Modulating Restriction Endonuclease Activities and Specificities Using Neutral Detergents


ABSTRACT

It is well known that type II restriction enzyme activities and specificities can be modulated by altering solution conditions. The addition of co-solvents such as dimethyl sulfoxide (DMSO), alcohols and polyols can promote star activity, which is the cleavage of non-cognate sequences. While neutral detergents are often used to control protein aggregation, little is known about the effect of neutral detergents on restriction enzyme activities and specificities. We report here that BamHI, BglI, BglII, EcoRI, EcoRV, HindIII, MluI, PvuII, SalI and XhoI restriction endonucleases are remarkably tolerant of high concentrations of neutral detergents Triton X-100, CHAPS and octyl glucoside. In most cases, λ DNA cleavage rates were comparable to those observed in the absence of detergent. Indeed, the specific activities of SalI and XhoI were appreciably increased in the presence of Triton X-100. For all enzymes active in the presence of detergents, sequence specificity toward λ DNA was not compromised. Assays of star cleavage of pUC18 by EcoRI, PvuII and BamHI endonucleases in equimolar concentrations of Triton X-100 and sucrose revealed reduced star activity in the detergent relative to the sucrose cosolvent. Interestingly, under star activity-promoting conditions, PvuII endonuclease displayed greater fidelity in Triton X-100 than in conventional buffer. Taken altogether, these results suggest that in some cases, neutral detergents can be used to manipulate restriction endonuclease reaction rates and specificities.

INTRODUCTION

Quite simply, type II restriction endonucleases (9) are the workhorses of molecular biology. The general utility of restriction enzymes lies in their exquisite specificity, i.e., their ability to recognize and cleave DNA at unique recognition sites typically 4–6 bp in length. This specificity of cleavage has been found to depend heavily on solution conditions. Relaxation in specificity, termed “star activity” (10), can be observed when ionic strength is low, pH is high, in the presence of Mn(II) (5) or in the presence of a variety of solvents, including dimethyl sulfoxide (DMSO), ethanol, glycerol and sucrose (12). Although little has been reported regarding the effects of neutral detergents on restriction enzyme activity, the tendency of restriction enzymes to aggregate (2–4,6–8,15) has prompted the application of Triton X-100 to control this behavior (6). Indeed, the optimized buffers for many commercial restriction enzyme preparations contain small amounts of Triton X-100 (typically 0.01%). This report describes a survey of the effects of neutral detergents on the activities and specificities of restriction enzymes. In general, our data demonstrate that neutral detergents are well tolerated by restriction enzymes and, in some cases, can be used to modulate both activity and specificity.

MATERIALS AND METHODS

Enzymes, Detergents and DNAs

Commercial enzymes and λ DNA were purchased from New England Biolabs (Beverly, MA, USA). In most cases, reaction buffers were prepared from concentrated stocks of commercial buffers. pUC18 was purchased from Life Technologies (Gaithersburg, MD, USA) and amplified using the Wizard Plus Maxiprep Kit (Promega Madison, WI, USA). Triton X-100, CHAPS and octyl glucoside (OG) were purchased from Pierce Chemical (Rockford, IL, USA).

Enzyme Activity Assays

Most enzymes were assayed at 1–6 U/µg λ DNA in reactions up to 35 min and quenched with loading dye in 100 mM EDTA. In reactions involving detergents, we added detergent from a concentrated stock to the reaction mixture. Resulting pH values were verified or adjusted as needed. As is customary for commercially prepared enzymes,
specific activities were computed using the units of enzyme and time required to completely cleave all natural sites on λ DNA for each enzyme. Enzymes that displayed λ DNA cleavage patterns not suitable for rate determinations (SalI, StyI and XhoI) were assayed against λ DNA predigested with HindIII endonuclease. Products were separated using agarose gel electrophoresis and visualized with ethidium bromide and UV light. Gels were documented with a documentation system from Alpha Innotech (San Leandro, CA, USA) and stored as digital files. Errors in relative rates were propagated according to sqrt[(err1/avg1)² + (err2/avg2)²] where err = relative variance.

For reactions involving cleavage of star sites, 1 μg pUC18 DNA was digested with 1–2 U of enzyme per microliter for 45 min. For EcoRI and BamHI endonucleases, star activity-promoting buffer (11) (25 mM Tris-HCl, 2 mM MgCl₂ at pH 8.2 and 8.5, respectively) was necessary to induce detectable amounts of star activity.

Vapor Pressure Osmometry

Osmometry was performed using a Model 5500 Vapro™ Vapor Pressure Osmometer (Wescor, Logan, UT, USA). The instrument was calibrated with standard solutions of known osmolality supplied by the manufacturer. Typical measurements involved placing 10 μL of sample on a sample disc and inserting it into the instrument, which then detected the vapor pressure above the disc in a closed chamber. The measurement is displayed and reported in mOsmol/kg of water.

