Basic peptides enhance protein/DNA interaction *in vitro*

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Recently many genes encoding DNA binding transcription factors have been isolated. In order to further characterise such factors, a pure protein preparation is often required. One commonly used bacterial expression system is the pGEX method (1) which allows the large scale production of highly purified, soluble protein. Upon purification the DNA binding ability of the protein can be characterised using electrophoretic mobility shift assays (2). In this type of analysis, conditions such as salt concentration, pH and redox state have been shown to affect the protein/DNA interaction. Here we show that the addition of basic peptides can dramatically increase the apparent DNA binding affinity of transcription factors which have been expressed and purified via the pGEX system.

Employing the pGEX system we expressed and purified the DNA binding domain of BZLF1 (an EBV transactivating transcription factor) as previously described (3). This factor specifically binds to DNA containing AP1 binding sites (4), and is a member of the bZIP transcription factor family. When employed in a band shift assay with an oligonucleotide containing an AP1 site a specific protein/DNA complex is observed (Figure 1 and (3)). We observed that the formation of this complex was significantly enhanced by the addition of the basic peptide poly L-Lys, but not by poly L-Asp. These peptides did not bind DNA themselves and did not change the specificity of binding (not shown). A similar enhancement of DNA binding was observed by the addition of a peptide encompassing the predominantly basic DNA binding region of the Fos protein (Fos_B) but not with a peptide encompassing the neutrally charged Fos leucine zipper (Fos_Z).

This effect of positively charged peptides is not restricted to BZLF1. We also expressed and purified the DNA binding domains of the transcription factors C/EBP (bZIP family) and GAL4 (Zn²⁺ finger family) and tested their DNA binding for sensitivity to basic peptides. Both DNA binding activities were significantly enhanced by the addition of poly L-Lysine (data not shown). However, we have found that factors which exhibit a high DNA binding affinity (e.g. Fos/Jun) are not significantly affected by Poly L-Lysine. Thus, this effect may be a general phenomenon affecting a wide selection of low abundance, or

weakly binding transcription factors and should be useful in the purification and analysis of such factors.

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REFERENCES

1. Smith and Johnson (1988) *Gene* **67**, 31–40.
2. Fried and Crothers (1981) *Nucleic Acids Res.* **9**, 6506–6525.
3. Bannister *et al.* (1991) *Oncogene* **6**, 1243–1250.
4. Farrell *et al.* (1989) *EMBO J.* **8**, 127–132.

Figure 1. Electrophoretic mobility shift assay with the DNA binding domain of BZLF1. The BZLF1 was purified using the pGEX system and used in a binding reaction (30 μ l) with a ³²P-labelled oligonucleotide containing an AP1 site as described previously (3). A. Poly Asp; Poly L-Aspartic Acid (Sigma # P5387) B. Poly Lys; Poly L-Lysine (Sigma # PO879) C. Fos_Z; Fos protein 161–199 D. Fos_B; Fos protein 139–160. Amounts shown in μ g.

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