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Short communication

Measurement of binding constants by capillary electrophoresis

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Abstract

The assumptions inherent in a capillary electrophoresis procedure for evaluating binding constants for interactions between lectins and charged polysaccharides [S. Honda et al., *J. Chromatogr.*, 597 (1992) 377] have been reappraised. Whereas the results were originally interpreted on the basis that the lectin–carbohydrate interaction was restricted to 1:1 complex formation, a more plausible interpretation is shown to be that an approximately constant incremental difference separates the mobilities of the successive complexes formed as the result of saccharide binding to equivalent and independent sites on the lectin. The parameter that is determined by capillary electrophoresis should thus be regarded as the intrinsic binding constant.

1. Introduction

Recent articles [1–6] have drawn attention to the potential of capillary zone electrophoresis as a method of evaluating binding constants for interactions between proteins and charged ligands. In that regard the first-mentioned two investigations [1,2] were concerned with the interactions of lectins with charged saccharides; and both investigations are therefore open to criticism on the grounds that the multivalence of the lectins in their interactions with carbohydrates has been ignored. Indeed, conformity of the experimental results with the quantitative expressions developed on the basis of 1:1 stoichiometry was taken to imply the operation of the lectin in monovalent mode. The aim of the present communication is to examine more closely the theoretical aspects of the quantification; and hence to identify the actual assumptions that were inherent in the analyses.

2. Theory

In both quantitative applications of capillary zone electrophoresis the interaction of the lectin with a charged saccharide has been quantified by determining the effect of ligand concentration upon the electrophoretic mobility of the protein (acceptor, A). The parameter being measured is the constituent electrophoretic mobility, $\bar{\mu}_A$, defined [7,8] by

$$\bar{\mu}_A = \left[\mu_A C_A + \sum_1^p \left(\mu_{AS_i} C_{AS_i} \right) \right] / \bar{C}_A \qquad (1a)$$

$$\bar{C}_A = C_A + \sum_1^p C_{AS_i} \qquad (1b)$$

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a system in which acceptor, A, characterized mobility μ_A and present at free molar concentration C_A, possesses p sites for interaction h ligand (saccharide), S. Complexes AS_i here $1 \leq i \leq p$), characterized by mobilities s_i, are present at molar concentrations C_{AS_i}, ereupon the total acceptor concentration, $\bar{C}_A$, obtained by summing the concentrations of all ceptor-containing species (Eq. 1b). As noted eviously [1–6], the binding constant for a stem restricted to 1:1 complex formation ($p =$ is readily determined from the dependence of upon C_S, the concentration of saccharide cluded in the electrophoretic medium. However, the decision [1,2] to ascribe p a value of ity is in conflict with the quaternary structure hibited by the various lectins investigated.

For acceptors with more than one binding site r ligand ($p > 1$), an analytical solution to Eq. 1 quires specification of μ_{AS_i}, the electrophoretic obility of each acceptor–ligand complex, AS_i $\leq i \leq p$), as well as its equilibrium concentation. Even under circumstances where all ceptor–ligand interactions are governed by a ngle intrinsic binding constant [9], K_A, the roblem of its determination is intractable without invoking a formal relationship between the agnitudes of the various μ_{AS_i}. By making the easonable approximation [8] that each successive attachment of a charged ligand to a protein ives rise to a constant incremental change in rotein migration rate, the constituent mobility $\bar{\mu}_A$) is related to the mobility of free acceptor μ_A) by the expression [10,11]

$$[(\bar{\mu}_A/\mu_A) - 1] = (p\delta)K_A C_S/(1 + K_A C_S) \qquad (2)$$

In this equation $\delta = (\mu_{AS_i} - \mu_{AS_{i-1}})/\mu_A$ is the ncremental change in mobility expressed as a raction of the mobility of free A. The two parameters that emanate from the rectangular yperbolic dependence of $[(\bar{\mu}_A/\mu_A) - 1]$ upon ree ligand concentration, C_S, thus define the ntrinsic binding constant, K_A, and $(p\delta) = [(\mu_{AS_p}/\mu_A) - 1]$. Multiplication of the latter parameter by μ_A yields the difference between the mobilities of ligand-saturated and free acceptor species. Because no account is taken in Eq. 2 of the consequences of endosmotic flow through the capillary, the mobilities incorporated into the analysis must be corrected for such effects.

In the original measurement of binding constants for lectin–carbohydrate interactions by capillary zone electrophoresis [1] the migration rate was defined in terms of time taken by the acceptor zone to migrate from the point of application to the detection point. On the grounds that the time taken to migrate a fixed distance is inversely proportional to mobility, the analogue of Eq. 2 becomes

$$[1 - (t_A/\bar{t}_A)] = [1 - (t_A/t_{AS_p})]K_A C_S/(1 + K_A C_S) \qquad (3)$$

where $\bar{t}_A$ is the retention time for acceptor in the presence of a given ligand concentration, C_S; and where t_A and t_{AS_p} are the corresponding retention times for free and ligand-saturated acceptor. Like their electrophoretic mobility counterparts in Eq. 2, these retention times need to be corrected for variations in endosmotic flow.

3. Applications

Because the fundamental quantitative expressions are initially derived in terms of electrophoretic mobilities, the first capillary electrophoresis study of ligand binding to be considered employs results presented in that format. Fig. 1a summarizes results obtained by capillary electrophoresis for the interaction of fucose 1-phosphate with the slowest-migrating component of *Tetragonolobus purpureas* lectin [2]. Non-linear regression analysis of the experimental results in terms of Eq. 2 yields an intrinsic association constant (± 2 standard error of the mean, SEM) of 5600 (± 800) M^{-1} and a value of 0.094 (± 0.001) for $p\delta$. If, for example, the lectin were tetrameric ($p = 4$), the latter parameter would imply that the incremental change in mobility for each successive ligand attachment is 2.35% of the mobility of free lectin.

