Binding of aldolase to actin-containing filaments

Quantitative reappraisal of the interactions

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Previously reported results of equilibrium-partition experiments on the interaction of aldolase with actin-containing filaments [Walsh, Winzor, Clarke, Masters & Morton (1980) *Biochem. J.* **186**, 89–98] have been subjected to a more rigorous theoretical analysis involving consideration of the consequences of cross-linking interactions between enzyme and filament. The experimental results obtained with F-actin–tropomyosin are best described by a model with one binding site per heptameric repeat unit of filament and a value of $39\,000\text{ M}^{-1}$ for the site binding constant, k . Similar analyses of the influence of Ca^{2+} on aldolase binding to F-actin–tropomyosin–troponin substantiate the existence of two equivalent binding sites ($k = 14\,900\text{ M}^{-1}$) for the enzyme on each repeat unit of the thin filament. The Ca^{2+} -sensitivity of this interaction reflects either a decrease in the strength of aldolase binding to these two sites ($k = 8200\text{ M}^{-1}$) or the elimination of one site.

In a recent publication (Walsh *et al.*, 1980), results of equilibrium-partition experiments were reported for the interaction of aldolase with re-constituted thin filaments of rabbit muscle. The experiments were considered in terms of the empirical expression,

$$r = r_{\text{max}} \cdot K[A]/(1 + K[A]) \quad (1)$$

where $[A]$ denotes the molar concentration of free aldolase, K the association constant for the enzyme–filament interaction, and r_{max} the value of the binding function r [expressed as the amount (g) of aldolase bound per g of filament] corresponding to saturation of the filaments with aldolase. Eqn. (1) accounts for the multiple binding of aldolase to the filaments in accordance with the binding theory developed by Klotz (1946) for a univalent ligand. In these terms, r_{max} may be identified as $M_A q/M_X$, where M_A is the molecular weight of aldolase and M_X is that of the repeating unit of the filaments, each of which bears q sites.

This treatment, however, may be questioned on the basis of the availability of multiple binding sites on aldolase (Walsh *et al.*, 1980), whereupon the possible formation of complexes with aldolase bridges between filament sites should also be

considered. Indeed, there is direct evidence from electron microscopy that such cross-linking does occur in the F-actin–tropomyosin–troponin–aldolase system (Clarke & Morton, 1976; Stewart *et al.*, 1980). The following revised treatment of the results places no restriction on the nature of the interactions between a p -valent protein and a q -valent matrix element, except that a single site binding constant, k , is assumed to govern all interactions, this assumption being common to several quantitative considerations of ligand binding in systems with an infinite array of complexes (Flory, 1941; Singer, 1965; Calvert *et al.*, 1979).

Theory

From reacted-site probability theory (Flory, 1941) it is possible to interrelate the free (c_A , c_X) and total weight concentrations of a p -valent ligand, A , interacting with a q -valent acceptor, X , by:

$$c_A = \bar{c}_A(1 - P_A)^p \quad (2a)$$

$$c_X = \bar{c}_X(1 - P_X)^q \quad (2b)$$

in which bar superscripts are used to denote total concentrations, and P_A , P_X to denote probabilities that a site on A or X (respectively) has interacted; A is thus visualized as aldolase and X as a muscle-filament repeat unit. If all interactions between A

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and X are assumed to be governed by the same site binding constant, k , it may be shown, by reasoning similar to that used previously (Calvert *et al.*, 1979), that:

$$k = P_A / \{ [q(\bar{c}_X/M_X) - pP_A(\bar{c}_A/M_A)](1 - P_A) \} \quad (3)$$

Since equilibrium-partition experiments involve measurement of the free protein concentration (c_A) in a system with predetermined values of $\bar{c}_A$ and $\bar{c}_X$, eqns. (2a) and (3), together with assigned values of p and q , provide a direct measure of k from each experiment. In principle, the appropriate combination of p and q may therefore be recognized by the degree of constancy observed in the k values so calculated from partition experiments with a wide range of $\bar{c}_A/\bar{c}_X$ ratios.

Results and discussion

Results of partition-equilibrium studies of the binding of aldolase to various actin-containing filaments in imidazole/HCl buffer, pH 6.8, I 0.088, were presented in Fig. 4 of Walsh *et al.* (1980). For consistency with that investigation, parameters have been assigned the following magnitudes: $p = 4$; $M_A = 160\,000$; $M_X = 362\,000$ and $432\,000$ for F-actin-tropomyosin and F-actin-tropomyosin-

troponin respectively. In Table 1, which summarizes the analysis of results for the binding of aldolase to F-actin-tropomyosin filaments, columns 1 and 2 list the experimental results, and the next two columns the values of the site binding constant, k , calculated from eqns. (2a) and (3) on the basis of interaction with one and two matrix binding sites per filament repeat unit. From the magnitudes of the relative errors associated with the mean k values, it is evident that the experimental results are described better by the model with one binding site per filament repeat unit ($q = 1$). Analysis of the results in terms of eqn. (1) with $r_{\max}$ taken as M_A/M_X yields the apparent intrinsic binding constants shown in the final column of Table 1, the mean value of $42\,700\text{ M}^{-1}$ corresponding to an equilibrium constant, $K (=4k)$, of $171\,000\text{ M}^{-1}$, in reasonable agreement with the value of $163\,000 \pm 12\,000\text{ M}^{-1}$ for K determined previously (Walsh *et al.*, 1980) on the basis of a Scatchard (1949) analysis of the same results; it is noted, however, that the previously evaluated quantity was incorrectly described as the intrinsic binding constant. It is also evident that the mean value of $42\,700\text{ M}^{-1}$ for k derived from eqn. (1) is indistinguishable (within the 95% confidence limits chosen for comparisons) from the value of $39\,000\text{ M}^{-1}$ deduced from eqns. (2a) and (3), an observation reflecting the fortuitous situation that eqn. (1), albeit empirically based, serves to approxi-

Table 1. *Interaction of aldolase with F-actin-tropomyosin filaments in imidazole/HCl buffer, pH 6.8, I 0.088*
The site binding constant, k , has been calculated from partition experiments, reported in Fig. 4 of Walsh *et al.* (1980), in which the concentration of filaments was 1 mg/ml.

