Short Communication

The Differential Adsorption of Aldolase Isoenzymes in Rat Brain

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Though most studies on the regulatory characteristics of enzymes have, to date, been concerned with monophasic aqueous systems, the interactions between the particulate components of the cell and enzyme activities in individual pathways have been attracting increased attention recently as a possible means of metabolic control. Contributing to this shift in emphasis has been a growing appreciation that free aqueous solution of enzymes may not be a common attainment in the cellular environment, and that even the so-called 'soluble' enzymes may be intimately associated with membranous components *in vivo* (Green *et al.* 1965; James & Auguinstein, 1966; Starlinger, 1967; Arnold & Pette, 1968). The feasibility of reversible adsorption of enzymes to cell-membrane structures as an allosteric control mechanism has been established by using a model system with an ion-exchange adsorbent as the solid phase (Masters, Sheedy, Winzor & Nichol, 1969). In an extension of these studies to a biological situation, the present communication describes the binding of the important glycolytic enzyme aldolase (EC 4.1.2.13) in rat brain and skeletal muscle. Brain was selected as the principal tissue for this investigation in view of the limiting nature of aldolase activity in this source (Scrutton & Utter, 1968), the critical importance of glycolysis in this tissue (Lowry & Passonneau, 1964) and the distinctive isoenzyme composition (Penhoet, Kochman, Valentine & Rutter, 1967; Masters, 1968).

Brain and muscle were excised from freshly slaughtered rats and homogenates of these tissues were prepared in 2 vol. of 0.25 M-sucrose buffered with 0.01 M-tris-HCl, pH 7.4; appropriate concentrations of lactic acid were added to portions of these homogenates as required. Samples were then centrifuged at 100 000 *g* at 2°C for 30 min, after which the supernatant extracts were analysed by zone electrophoresis on agar gels (1.0%) with 0.06 M-sodium barbitone buffer, pH 8.6, and a constant current of 30 mA for 5 h at 4°C. The gels were then stained for aldolase activity, the resulting zymograms scanned on an integrating densitometer and the percentages of aldolase A and C activities calculated; this methodology has been described in

detail previously (Herskovits, Masters, Wassarman & Kaplan, 1967; Masters, 1968). Tissue-extract supernatants were also assayed for aldolase activity by the method of Richards & Rutter (1961), the results of these analyses being illustrated in Fig. 1.

Several features of these results are particularly noteworthy. From Fig. 1(a) it is evident (a) that a large proportion of the total aldolase activity extractable from rat brain and muscle is bound to the particulate fraction in the resting condition, and (b) that additions of the function-dependent metabolite, lactic acid, markedly influence the extent of binding, since activities of supernatants are unaffected by these additions of the metabolite; pH-dependence of enzymic activity is thus precluded as the source of the observed effects. In the presence of 10 mM-lactic acid the amount of aldolase activity in the solution phase is decreased markedly in brain extracts, but increased in muscle extracts. An explanation of this difference in response of the two extracts is provided by Fig. 1(b), in which the total aldolase activity of the brain supernatants has been divided between the two types of this enzyme (A and C) that coexist in this tissue. Thus the effect of lactic acid on the binding of aldolase in muscle extracts parallels that of the A variant (the classical muscle enzyme), whereas the overall response in brain tissue typifies that of aldolase C. Additional data of relevance in the present context are (i) the failure to observe this characteristic response with acetic acid, a metabolite producing similar pH variation (7.4–5.5), nor with homogenates maintained at pH 7.4, (ii) the recovery of activity equivalent to that lost from the soluble fraction in the presence of lactic acid on removal of the metabolite either by elution of the particulate matter with 0.25 M-NaCl or by dialysis, (iii) the observation of qualitatively similar effects with tissue fractions prepared under conditions physiological with respect to temperature and salinity, and (iv) that the aldolase activity of whole-brain homogenate, as well as that of the soluble fraction, is decreased by the addition of lactic acid.

With regard to the binding of aldolase to particulate matter in these tissues, it seems evident that an equilibrium between soluble and bound forms

Fig. 1. Effect of lactic acid concentration on the reversible adsorption of aldolase to the particulate fraction of rat tissue: (a) its effect on the total activity of enzyme in the solution phases of muscle (○) and brain (Δ) homogenates; (b) its differential effect on A-type (●) and C-type (▲) activities in the soluble fraction of brain.

of the enzyme may have significant regulatory implications. As indicated previously (Masters *et al.* 1969), reversible binding to structural components may afford the inherent characteristics of an allosteric control mechanism, and the likelihood of a regulatory function for aldolase in nervous tissue especially is compounded by the marked influence of lactic acid on the extent of aldolase binding in this location. Although hexokinase and phosphofructokinase have generally been considered as the main glycolytic control points in brain, aldolase is in fact the enzyme with the lowest maximal capacity in this tissue, and hence must be considered as an eminently suitable site for the application of regulatory controls (Scrutton & Utter, 1968). In the event that lactic acid concentrations in the brain (produced, for example, by anoxia in this tissue, or by abstraction from the circulation) are sufficient to disturb the rigid maintenance of physiological pH (7.4), the influence of this metabolite provides a specific mechanism for further limitation of activity at this point; a mechanism that, furthermore, would possess the advantage of fine correlation between the degree of anaerobic glycolysis in the tissue and the extent of accumulation of end product of this pathway. As further support for this argument it may be noted that Lowry & Passonneau (1964) found a marked discrepancy between the detected concentrations of fructose 1,6-diphosphate and those

necessary for the observed glycolytic flux. Though they did not connect this finding with a limitation of glycolysis at the aldolase step, their data are consistent with such a limitation.

Another observation of considerable interest in the present study is the differential response in partitioning of aldolases A and C to the presence of lactic acid (Fig. 1b), a finding that may well provide new insight into the biological role of aldolase C. Whereas the physiological significance of aldolase A (the muscle enzyme) and of aldolase B (its liver counterpart) have been rationalized in relation to the substrate specificities and tissue distributions of these multiple enzyme forms (Rutter, Blostein, Woodfin & Weber, 1963), no adequate explanation of a separate and unique role for the C variant has been forthcoming, despite extensive investigation of the catalytic properties of the purified enzymes (Penhoet *et al.* 1967; Masters, 1968).

In the present context, however, additional information about the individual characteristics of aldolase C is provided by the specific sensitivity of this type of activity in nervous tissue to increasing concentrations of lactic acid. C-type activity is observed to differ distinctly from that of A-type activity in this respect (Fig. 1b). One of the known distinguishing features of aldolase C distribution is its invariant and major contribution to the aldolase activity in the brains of vertebrate species (Penhoet *et al.* 1967; Masters, 1968). The rationale for this

specific localization may well be associated with the existence of the aldolase control mechanism discussed above. A feedback circuit provided by the lactic acid-aldolase C-tissue interaction would allow a measure of self-regulation in the rate of glycolysis in brain, and yet, because of the coexistence of aldolase A, favour a continuation of a basic activity in response to mild anoxia. Clearly, such a control mechanism would be eminently suited to the inherent requirements of continuing glucose catabolism in nervous tissue.

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