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Note

Improved chromatography: prearranged distances between boronate groups by the molecular imprinting approach

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In our laboratory, as in others, the potential of molecular imprinting has been studied in the past[1–5]. In one of these studies, silica particles with a coating of polymerized silanes were used and the print molecule was held covalently bound to a boronate silane molecule during polymerization[1]. The preparations obtained showed preferential binding for the original print molecules including a glycoprotein such as transferrin. The binding was much more effective and specific compared with silica particles containing the corresponding amount of boronate groups randomly distributed.

In this paper we report on studies of the distance between binding points, comparing the binding of NAD and that of the corresponding bis-NAD, a molecule previously used for affinity precipitation of enzymes[6] and directed enzyme immobilization[7]. The binding of two bis-NAD molecules with different lengths of the interconnecting spacer was also investigated.

EXPERIMENTAL

A typical polymerization/silanization was carried out as follows. Boronate silane (4 μmol) in 0.5 ml of methanol containing 0.15 M hydrochloric acid was heated at 80°C for 30 min to remove the propyl groups with which the boronate silane was originally protected. The template molecule used (4 μmol of dATP, 2 μmol of NAD or 1 μmol of bis-NAD), dissolved in 2.0 ml of distilled water, was thoroughly mixed with the above boronate silane solution, followed by addition of dodecyltriethoxysilane (20 μmol), phenyltriethoxysilane (31 μmol), N-2-aminoethyl-3-aminopropyltrimethoxysilane (113 μmol) and bis(2-hydroxyethyl)aminopropyltriethoxysilane (138 μmol). Finally, 0.5 g of silica (LiChrospher Si 1000, 10 μm) was added and, after mixing, the pH was adjusted to 7.0. The silanization was allowed to take place at room temperature for 48 h with occasional pH adjustments.

The samples (approximately 50 nmol) were injected via a 20-μl loop and eluted at 0.07 ml/min with 0.1 M glycine–0.1 M magnesium sulphate (pH 8.0). A void volume of 0.48 ml was obtained for each column.

Boronate analysis was performed according to ref. 8.

Bis-NAD II was synthesised from N^6-carboxymethyl-NAD[9] as described earlier[6]; bis-NAD I can be synthesised in an analogous manner, substituting hydrazine for adipic acid dihydrazide.

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RESULTS AND DISCUSSION

A mixture of organic silanes was polymerized around porous silica particles, leading to silica particles now coated with an additional layer of silanes. During this process a template molecule, NAD (two riboses) or bis-NAD (four riboses) was bound with reversible covalent bonds to the boronate silane through their ribose units. After polymerization, the template molecule was removed, exposing the boronate groups now fixed at a distance from each other defined by the template (Fig. 1).

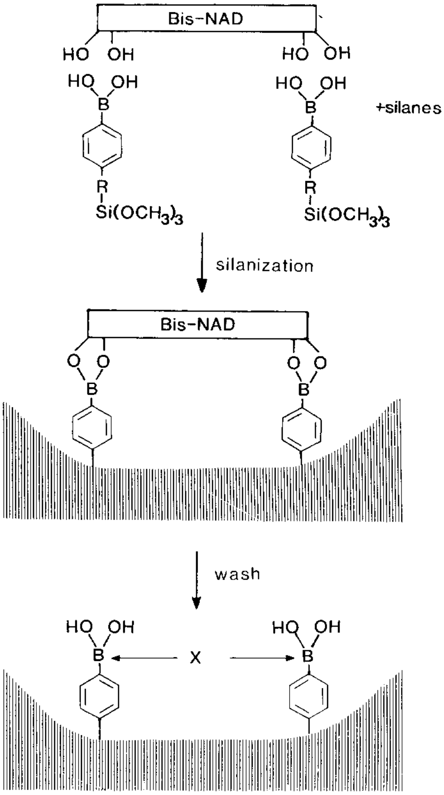

Fig. 1. Schematic representation of the preparation of silica particles with a prearranged distance (X) between boronate groups.

Packing the silica particles in a high-performance liquid chromatographic (HPLC) column allowed rapid evaluation of the chromatographic properties of the material. The results of the chromatographic separations are summarized in Table I. First deoxy-ATP was used as an imprinting reference substance, as owing to its lack of vicinal hydroxy groups it does not form an ester linkage with the boronate groups. The fact that NAD is retarded more is obvious, as here we have ribose molecules available; the two bis-NAD molecules (Fig. 2) bind even more tightly owing to the higher number of available ribose units.