RESULTS AND DISCUSSION

Survey of Restriction Activities in Various Detergents

As shown in Figure 1, 11 common restriction enzymes (BamHI, BglII, EcoRI, EcoRV, HindIII, MluI, PvuII, SalI, StyI and XhoI endonucleases) were assayed toward λ DNA in 10% Triton X-100. All enzymes were active in the presence of 10% Triton X-100 except StyI endonuclease, the activity of which could not be detected even after overnight digestions (16 h). To quantitate the effect of Triton X-100 and similar non-denaturing detergents on enzyme activity, assays were conducted in 10% (wt/vol) solutions of Triton X-100, CHAPS (zwitterionic) and OG (neutral). Table 1 is a summary of specific activities relative to those observed in the absence of detergent. With the exception of StyI endonuclease, reaction rates in the presence of Triton X-100 ranged from 68%–160% of the rate in the absence of detergent, with XhoI and SalII endonucleases cleaving DNA at rates appreciably above the control rate. Behavior in the presence of OG and CHAPS was more variable. For example, while XhoI activity was quite robust in the presence of OG, PvuII and EcoRI endonucleases exhibited only residual activity in the presence of this detergent.

The enhanced rates of cleavage for XhoI and SalI endonucleases in the presence of 10% Triton X-100 prompted determination of the dependence of the cleavage rate on detergent concentration. As summarized in Figure 2, XhoI and SalI cleavage rates increased with increasing Triton X-100. XhoI activity maximized between 5%–10% detergent; for SalI, maximum activity occurred at concentrations between 2%–5% Triton X-100. In contrast, SalI activity decreased with increasing CHAPS, indicating no activity benefit from any concentration of this detergent (data not shown).

Assays of Star Activities in Equimolar Triton X-100 and Sucrose

Distinctive λ cleavage patterns were retained for all enzymes active in the presence of detergents (Figure 1). Star activity, or relaxed specificity in cleavage, can be induced in many restriction enzymes with the introduction of high concentrations of co-solvents such as glycerol, sucrose, ethylene glycol, etc. (12). Such co-solvents have been used to quantitate and compare water release in specific and nonspecific DNA binding (13,14) as a function of osmotic pressure. Because the colligative property osmotic pressure is dependent on concentration, the contributions to osmotic pressure by detergents used in the above assays are certainly higher than in more typically dilute (0.01%) detergent solutions. However, both literature values (1,16) and our vapor pressure osmometry measurements (Table 2) indicate that high concentrations (up to 30%) of Triton X-100, CHAPS or OG

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Triton X-100</th>
<th>CHAPS</th>
<th>Octyl glucoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BamHI</td>
<td>1.06 ± 0.26</td>
<td>≤0.06</td>
<td>nd</td>
</tr>
<tr>
<td>BglII</td>
<td>0.89 ± 0.18</td>
<td>0.82 ± 0.20</td>
<td>≤0.01</td>
</tr>
<tr>
<td>BglII</td>
<td>0.98 ± 0.17</td>
<td>0.80 ± 0.18</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td>EcoRI</td>
<td>0.68 ± 0.20</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>EcoRV</td>
<td>1.32 ± 0.28</td>
<td>1.04 ± 0.26</td>
<td>0.97 ± 0.29</td>
</tr>
<tr>
<td>HindIII</td>
<td>1.00 ± 0.18</td>
<td>1.00 ± 0.18</td>
<td>0.51 ± 0.09</td>
</tr>
<tr>
<td>MluI</td>
<td>0.94 ± 0.25</td>
<td>0.56 ± 0.12</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>PvuII</td>
<td>0.88 ± 0.09</td>
<td>≤0.02</td>
<td>≤0.02</td>
</tr>
<tr>
<td>SalI</td>
<td>1.43 ± 0.27</td>
<td>0.62 ± 0.10</td>
<td>1.08 ± 0.28</td>
</tr>
<tr>
<td>StyI</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>XhoI</td>
<td>1.60 ± 0.30</td>
<td>0.59 ± 0.05</td>
<td>1.14 ± 0.11</td>
</tr>
</tbody>
</table>

Rates are relative to those observed in the absence of detergent. Errors in relative rates were propagated as outlined in Materials and Methods. nd: no rate detected.
exhibit osmotic pressures less than half of those observed with equimolar concentrations of sucrose.

Due to the higher molecular weights of Triton X-100 and CHAPS (ca. 600 g/mol), even 100% solutions of these detergents are \(< \, 2 \, M\) in concentration. Since concentrations of common osmolytes necessary to induce large fractions of star activity in restriction enzymes are typically 1-5 M (11,12), it is unlikely that star activity would dominate over cleavage of canonical sites in the presence of these detergents.

To determine if this is true, we compared star activities of EcoRI, PvuII and BsmHI endonucleases toward pUC18 in the presence of equimolar sucrose and Triton X-100, the detergent that best supported the cleavage activities of all three enzymes. pUC18 was chosen because star sites for these enzymes on this plasmid are well defined and can be easily distinguished on an agarose gel (12). There is one canonical BsmHI site and one canonical EcoRI site in pUC18, which separately produce a linearized DNA 2686 bp in length.

