The Origin of Biphasic Arrhenius plots of Rat Liver Plasma-Membrane 5'-Nucleotidase

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Intrinsic membrane proteins, which may only be solubilized by procedures that disrupt membrane structure, must all have domains that interact with their non-polar environment. The degree of interaction, however, varies from a strict requirement of phospholipid for activity (e.g. Ca²⁺-activated adenosine triphosphatase; Warren et al., 1974) to a requirement for structural support only (e.g. cytochrome bs; Spatz & Strittmatter, 1971). One of the criteria for a membrane-bound enzyme that interacts with its lipid environment has been a biphasic Arrhenius plot. This is interpreted as a perturbation of the activation energy of the enzyme by a change in the physical structure of the lipid molecules in the membrane bilayer. There are, however, several other possibilities (Dixon & Webb, 1958; Silvius et al., 1978; Wynn-Williams, 1976), of which the principal alternative is that the membrane protein itself undergoes a conformational change at the break temperature which modifies its activation energy. This view has been supported by Kreiner et al. (1973) on the basis of the high cholesterol content of mammalian plasma membranes, which might be expected to damp out any rapid phase changes of membrane phospholipids. It has also been noted by Houslay et al. (1976) that the discontinuities in their e.s.r. data from plasma membranes were dependent on the presence of membrane protein and were not observed in vesicles made from lipids extracted from the same membranes but without protein. Even with Ca²⁺-activated adenosine triphosphatase, reconstitution of activity in phospholipid-free detergent micelles has been achieved and biphasic Arrhenius plots obtained similar to those for sarcoplasmic reticulum (Dean & Tanford, 1977; Tanford, 1978).

5'-Nucleotidase may be defined as an intrinsic membrane protein by its lack of solubility except in high detergent concentrations. The fact that it is an ectoenzyme (DePierre & Karnovsky, 1974; Newby et al., 1975) synthesized on the rough endoplasmic reticulum (Bergeron et al., 1975) might also suggest that it is a transmembrane protein (Rothman & Lenard, 1977). Since the enzyme may be purified as a complex with sphingomyelin (Widnell, 1975) and also in a phospholipid-free form (Evans & Gurd, 1973; Slavik et al., 1977), it provides an example where the relationship between Arrhenius plot and lipid environment may be directly tested.

Fig. 1 shows the Arrhenius plot of the sphingomyelin complex of 5'-nucleotidase compared with partially purified forms of the enzyme containing no measurable phospholipid (less than 0.4 mol of phospholipid/mol of protein, based on a protein mol.wt. of 140000). Not only was biphasicity retained in all preparations, but the break temperatures and activation energies were indistinguishable.

The cholesterol content of the sphingomyelin–enzyme complex was similar to that of plasma membranes on a protein basis, and no discontinuity over the temperature range 20–40°C was observed in the e.s.r. data obtained from the spin label 5-doxylstearic acid when incorporated into this material. The 5'-nucleotidase activity, however, shows a 50% change in activation energy at 30°C.
5'-Nucleotidase activity was measured by the spectrophotometric assay of Ipata (1968). Initial rates were measured and the activity at each temperature (\(v\); arbitrary units) assayed in triplicate. Purified sphingomyelin complex of 5'-nucleotidase (\(\circ\); specific activity 72 units/mg) is compared with partially purified preparations from plasma membranes solubilized in Lubrol (\(\square\)) and Sarkosyl (\(\triangle\)) (specific activity about 2 units/mg). The detergent concentration in the assays was less than 0.001%. The mean break temperature was 30.4 ± 0.5°C for all preparations and the activation energies were 65 ± 3 kJ/mol below and 42 ± 3 kJ/mol above the break temperature (S.E.M., 11 observations).

When the Arrhenius plot of 5'-nucleotidase was measured with CMP rather than AMP as substrate, the break temperature was lowered to 20.8 ± 0.7°C (S.E.M., four measurements) and the activation energy below the break temperature was raised to 87 kJ/mol. Therefore not only was the Arrhenius-plot behaviour insensitive to the lipid composition of the preparation and physical state of the lipid as measured by e.s.r., but it could also be significantly altered simply by changing the substrate.
We conclude that 5’-nucleotidase is an example of a membrane enzyme which displays a biphasic Arrhenius plot independent of its lipid environment.


Chloride-Independent Transport of Pyruvate and Lactate across the Erythrocyte Membrane

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Previous work from this laboratory has demonstrated the presence of two carrier mechanisms for the transport of pyruvate and lactate across the erythrocyte membrane, both being inhibited by α-cyano-4-hydroxycinnamate and one, identified as the well-characterized Cl⁻ carrier, inhibited by isothiocyanostilbene derivatives (Halestrap, 1976). Evidence has been presented for the existence of a transport mechanism for pyruvate and lactate in Ehrlich ascites cells which is inhibited by α-cyanocinnamate derivatives and, more powerfully, by thiol reagents such as mersalyl (Spencer & Lehninger, 1976). It seemed likely that the carrier of the ascites cell might be identical with the Cl⁻-independent carrier of the erythrocyte. To test this possibility the effects of thiol reagents on pyruvate and lactate transport into the erythrocyte have been studied and the kinetics of the Cl⁻-independent transport process studied in more detail. Evidence is presented that shows that this carrier is sensitive to thiol reagents such as mersalyl and, more specifically, p-chloromercuribenzenesulphonate. Similar conclusions have been reached, in part, by Deuticke et al. (1978). The properties of this carrier suggest it is similar to that present in ascites cells and experiments on other tissues suggest that it is the normal plasma-membrane transport mechanism for pyruvate and lactate. A summary of some of this work has been presented previously (Halestrap, 1978).

In these studies pyruvate and lactate transport across the erythrocyte membrane have been studied by three methods. In all cases 4-acetamido-4’-isothiocyanostilbene-2,2’-disulphonate (0.1 mM) was present to inhibit totally the Cl⁻-dependent transport mechanism (Halestrap, 1976). Firstly the loss of lactate from cells previously loaded with L-lactate by metabolism of glucose was investigated as described by Halestrap (1976). Even in the absence of added exchangeable ions to the cells, lactate...