

# **Neutral additives enhance the metal-chelate affinity adsorption of nucleic acids: role of water activity.**

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Immobilized metal-chelate affinity chromatography has been widely used in the purification of proteins, and we have recently found that it can also be applied to purification of nucleic acids through interactions involving exposed bases, especially purines. Here we report that the inclusion of moderate quantities of neutral solutes in the buffer substantially enhances the binding affinity of nucleic acids for immobilized metal-chelate affinity adsorbents. Addition of 20% (v/v) of solutes such as ethanol, methanol, isopropanol, n-propanol, and dimethyl sulfoxide enhances the initial affinity of binding of total yeast RNA by 4.4-, 3.8-, 3.7-, 3.0-, and 2.8-fold, respectively for Cu(II)-iminodiacetic acid (IDA) agarose adsorbent, and the weaker adsorption by Cu(II)-nitrilotriacetic acid (NTA) agarose was even more strongly enhanced. The adsorption affinities of the smaller oligodeoxynucleotide molecules A20, G20, C20 and T20 also increase with the addition of ethanol, suggesting that the effect is not significantly mediated by conformational changes. Binding enhancement generally correlates with reduction of water activity by the various solutes, as predicted by several models of solution thermodynamics, consistent with an entropic contribution by displacement of waters from the metal-chelate. Interestingly, the enhancement was not seen with the proteins bovine serum albumin and lysozyme.

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