Further cleavage at the eight BsmHI star sites produces a characteristic series of smaller fragments; under star conditions for EcoRI endonuclease, fragments at 1869, 1677, 1009 and 817 bp are observed. There are two canonical PvuII sites in pUC18, producing two fragments 2368 and 318 bp in size. Under PvuII star conditions, one additional cleavage site produces fragments at 2174, 318 and 194 bp in length. Sucrose was chosen as the control co-solvent for these experiments because the high molecular weight of sucrose (342.3 g/mol) relative to other common co-solvents (ca. 100 g/mol) simplifies the preparation of solutions of equal molarity (0.4 M) and comparable wt/vol percentage (15%) to 25% Triton X-100. Finally, reaction conditions were

Table 2. Osmotic Pressures of Co-solvents

<table>
<thead>
<tr>
<th>Solution</th>
<th>Sucrose (g/mol)</th>
<th>Triton X-100 (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWT</td>
<td>342.3</td>
<td>602</td>
</tr>
<tr>
<td>M (mol/L)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>% wt/vol</td>
<td>15%</td>
<td>25%</td>
</tr>
<tr>
<td>mOsomol/L</td>
<td>544a</td>
<td>129b</td>
</tr>
<tr>
<td>mOsomol</td>
<td>515</td>
<td>110</td>
</tr>
</tbody>
</table>

To facilitate comparison with literature values, osmotic pressure measurements (in mOsomol/kg) were conducted in water. Star assay buffer (25 mM Tris-HCl, 2 mM MgCl₂, pH 8.2) contributes 44 mOsomol/kg of osmotic pressure.

aReference 16.
bReference 1.
chosen that encouraged enough star activity to conveniently detect it in an agarose gel. For EcoRI and BamHI endonucleases, this involved the use of star activity promoting buffer (11). In all cases, star activity was also promoted with high concentrations of enzyme (1–2 U/µL). Indeed, this was all that was required to induce star activity for PvuII endonuclease.

As shown in Figure 3, A and B, for both BamHI and EcoRI endonucleases, star activity is significantly higher in star-promoting buffers containing 15% (0.4 M) sucrose than in the detergent and conventional buffers. In the presence of 25% (0.4 M) Triton X-100, no additional BamHI or EcoRI star activity is observed relative to the conventional buffer. The cleavage patterns are more complex for PvuII endonuclease. For this enzyme, substantial star activity is observed in the conventional buffer, most likely because star behavior by this enzyme is particularly sensitive to high enzyme concentrations (1 U/µL) (C.M. Dupureur, unpublished observations). As expected (12), cleavage at the PvuII star site (2174 bp product) is greater in sucrose than in the control buffer. However, in the presence of Triton X-100 under star-promoting conditions, very little PvuII star cleavage was observed (Figure 3C), indicating that this enzyme exhibits greater fidelity in the presence of Triton X-100 under star activity-promoting conditions than in the absence of detergent.

Restriction Enzymes in Detergents

Neutral detergents are often used to lyse cells, solubilize membrane proteins and control protein aggregation (6). The tendency toward aggregation by a number of restriction enzymes has prompted the use of Triton X-100 in characterization studies. Our results indicate that for most of the enzymes surveyed, the presence of neutral detergents in buffer solutions does not significantly compromise the use and/or study of restriction enzymes.
Such tolerance has been reported for other soluble hydrolases (17) and may challenge the common assumption that most soluble proteins are not active in the presence of neutral detergents. Either way, our findings suggest that restriction digests can be performed in and with detergent-containing cell lysates. This finding has been useful in detecting plasmid DNAs in cell cultures without having to perform a maxi- or miniprep of DNA. It could also be useful in screening bacterial cells for new restriction activities.

Also of interest is the observation that the specific activities and specifici- ties of some restriction enzymes can be modestly improved by the addition of high concentrations of Triton X-100. One possible explanation for this phe- nomenon is that the detergent exerts some influence on the interaction be- tween the enzyme and target DNA. How this influence is distributed (e.g., if there are interactions between either or both reactants or with bulk water) re-quires additional study.

In the meantime, the finding that Triton X-100 can improve the fidelity of PvuII endonuclease (and perhaps other enzymes) will prove useful in curbing star activity in those situations that require suboptimal conditions. These include double digests in which the optimal conditions of one enzyme are compromised to accommodate an- other enzyme. In the presence of deter- gents, higher concentrations of enzyme could be more safely used to cleave stubborn cleavage sites.

REFERENCES


We thank Dr. William Neill for the use of his vapor pressure osmometer. This work is supported in part by the Robert Welch Foun- dation (Grant No. A-1315). Address correspond- ence to Cynthia M. Dupureur, Depart- ment of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843-2128, USA. Internet: cdup@tamu.edu

Received 3 June 1999; accepted 24 June 1999.

Lori H. Conlan, Thomas J. José, Kevin C. Thornton and Cynthia M. Dupureur
Texas A&M University
College Station, TX, USA