BIOCHEMICAL LETTERS

Biospecific elution and multivalency in affinity chromatography: reappraisal of the biospecific desorption of rat liver lactate dehydrogenase from 10-carboxydecylamino-Sepharose

A recent publication (Kyprianou & Yon, 1982) reported results of frontal chromatographic studies on the interaction of the M_4 isoenzyme of rat liver lactate dehydrogenase with 10-carboxydecylamino-Sepharose (10-CD-Sepharose), and on its biospecific elution by NADH. It was concluded that interaction with the matrix led to the simultaneous blocking of two NADH-binding sites on the enzyme (Fig. 5 of Kyprianou & Yon, 1982). However, the conclusion may be questioned on the grounds that the theoretical treatment disregarded the possible formation of cross-linked complexes in which the effectively bivalent lactate dehydrogenase molecule formed bridges between two different matrix sites on the 10-CD-Sepharose. The results are therefore re-examined in terms of quantitative affinity chromatography theory (Nichol *et al.*, 1981) which does take into account such interactions, and are shown to be consistent with the simpler concept that the biospecific desorption of lactate dehydrogenase reflects competition between 10-CD-Sepharose sites and NADH for the enzyme's four coenzyme-binding sites, each of which is equivalent and independent with respect to either type of interaction.

In frontal chromatography of a mixture of partitioning enzyme, A, and a ligand, S, that competes with matrix sites, X, for f equivalent and independent sites on the enzyme, the quantitative relationship describing such affinity chromatography has been reported (Nichol *et al.*, 1981) as:

$$k_{AX} = \frac{(1 + k_{AS}m_S)[(1 + K_{av} - K_{av}^*)^{1/f} - 1]}{\bar{m}_X - f\bar{m}_A(1 + K_{av} - K_{av}^*)^{(f-1)/f}[(1 + K_{av} - K_{av}^*)^{1/f} - 1]} \quad (1)$$

where k_{AX} and k_{AS} denote intrinsic association constants (Klotz, 1946) for the interactions of enzyme with matrix sites and ligand respectively; $\bar{m}_A$ refers to the total concentration in the mixture subjected to frontal chromatography; and m_S , the free ligand concentration, may be equated with $\bar{m}_S$,

its total concentration in the mixture, if $\bar{m}_S \gg f\bar{m}_A$. The partition coefficient, K_{av} , of the enzyme constituent may be obtained (Laurent & Killander, 1964) from the measured weight-average elution volume, $\bar{V}_A$. Indeed, the required difference between partition coefficients is given by:

$$(K_{av} - K_{av}^*) = (\bar{V}_A - V_A^*)/V_S \quad (2)$$

where V_S is the volume of the stationary phase, and V_A^* the elution volume of enzyme in the absence of any interaction with matrix sites. $\bar{m}_X$ refers to the concentration of accessible matrix sites.

As noted by the authors, Figs. 2 and 3 of Kyprianou & Yon (1982) refer to frontal chromatographic experiments on a column (0.1 ml) of 10-CD-Sepharose for which $V_S = 0.066$ ml and $V_A^* = 0.086$ ml. These values, taken in conjunction with the elution volumes ($\bar{V}_A$) or differences ($\bar{V}_A - V_A^*$) inferred from the above-mentioned Figures, clearly allow specification of $(K_{av} - K_{av}^*)$. Furthermore, in view of the subunit structure of lactate dehydrogenase, f may reasonably be ascribed a value of 4, or of 2 to encompass the situation envisaged previously (Kyprianou & Yon, 1982). Consequently, eqn. (1) may be used to evaluate k_{AX} and k_{AS} provided that a magnitude can be assigned to $\bar{m}_X$, the concentration of accessible matrix sites. This parameter is usually obtained (Nichol *et al.*, 1981; Kuter *et al.*, 1983) from the ordinate intercept of a double-reciprocal plot of the amount of enzyme bound as a function of enzyme concentration, $\bar{m}_A$, in experiments with no ligand present. However, since the range of enzyme concentrations examined in Fig. 2 of Kyprianou & Yon (1982) is too small to allow precise delineation of the ordinate intercept of that plot, apparent magnitudes of k_{AX} were determined from each of

the six experimental points for values of $\bar{m}_X$ in the range 40–230 μM , the appropriate value being identified as that giving the smallest standard error in k_{AX} . With $f = 4$ the relative errors (twice the

Table 1. Evaluation of k_{AX} and k_{AS} for the 10-CD-Sepharose-lactate dehydrogenase-NADH system
 k_{AX} and k_{AS} were determined by application of eqn. (1) to frontal chromatographic results (Figs. 2 and 3 of Kyprianou & Yon, 1982) obtained in the absence and presence, respectively, of NADH.

