-ligated species in each sample, corrected for the efficiency of the DNA ligase preparation used (55% under these conditions). (c) To the remainder of each sample in (b), 10 units of *SalGI* enzyme were added and each was incubated at 37°C for 30 min. The products were analysed by electrophoresis and scintillation counting as above. Values represent the percentage of the re-ligated forms in (b) which were cleaved by the *SalGI* enzyme.

Fraction	(a) Amount of enzyme to cleave pMB8 DNA over amount required to cleave pMB9 DNA	Re-ligation and re-cleavage efficiencies of pMB9 DNA digested with 10-fold excess of <i>SalGI</i> enzyme	
		(b) Re-ligation (%)	(c) Re-cleavage (%)
100 000g supernatant	5	8	17
Phosphocellulose pool	10	20	12
DEAE-Sephadex pool	>200	37	19
Heparin-agarose pool	>200	90	85
Hydroxyapatite pool	>2000	100	85

or for DNA sequencing, preparation of the *Sal*GI enzyme to the heparin-agarose stage would be of sufficient purity.

Enzyme stability and activity

As mentioned above, the *Sal*GI restriction endonuclease is unstable under certain buffer conditions. The optimal conditions for enzyme stability have been determined by storing the enzyme in a variety of buffers at 4°C and assaying the restriction endonuclease after periods varying from a few hours to several weeks. The optimal pH for enzyme stability is pH 8.0; storage below pH 7.0 or above pH 9.0 leads to major losses in activity (75% over 2 weeks). The optimal NaCl concentration for enzyme stability is in the range 0.05–0.35 M-NaCl. Enzyme stored for 3 days with no NaCl lost 40% of its original activity whilst enzyme stored with 0.5 M-NaCl lost over 90%. In addition, total protein concentration affects the stability of the enzyme. If the concentration of purified enzyme was less than 1×10^5 units $\cdot$ ml⁻¹, then bovine serum albumin (0.5 mg $\cdot$ ml⁻¹) was required to maintain enzyme stability. We find that the *Sal*GI restriction endonuclease may be stored in 10 mM-KH₂PO₄/10 mM- β -mercaptoethanol/0.2 M-NaCl/1 mM-EDTA/50% (v/v) glycerol/0.5 mg $\cdot$ ml⁻¹ bovine serum albumin, pH 8.0, at -20°C for longer than 18 months without loss of enzyme activity.

The optimal reaction conditions for *Sal*GI restriction endonuclease activity were determined by assaying the enzyme with λ TF DNA, as in Fig. 1, but under a range of buffers. The optimal conditions were found to be 50 mM-Tris/10 mM-MgCl₂/125 mM-NaCl/10 mM- β -mercaptoethanol/0.5 mg $\cdot$ ml⁻¹ bovine serum albumin, pH 8.0; alterations from these values to the concentrations of either NaCl or MgCl₂, or to the pH, caused a reduction in the rate of DNA cleavage. However, the cleavage of λ TF DNA measures only the formation of products cleaved in both strands of the DNA at the recognition site, whereas products cleaved in one strand only remain undetected. Maxwell & Halford (1982a) report that alterations to the pH or reductions in the MgCl₂ concentrations caused not only a decrease in the overall rates of the reactions but also the formation of products cleaved in only one strand of the DNA. Changes to the ionic strength of the reaction mixtures did not alter the nature of the reaction products but only reduced the overall rate; slow rates observed at concentrations of MgCl₂ above 10 mM were found to be due to a general ionic strength effect.

The effect of other divalent cations on the cleavage of DNA by *Sal*GI restriction endonuclease has been investigated. Manganese can replace magnesium although the overall rate of DNA cleavage is slower. A very slow rate is

obtained with cobalt but neither zinc nor calcium will support the reaction of the *Sal*GI enzyme. Low concentrations (1 mM) of *p*-chloromercuribenzoate and mercuric chloride completely inactivate the *Sal*GI restriction endonuclease, indicating that thiol groups may be involved in maintaining enzyme activity; β -mercaptoethanol is included in all buffers to counteract the possible effects of such agents. As found in studies of enzyme stability, the addition of bovine serum albumin (0.5 mg $\cdot$ ml⁻¹) to reaction mixtures is required to maintain enzyme activity at high dilutions. Arrand *et al.* (1978) and Bingham & Atkinson (1978) have reported the appearance of two other restriction endonucleases (designated *Sal*II and *Sal*III) during preparations of the *Sal*GI enzyme from *S. albus* G. We have not observed these activities during our preparations.

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