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Note

Binding of zinc and copper to some gel filtration media

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Gel filtration chromatography is a widely used technique. Many of the substances purified by this method are metalloenzymes or other metalloproteins. In addition to its use as a method of purification, gel filtration has been used to determine the stability constants of various metal–ligand or metal–protein complexes[1–5].

Chromatography of metal-containing substances is complicated by two problems. The first is that complexes of some metals such as zinc are labile, and dissociation of the complex may occur during chromatography. This can be overcome by the use of a modified gel filtration technique[1,6] in which the eluting buffer contains a constant level of the metal in question. A second complication is that many commonly used gels are themselves avid metal binders. Morgan *et al.*[7] reported in 1972 that some Sephadex gels bind zinc; Sephadex G-15 binds as much as 108 μg Zn/g gel, depending on the buffer used[6].

This paper is a short report of the zinc- and copper-binding capacities of various types of gel chromatographic media.

EXPERIMENTAL

Zinc and copper binding to the following gels was measured*: Sephadex G-10, G-15, G-25 and G-75; Biogel P-2, P-10, P-100; LKB Ultragel AcA-54 and Biogel A-5 m. Sephadex is a cross-linked dextran gel. Biogel P gels are polyacrylamide gels. Biogel A-5 m is an agarose gel and LKB Ultragel AcA-54 is a mixture of agarose and acrylamide. Biogel A-5 m and LKB Ultragel AcA-54 were washed with water before use to remove sodium azide, Tris, and EDTA.

Known weights of each gel were swollen in distilled deionized water and packed into 30 × 1.5 cm glass columns. The void volume of each gel column was determined with blue dextran and the total volume was defined as the elution volume of tritiated water (^3H_2O), which was measured by liquid scintillation counting.

Each gel was washed with a solution containing 10 ppm (1.54×10^{-4} *M*) Zn as $Zn(NO_3)_2$ and either no buffer, 10 m*M* acetate buffer pH 5.5, or 10 m*M* Tris–acetate

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buffer pH 7.4. Fractions of 0.8 ml were collected and the zinc or copper content was determined by atomic absorption spectrophotometry.

The metal-binding capacity of the gels was calculated as follows: μg metal/g gel = (elution volume in ml required to reach equilibrium — elution volume of ^3H_2O [V_T])/g of dry gel. Two gels, LKB AcA-54 and Biogel A-5 m, are not available in a dry form. Zinc binding to these gels was expressed as μg Zn/ml settled bed volume of gel. For comparison, binding of zinc to other gels was expressed in the same fashion.

RESULTS AND DISCUSSION

The amounts of zinc and copper bound by each of the gels tested is listed in Table I. For the dextran and polyacrylamide gels, the amount of metal bound tended to increase with increased degrees of cross-linking in the gel; thus Sephadex G-10 and Biogel P-2 are the most avid binders. The two agarose-containing gels are not highly cross-linked, having exclusion limits of 5,000,000 (Biogel A-5 m) and 70,000 (LKB AcA-54) daltons, but they bind more zinc than any of the other gels examined except Biogel P-2. Sepharose 4B, another agarose gel, also binds zinc, as shown by the fact that ^{65}Zn applied to a column of Sepharose 4B cannot be eluted from the column.

TABLE I

BINDING OF ZINC AND COPPER TO VARIOUS GELS

	μg Zn/g gel			*μg Zn/ml bed vol.*	*μg Cu/g gel*	
	No buffer	*10 mM Tris, pH 7.4*	*10 mM Ac, pH 5.5*	*No buffer*	*No buffer*	*10 mM Ac, pH 5.5*
Sephadex						
G-10	154	43		46	143	44
G-15 (ref. 6)	108	29				
G-25	144	32	9	20	123	21
G-75	45	8		2	33	3
Biogel						
P-2	569	210		164	337	337
P-10	403	30		31	275	36
P-100	57	11		3	34	26
LKB AcA-54				85		
Biogel A-5 m				83		

Increasing buffer strength decreased the amount of metal bound, except for copper binding to Biogel P-2. Increasing buffer strength to 50 m*M* (ref. 6) further decreased zinc binding to Sephadex to G-15.

The zinc experiments were originally done at pH 7.4 because many biochemists prefer to operate at physiological pH; because of solubility limitations, it was more practical to use pH 5.5 for copper. Copper is generally a better ligand than zinc if conditions are equal. At pH 7.4 for zinc and pH 5.5 for copper, the amount bound is similar for zinc and copper. Sephadex G-25 bound about three times as much zinc at pH 7.4 as at pH 5.5. It seems likely that at pH 7.4, copper binding to these gels would be higher than copper binding at pH 5.5, and would exceed that of zinc at pH 7.4.

When the experiments were completed and the metal-saturated gels dried for storage, all of the copper-containing gels were distinctly blue. Biogel P-2 was noticeably blue even in the swollen state at the end of the experiment.

Examination of the binding of every metal to every possible gel would be an endless project. This sampling of common gels shows they are good binders of zinc and copper. Other metals, such as calcium, magnesium, sodium, potassium, and lithium are known to displace zinc from Sephadex gels[6]. It seems reasonable to expect that most transition metals would bind to these gels, in varying degrees, depending upon the experimental conditions.

Biochemists who work with both metalloproteins and low-molecular-weight metal compounds would do well to check the metal-binding properties of the gels they use, particularly if the metal in question is one (such as zinc) that forms labile complexes that may dissociate during chromatography. The agarose gels and the highly cross-linked gels appear to have the greatest potential for causing problems. In the determination of stability constants[1,3–5], a constant level of metal is passed through the column in the buffer, which eliminates any problem that might arise from metal binding by the gel. However, purification procedures may benefit considerably if metal binding by the gels is taken into account.

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