ADDENDUM

The effect of nucleotide presaturation on the chromatographic behaviour of 6-phosphogluconate dehydrogenase from *Bacillus stearothermophilus* on immobilized triazine dyes

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We have extended the work of Metcalf *et al.* (1981) to study the effect of ligand (nucleotide) presaturation on the chromatographic behaviour of proteins on triazine-dye matrices. We have examined the effect of nucleotide presaturation on the binding of NADP⁺-dependent 6-phosphogluconate dehydrogenase to Procion Red HE-3B-agarose and Cibacron Blue 3G-A-agarose.

In view of the claims by several authors that the triazine dye Cibacron Blue 3G-A binds to the nucleotide-binding sites of proteins, it seemed to us important to know whether nucleotide presaturation affected the binding of proteins to Cibacron Blue 3G-A-agarose and other dye columns and whether these results would give some insight into the anomalous behaviour of triazine-dye columns when no care was taken to remove presaturating ligands (nucleotides) before chromatography.

Materials and methods

Materials

Procion Red HE-3B was a gift from Dr. C. V. Stead, I.C.I. Organics Division, Blackley, Manchester, U.K. Cibacron Blue 3G-A was from CIBA-GEIGY (U.K.), Manchester, U.K. Sepharose 6B was obtained from Pharmacia Fine Chemicals (G.B.), London, U.K. *Bacillus stearothermophilus* was supplied by Dr. A. Atkinson, Centre for Applied Microbiological Research, Porton Down, Wilts., U.K. Other reagents were purchased from BDH Chemicals, Poole, Dorset, U.K.

Methods

Affinity gel electrophoresis was used to determine K_{diss} values as described by Johnson *et al.* (1980). Other details are given in Table 1.

Results and discussion

Table 1 shows the effect of the addition of presaturating NADP⁺ on the dissociation constant for the interaction between 6-phosphogluconate dehydrogenase from *B. stearothermophilus* and either Procion Red HE-3B-Sepharose 6B or Cibacron Blue 3G-A-Sepharose 6B.

Table 1. Effect of the addition of NADP⁺ on the dissociation constant for the interaction between 6-phosphogluconate dehydrogenase (*B. stearothermophilus*) and immobilized triazine dyes

Dissociation constants were measured for the purified protein by affinity gel electrophoresis as described by Johnson *et al.* (1980). Determinations of K_{diss} were made from three separate measurements for at least six different ligand concentrations. The pure enzyme was isolated by the method described by Qadri & Dean (1980). The charcoal-treated enzyme (Eby & Kirtley, 1971) [5 units ($\mu\text{mol}/\text{min}$) of activity] was treated with 0.5 mM-NADP⁺ (final concn.) and the solution was exhaustively dialysed against 50 mM-Tris/HCl buffer, pH 7.2, containing 1 mM-EDTA and 1 mM-mercaptoethanol; 10 μg of enzyme was used for each determination. The ligand concentrations used were 3–30 μM (Procion Red HE-3B) and 30–150 μM (Cibacron Blue 3G-A). $R_{\text{m0}}/R_{\text{m1}}$ varied between 1 and 6. K_{diss} values are means $\pm$ S.D.

Added nucleotide	$K_{\text{diss.}} (\mu\text{M})$	
	Procion Red HE-3B	Cibacron Blue 3G-A
None	9.5 $\pm$ 3	39.3 $\pm$ 3.5
NADP ⁺	28.5 $\pm$ 4	>3000

The enzyme showed a lower (weaker) affinity for the blue dye than for the red, which is consistent with the lower salt concentration required to elute this enzyme from Cibacron Blue 3G-A-agarose than from Procion Red HE-3B-agarose columns (Qadri & Dean, 1980). Furthermore, the binding to Cibacron Blue 3G-A-agarose could be completely abolished by preincubation with NADP⁺ (no observable retardation on affinity-gel electrophoresis). The above effect, which occurred on adding NADP⁺, was observed even after the enzyme had been exhaustively dialysed (after preincubation with nucleotide). On the other hand, Table 1 also shows that nucleotide presaturation of the enzyme only altered the K_{diss} for immobilized Procion Red HE-3B 3-fold.

Once 6-phosphogluconate dehydrogenase had been exposed to NADP⁺, it was only possible to

bind the enzyme to Cibacron Blue 3G-A-agarose after either charcoal treatment (Eby & Kirtley, 1971) or by prior chromatography on Procion Red HE-3B-agarose, with salt (KCl) as eluent (Qadri & Dean, 1980).

The above effects have important implications with respect to dye-ligand chromatography in general: presaturating ligands such as fatty acids, nucleotides and lipids must be removed from proteins before dye-ligand or affinity chromatography in order to produce reliable and reproducible results.

These findings also show that affinity electrophoresis can be a useful tool in assessing the effect

of presaturating ligands on enzyme (or protein) binding to affinity columns.

We thank Powell and Scholefield Ltd. for financial support for F. Q.

References

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