New technique for thermostability of restriction and modifying enzymes

A. K. Handique

No other discipline of science witnessed as much spectacular and massive growth as biotechnology has in terms of generation of basic knowledge, new techniques and newer concepts and applications, within a span of a decade. The paramount need for a fast-growing subject is development of new techniques to facilitate not only newer applications but also speed up generation of basic knowledge. The backbone of biotechnology is recombinant DNA technology by which a researcher can make and break a tailormade DNA molecule. What a scissor and sewing machine are to a tailor, their equivalent to a biotechnologist are a group of enzymes called restriction endonucleases and ligases. A problem that was worrying biotechnologists was the instability of these enzymes – they are notoriously fragile and had to be transported and stored at –20°C. Obviously this involves additional cost not only for researchers but for manufacturers and dealers. In a country with acute power shortage like India a sudden power cut for two days could mean that these expensive enzymes will find their way from deep freeze to dust bin!

A recent exciting discovery has shown that the problem of instability of these valuable enzymes may be over (*Biotechnology*, Sept. 1992). A team of researchers comprising of Camilo Colaco, Shevanti Sen, Madan Thangavelu, Stephen Pinder and Bruce Roser from Quadrant Research Foundat-

Table 1. Stability of restriction enzyme Pst I dried in various sugars

Carbohydrates	Reducing property	Temperature (°C)	Time (Days)	Activity recovered
Monosaccharides				
Threitol	–	37°	14	–
Erythritol	–	37°	14	–
Glucose	+	37°	1	+
			14	–
Sorbitol	–	37°	14	+
			35	+
			70	–
Di- and Trisaccharide				
Trehalose	–	37°	98	+++
		55°	70	+++
		70°	35	+++
Maltose	+	37°	14	–
		37°	7	+
Maltotriose	+	37°	14	–
Lactose	+	37°	14	+
		37°	35	–
Lactulose	+	37°	14	+
		37°	35	–
Palatinose	+	37°	14	–
Sucrose	–	37°	14	++
		37°	35	–
Polymers				
Inulin	–	37°	7	–
Dextran	+	37°	7	–
Ficoll	–	37°	7	+

Reducing property: (+) reducing sugar; (–) non-reducing sugar
Quantification of enzyme activity: (–) No detectable activity; (+) Some activity (10–20% of titer); (++) Partial activity (25–50% of titer); (+++) Full activity (90–100% of titer).
Other monosaccharides with no detectable activity are galactose, galactitol, mannose, mannitol and myo-inositol.
Source. Biotechnology, Sept. 1992 (p. 1009).

ion, Cambridge, England have demonstrated to the amazement of all that such restriction enzymes as well as modifying enzymes like ligases and polymerases when dried at 37°C or ambient temperature with a simple disaccharide called trehalose become extraordinarily stable. Extraordinary in the sense that such enzymes dried with trehalose remain perfectly intact even up to a temperature of +70°C and for prolonged period. The researchers showed that at 37°C (i.e. average tropical temperature) such enzymes dried with trehalose remain intact for up to nine months. Moreover, at a temperature of 70°C they remain intact for 35 days. Observations were not recorded beyond this period as it was not necessary because under normal environmental conditions such a high temperature for such a long duration is just unlikely. The restriction enzymes tested were Eco RI, Bgl II, Pst I, Hind III, Alu I, Bam HI, Bss HII, Bst XI, Cla I, Dde I, Eco RV, Hae III, Hin CII, Hin fI, Hpa II, Kpn I, Mlu I, Msp I, Not I, Nru I, Sae II, Sal I, Sfi I, Sma I, Taq I and Xba I. The modifying enzymes tested were T4 ligase and T7 DNA polymerase. If a simple disaccharide could do such wonder why not try other similar disaccharides, monosaccharides and polymers? Researchers tried that as well but found that none of them could impart the stability that trehalose imparted indicating the unique property of trehalose (Table 1).

The question is how did trehalose attract the attention of scientists as a potential stabilizing agent? Fundamental studies over decades on some curious biological phenomenon like cryptobiosis provided valuable clues in this regard. Cryptobiosis is a phenomenon whereby certain organisms during extreme desiccation and unfavourable condition remain dormant with nominal existence and upon rehydration resume normal life and flourish. Good examples are bakers yeast *Saccharomyces cerevisiae*, brine shrimp *Artemia salina*, soil nematode *Ditylenchus dipsaci*; lower group of plants such as Selaginella, moss, etc. Although the molecular mechanism associated with cryptobiosis is still not understood, yet as early as 1965 J. S. Clegg discovered a high concentration of trehalose in the tissues of organisms exhibiting cryptobiosis, i.e. encysted embryos of *Artemia salina* during desiccation. Subsequently several others demonstrated the high concentration of trehalose in the tissues of other organisms exhibiting cryptobiosis. High concentration of trehalose was found during desiccation and not when their normal life flourished under condition of adequate moisture. This strong positive co-relation between trehalose concentration and cryptobiosis indicates that trehalose somehow protects the vital enzymes and particularly genetic materials from degradation during desiccation. The entire development illustrates how knowledge generated through fundamental research leads to discoveries of great applied value. This should serve as an eye-opener for those who have scant regard for basic research.

It is also not understood how trehalose provides stability to the restriction and modifying enzymes. According to J. S. Clegg (1985) who proposed the water replacement hypothesis; trehalose can make multiple external hydrogen bonds and could therefore replace the essential water molecules that are involved in maintenance of tertiary structure. Another theory proposed by M. J. Burke (1985), known as glass state theory, states that the tendency of trehalose solutions to undergo glass transformation results in an amorphous continuous phase, similar in structure to vitreous ice, in which molecular motion and thus degradative molecular reactions would kinetically be insignificant. However, Colaco *et al.* (1992) opine that their observations are not consistent with either theory although it is closer to the water replacement theory.

Whatever may be the molecular mechanism of stability, the discovery has many immediate applications. First, the cost of restriction enzymes and modifying enzymes should come down because their transportation and storage will be less expensive as they can be transported like any other commodity and stored in any ordinary shelf like other common reagents. According to Colaco *et al*, their finding raises the possibility of developing simplified, pre-aliquotted kits for research in molecular biology as well as novel formats for application, such as automation of techniques for genome mapping and sequencing. Moreover it will be of great help in clinical diagnostics and education. Already there are reports of anti-blood group antibodies dried with trehalose remaining intact for prolonged periods, even for several years under room temperature. Similar observations are reported with respect to a variety of antibodies and blood coagulation factors (used for treatment of haemophiliacs).

Like PCR, which when it came to notice in 1985 made gene-cloning easy and simple overnight, trehalose should be able to convert restriction enzymes and modifying enzymes into stable reagents in no time. About PCR, its discoverer Karry B. Mullis commented, 'It (PCR) is going to make the life of molecular biologists easy'. Now trehalose will also make life easy not only for researchers but also for manufacturers and dealers of restriction and modifying enzymes.

A. K. Handique is in the Department of Applied Botany and Biotechnology, Gauhati University, Guwahati 781 014, India