The second illustration of the present approach (Fig. 1b) employs retention time data for

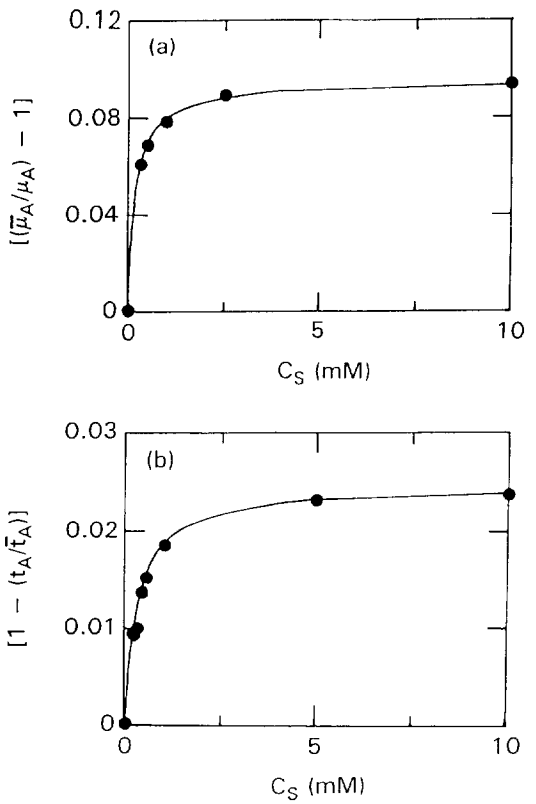

Fig. 1. Evaluation of binding constants by capillary zone electrophoresis. (a) Plot, in accordance with Eq. 2, of published electrophoretic mobility data [2] for the interaction of fucose 1-phosphate with the slowest-migrating component of *T. purpureas* lectin. (b) Plot, in accordance with Eq. 3, of published retention time data [1] for the interaction of lactobionic acid with *R. communis* agglutinin.

the interaction of lactobionic acid with *Ricinus communis* agglutinin, the results being taken from the original study of ligand binding by capillary electrophoresis [1]. Values (± 2 SEM) of 2900 (± 500) M^{-1} and 0.025 (± 0.001) are obtained for the intrinsic binding constant and $(t_A - t_{AS_p})$, respectively, by non-linear regression analysis of the results in terms of Eq. 3.

4. Discussion

The present consideration of the use of capillary electrophoresis for the characterization of interactions between lectins and charged saccharides has eliminated the invalid assumption, inherent in the earlier analyses [1,2], that the lectins exhibit univalence towards carbohydrates. Nevertheless, despite its elimination of this undesirable feature of the earlier analyses, the present approach is readily shown to differ very little from its predecessors. Although linear transformation of rectangular hyperbolic expressions is not recommended because of the consequent statistical distortion of the data distribution that results from such transformation [12], Eqs. 2 and 3 may be written in double-reciprocal format as

$$\frac{1}{[(\bar{\mu}_A/\mu_A) - 1]} = \frac{1}{[(\mu_{AS_p}/\mu_A) - 1]} + \frac{1}{K_A[(\mu_{AS_p}/\mu_A) - 1]C_S} \quad (4a)$$

$$\frac{1}{[1 - (t_A/\bar{t}_A)]} = \frac{1}{[1 - (t_A/t_{AS_p})]} + \frac{1}{K_A[1 - (t_A/t_{AS_p})]C_S} \quad (4b)$$

to conform with the practices adopted earlier [1,2]. As required, these equations are essentially identical with the expressions deduced in those publications if a value of unity is assigned to p. Indeed, the magnitudes of the binding constants deduced from the present analysis must also duplicate those reported in the original publications [1,2]. However, whereas conformity of the results with these expressions was taken to justify an assumption that the lectin–carbohydrate interactions were restricted to 1:1 complex formation, a more plausible interpretation is that the linear double-reciprocal plots (Figs. 3 and 2 of [1] and [2], respectively) signify an approximately constant incremental difference between the mobilities of successive AS_i complexes. On that basis the constants reported for the binding of lactobionic acid to *R. communis* agglutinin, peanut agglutinin and soybean agglutinin [1], and of fucose 1-phosphate to *T. purpureas* lectins [2] should simply be regarded as intrinsic association constants for the interactions of the charged saccharides with equivalent and independent binding sites on the various lectins. In other

words, the importance of this reappraisal of the earlier quantitative investigations [1,2] is its identification of the parameter (the intrinsic binding constant) that is actually measured; and its identification of the assumptions inherent in the application of capillary electrophoresis [1–6] as well as conventional gel electrophoresis [13] to the quantitative characterization of interactions between charged ligands and multivalent acceptor systems.

In summary, this reappraisal of the evaluation of binding constants by capillary electrophoresis serves to bring the method into line with earlier electrophoretic procedures [8,10,11], in which the inherent assumptions/approximations associated with the interpretation of constituent migration rates for multivalent acceptors had already been identified. Such strengthening of its theoretical basis adds considerably to the quantitative potential of capillary electrophoresis, which has great advantages over its electrophoretic predecessors from the viewpoints of experiment duration, material requirements, and resolving power.

Acknowledgement

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