$\bar{m}_A$ (nM)	m_S (μ M)	$(\bar{V}_A - V_A^*)$ (ml)	$(K_{av} - K_{av}^*)$	$10^{-4} \times k_{AX}$ (M^{-1})	$10^{-5} \times k_{AS}$ (M^{-1})
238	—	2.31	35.0	1.37	—
178	—	2.34	35.5	1.32	—
142	—	2.61	39.5	1.36	—
119	—	3.22	48.8	1.49	—
102	—	3.51	53.2	1.53	—
9	—	3.58	54.2	1.38	—
9	2	1.04	15.8	1.41	3.65
9	3	0.74	11.2		3.47
9	4	0.59	8.9		3.23
9	5	0.39	5.9		3.72
9	6	0.37	5.6		3.24

S.E.M. expressed as a percentage of the mean) ranged from 13.4%, for $\bar{m}_X = 40 \mu$ M, to a minimum of 4.8%, for $\bar{m}_X = 126 \mu$ M: with $f = 2$ the corresponding values ranged from 7.9%, for $\bar{m}_X = 40 \mu$ M, to 6.7%, for $\bar{m}_X = 66 \mu$ M. The results thus favour the concept that the four coenzyme-binding sites of the enzyme interact independently with the 10-CD-Sepharose, rather than that involving their interaction in pairs. It is also noted that the small magnitude of the relative error in k_{AX} seemingly justifies the approximation, inherent in eqn. (1) (Nichol *et al.*, 1981), that the enzyme-matrix interactions for this system may be considered in terms of a single intrinsic association constant.

The first part of Table 1 summarizes the experimental results from Fig. 2 of Kyprianou & Yon (1982), and also the values of k_{AX} obtained therefrom on the basis that $f = 4$ and $\bar{m}_X = 126 \mu$ M: a value of $1.41 (\pm 0.07) \times 10^4 M^{-1}$ is obtained for k_{AX} . Application of eqn. (1) with this value of k_{AX} to the results reported in Fig. 3 of Kyprianou & Yon (1982), which refers to the elution of lactate dehydrogenase (9 nM) in the presence of 2–6 μ M-NADH, then yields the magnitudes of k_{AS} listed in the final column of Table 1. The present estimate of $3.5 (\pm 0.2) \times 10^5 M^{-1}$ for k_{AS} compares very favourably with literature values for the binding of NADH to mammalian M_4 isoenzymes of lactate dehydrogenase, the majority of which are in the range 1×10^5 – $5 \times 10^5 M^{-1}$ (Stinson & Holbrook, 1973; Brinkworth *et al.*, 1975; Brodelius & Mosbach, 1976).

The important conclusion is therefore reached that the elution by NADH of rat liver lactate dehydrogenase from 10-CD-Sepharose is indeed a biospecific phenomenon, reflecting direct competition between matrix sites and NADH for the four coenzyme-binding sites of the enzyme. It is hoped that this reappraisal of results for this system may focus attention on the need (Eilat & Chaiken, 1979; Nichol *et al.*, 1981) to consider the

formation of crosslinked complexes in the quantitative interpretation of affinity chromatographic data obtained with solutes that exhibit multivalency towards matrix sites; and on the availability of theoretical relationships for a wide variety of possible affinity systems (Winzor *et al.*, 1982). On the other hand, non-uniformity in the distribution of matrix sites may impose restrictions on the freedom of the multivalent solute to form all possible cross-linked complexes, a freedom implicit in eqn. (1). Consequently it is also possible to envisage situations where severe clustering of matrix sites may render appropriate the more restrictive analysis used by Kyprianou & Yon (1982).

Donald J. WINZOR* and Robert J. YON†

*Department of Biochemistry, University of Queensland, St. Lucia, Queensland 4067, Australia; and †School of Biological Sciences and Environmental Health, Thames Polytechnic, Wellington Street, London SE18 6PF, U.K.

(Received 2 November 1983)

- Brinkworth, R. I., Masters, C. J. & Winzor, D. J. (1975) *Biochem. J.* **151**, 631–636
 Brodelius, P. & Mosbach, K. (1976) *Anal. Biochem.* **72**, 629–636
 Eilat, D. & Chaiken, I. M. (1979) *Biochemistry* **18**, 790–794
 Klotz, I. M. (1946) *Arch. Biochem.* **9**, 109–117
 Kuter, M. R., Masters, C. J. & Winzor, D. J. (1983) *Arch. Biochem. Biophys.* **225**, 384–389
 Kyprianou, P. & Yon, R. J. (1982) *Biochem. J.* **207**, 549–556
 Laurent, T. C. & Killander, J. (1964) *J. Chromatogr.* **14**, 317–330
 Nichol, L. W., Ward, L. D. & Winzor, D. J. (1981) *Biochemistry* **20**, 4856–4860
 Winzor, D. J., Ward, L. D. & Nichol, L. W. (1982) *J. Theor. Biol.* **98**, 171–187
 Stinson, R. A. & Holbrook, J. J. (1973) *Biochem. J.* **131**, 719–728