TABLE I

RESULTS OF CHROMATOGRAPHIC SEPARATIONS

Parameter	*Template*			
	dATP	*NAD*	*Bis-NAD I*	*Bis-NAD II*
μmol boronate/g silica	1.08	1.19	0.96	0.98
Retention of dATP (ml)	0.58	0.58	0.58	0.58
Retention of NAD (ml)	1.05	1.40	0.77	0.77
Retention of bis-NAD I (ml)	4.10	4.38	4.03	1.82
Retention of bis-NAD II (ml)	4.45	5.15	1.93	4.27

When NAD was used as a template, as expected dATP did not bind well compared with NAD and the two bis-NAD molecules. Finally, when molecular imprinting was carried out with the shorter bis-NAD I (spacer length 7 Å) or the longer bis-NAD II (spacer length 17 Å), the former was far more retarded on its "own" silica particles as was the latter. This case clearly illustrates that the geometry of the position of the boronate groups is instrumental in the observed retardation pattern (and not only the number of possible interactions between available boronate groups on the surface of the silica particles and ribose moieties).

As seen from the above discussion, comparisons should be made within each preparation, as it is most difficult to obtain totally reproducible preparations, owing partly to the fact that print molecules are used.These experiments clearly demonstrate the possibility of obtaining highly specific (tailor-made) preparations by proper arrangement of the binding groups. Such preparations may have great potential in chromatography.

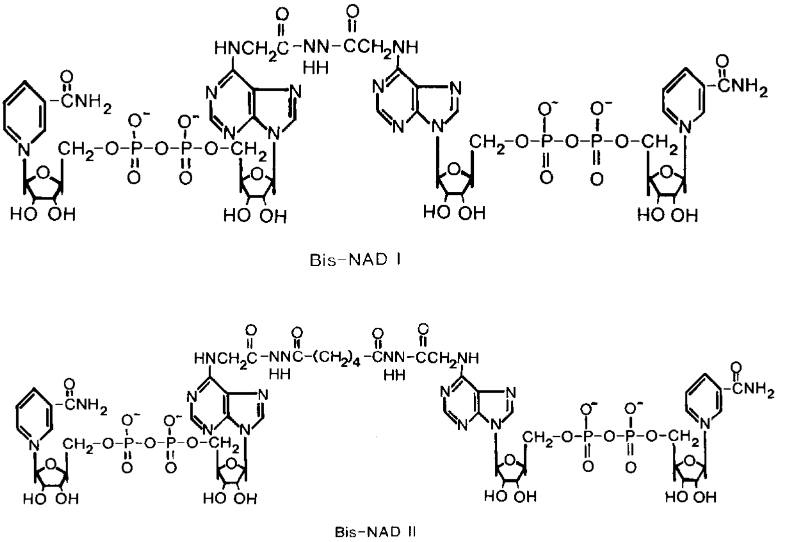

Fig. 2. Structure of the bis-NAD molecules used as template.

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REFERENCES

1 M. Glad, O. Norrlöw, B. Sellergren, N. Siegbahn and K. Mosbach, *J. Chromatogr.*, 347 (1985) 11.
2 O. Norrlöw, M. Glad and K. Mosbach, *J. Chromatogr.*, 299 (1984) 29.
3 L. Andersson, B. Sellergren and K. Mosbach, *Tetrahedron Lett.*, 25 (1984) 5211.
4 G. Wulff, B. Heide and G. Helfmeier, *J. Am. Chem. Soc.*, 108 (1986) 1089.
5 K. Shea and T. Dougherty, *J. Am. Chem. Soc.*, 108 (1986) 1091.
6 P. O. Larsson and K. Mosbach, *FEBS Lett.*, 98 (1979) 333.
7 M. O. Månsson, N. Siegbahn and K. Mosbach, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1984) 1487.
8 M. K. John, H. H. Chuah and J. H. Neufeld, *Anal. Lett.*, 8 (1975) 559.
9 M. Lindberg, P. O. Larsson and K. Mosbach, *Eur. J. Biochem.*, 40 (1973) 187.