Aldolase concn. (mg/ml)		k (M^{-1})		
$\bar{c}_A$	c_A	if $q = 1$	if $q = 2$	from eqn. (1)
0.039	0.023	53 400	26 100	65 300
0.061	0.041	39 900	19 400	46 200
0.100	0.068	40 000	19 100	45 900
0.121	0.086	35 400	16 900	40 000
0.150	0.098	47 100	21 800	54 400
0.193	0.136	38 800	17 900	43 500
0.246	0.168	45 400	20 100	51 000
0.281	0.209	34 000	15 300	37 200
0.297	0.209	42 900	18 700	47 600
0.400	0.303	34 400	14 800	37 100
0.506	0.384	37 300	15 300	39 700
0.665	0.532	30 800	12 400	32 400
0.673	0.525	36 600	14 200	38 400
0.773	0.629	29 500	11 600	30 700
0.877	0.709	33 500	12 400	34 600
0.992	0.789	42 600	14 200	43 100
1.004	0.805	40 200	13 600	40 700
1.222	1.000	40 500	12 800	40 400
Mean		39 000	16 500	42 700
Relative error* (%)		7	11	9

* Twice the S.E.M., expressed as a percentage of the mean.

mate the dependence of r upon $[A]$ for a cross-linking system with $p = 4$ and $q = 1$.

Analysis of the results for the interaction of aldolase with F-actin filaments (Fig. 4a of Walsh *et al.*, 1980) yields a site binding constant of $18\,600 \pm 2800 \text{ M}^{-1}$ for the interaction of tetravalent enzyme with a single matrix site per seven actin residues. In view of this finding and the detection of only a single binding site (with $k = 39\,000 \text{ M}^{-1}$) per repeat unit of F-actin-tropomyosin filament, the attachment of tropomyosin to F-actin must effectively eliminate the aldolase-actin interaction; the binding of aldolase to tropomyosin has been demonstrated (Walsh *et al.*, 1980), and the good conformity of the experimental results obtained with F-actin-tropomyosin to the model with $q = 1$ seemingly precludes coexistence of two separate matrix sites with binding constants differing by a factor of two.

In agreement with the conclusion of Walsh *et al.* (1980), the present analysis of results on the interaction of aldolase with F-actin-tropomyosin-troponin also favours the existence of two equivalent and independent binding sites for enzyme on the repeat unit of thin filaments in the absence of Ca^{2+} . The relative error associated with the mean value of k for the model with $q = 2$ (5%) is substantially smaller than that (11%) for the system with $q = 1$. However, the coexistence of tropomyosin and troponin, both of which possess a site for interaction with aldolase (Walsh *et al.*, 1980) on the filament, lowers the site binding constant from $39\,000 \text{ M}^{-1}$ (Table 1) to $14\,900 \text{ M}^{-1}$, possibly as a consequence of steric effects in both binding and cross-linking processes. Unequivocal designation of the phenomenon responsible for the Ca^{2+} -sensitivity of the interaction of aldolase with F-actin-tropomyosin-troponin filaments cannot be made, since the relative error associated with the mean k value (6%) is the same for models with $q = 1$ and $q = 2$. Thus the addition of CaCl_2 (0.2 mM) leads either to essentially a twofold decrease in the strength of aldolase binding to the two matrix sites ($k = 8200 \text{ M}^{-1}$), or to the elimination of one of the two sites per filament repeat unit ($k = 18\,900 \text{ M}^{-1}$). The previous inference, based on empirical analysis in terms of eqn. (1), that one matrix binding site per 14 actin residues (two repeat units) is eliminated by

Ca^{2+} , seems less tenable on physical grounds than either of the above two propositions.

In addition to providing a more rigorous basis for the interpretation placed on results for the interaction of aldolase with actin-containing filaments, the present paper has also illustrated a method for direct evaluation of the site binding constant from binding data for interactions of acceptors and ligands that are both multivalent. A suggested alternative method of analysis (Steensgaard *et al.*, 1977), based on calculation of the equilibrium mixture composition, is to use the experimentally determined concentration of unbound ligand and the known total concentrations of acceptor and ligand in a numerical-simulation procedure aimed at evaluating the interaction constant. However, inspection of eqn. (1) of Steensgaard *et al.* (1977) shows that the suggested procedure neglects the Flory (1941) statistical factors and thus assumes that all equilibria are governed by a single stoichiometric equilibrium constant, a situation that imposes unusual restraints on the standard free-energy changes associated with successive additions of ligand to acceptor. Although adaptation of that numerical-simulation approach to conform with the concept of a single site binding constant is feasible, the present analysis of binding data in terms of reacted-site probability theory is clearly to be favoured since it provides a more tractable expression, amenable to analytical solution, for the evaluation of k .

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