COMMENTARY
That zinging feeling: the effects of EDTA on the behaviour of zinc-binding transcriptional regulators

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Zinc-binding proteins account for nearly half of the transcription regulatory proteins in the human genome and are the most abundant class of proteins in the human proteome. The zinc-binding transcriptional regulatory proteins utilize Zn\(^{2+}\) to fold structural domains that participate in intermolecular interactions. A study by Matt et al. in this issue of the Biochemical Journal has examined the transcription factor binding properties of the zinc-binding module C/H1 (cysteine/histidine-rich region 1) found in the transcriptional co-activator proteins CBP (CREB-binding protein) and p300. Their studies revealed that EDTA treatment of native C/H1 leads to irreversible denaturation and aggregation. Of particular concern is their finding that unfolded C/H1 participate in non-specific protein–protein interactions. The implications of these results are significant. EDTA is a very potent zinc-chelating agent that is used ubiquitously in protein interaction studies and in molecular biology in general. The potentially detrimental effects of EDTA on the structure and interactions of zinc-binding proteins should be taken into account in the interpretation of a sizeable number of published studies and must be considered in future experiments.

Key words: CREB-binding protein (CBP), p300, chelation, denaturation, EDTA, transcription factor, zinc-binding domain, zinc finger.

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expression, questions regarding protein–protein interactions with the zinc-binding modules of CBP/p300 are likely just the tip of the iceberg. A cursory review of approx. 100 papers from the recent literature revealed that over 80% of the reported protein–protein and protein–DNA interactions with known zinc-binding transcription factors were studied using EDTA-containing reaction buffers. These include GST pull-down, electrophoretic mobility-shift and co-immunoprecipitation assays. The work presented by the Matt et al. [5] leads to the unavoidable conclusion that at least some of these published experiments were performed with partially or fully unfolded proteins.

The prevalence of EDTA in protein–protein interaction assays is not surprising, because its use has become nearly ubiquitous in the field of molecular biology. EDTA has been used for many decades as an inhibitor of metalloproteases and DNases and as a scavenger of trace heavy metals. EDTA concentrations of 0.1–5 mM are common in buffers used for protein purification, DNA and nuclear extract preparations, and a wide variety of protein–protein and protein–DNA interaction assays. EDTA is also frequently used in running buffers (e.g. Tris/borate/EDTA) for ‘native’ electrophoretic mobility-shift assays. Additionally, EDTA may be inadvertently introduced with some component of a reaction mixture, resulting in a low concentration of EDTA that may be present in reagents. False-negative results arise when the zinc-binding protein unfolds in the presence of EDTA and thus are unable to interact with their legitimate binding partners. False-positive results arise when the zinc-binding protein unfolds in the presence of EDTA and subsequently interacts non specifically with other proteins. Given the prevalence of zinc-binding motifs in transcription regulatory proteins, one would be wise to empirically determine the effect of EDTA on molecular interactions observed in gene expression studies in vitro.

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REFERENCES