Thermodependence of Basal and Stimulated Rat Liver Adenylate Cyclase

A RE-EVALUATION* (Received for publication, May 10, 1977, and in revised form, September 27, 1977)

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Adenylate cyclase activity from a purified rat liver plasma membrane preparation was assayed at 15 to 20 temperatures between 17° and 39°. Reaction conditions were such that: (a) the substrate concentration was saturating (ATP 2 mM; MgCl₂, 5 mM); (b) ATP⁺, a possible adenylate cyclase inhibitor, was absent (pH: 8.2); and (c) linearity of reaction velocity was effective throughout the 5-min incubation. The Arrhenius plot of basal activity exhibited two slopes with a break at 25°. The apparent energy of activation (Eₐ) was 29 kcal/mol below 25° and 0.8 kcal/mol above 25°. Irreversible thermodenaturation did not explain the low Eₐ above 25°. When the experiment was performed with increasing concentrations of NaF (from 2.5 to 15 mM), the break at 25° persisted. Below 25°, Eₐ was always equal to 29 kcal/mol. However, for temperatures above 25°, Eₐ increased hyperbolically from 0.8 to 18 kcal/mol as a function of increasing NaF concentrations. Similar data were obtained in the presence of increasing concentrations of glucagon (from 0.5 nM to 1 μM), except that the break point shifted toward a higher temperature. At a saturating concentration, guanyl-5'-yl imidodiphosphate, another adenylate cyclase activator, drastically modified the temperature-activity relationship so that the Arrhenius plot showed no break, and Eₐ was equal to 24 kcal/mol both above and below 25°. We demonstrate here that the increase in Eₐ produced by all the activators tested is related to an increase in entropy of activation.

In contrast to most enzyme systems, adenylate cyclase (ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1) possesses the double privilege of being a membrane-bound and multi-regulated enzyme system (1). However, despite extensive research, its mechanism remains obscure. The present study was undertaken to assess the functional relationship of the various membrane components during activation by hormonal or nonhormonal ligands in purified membranes from rat liver. It is based on the fact that membrane-bound enzymes are characterized by a particular thermodependence as evidenced by a typical, two-sloped Arrhenius plot. Changes in this thermodependence have been reported as a function of the nature of the surrounding fatty acids, or in the presence of various agonists (2-8). We, therefore, explored the influence of some effectors on the thermodependence of adenylate cyclase in rat liver plasma membrane.

The present study demonstrates that: (a) basal adenylate cyclase displays a two-sloped Arrhenius plot; (b) stimulation of basal adenylate cyclase activity by hormonal or nonhormonal ligands at temperatures above 25° is paralleled by an apparently paradoxical increase in the enzyme Eₐ; (c) this increase in Eₐ is related to an increase in the entropy of activation; (d) fluoride, glucagon, and guanine nucleotides appear to transform the enzyme system into the same final state. Furthermore, in the presence of these activators, the thermodynamic state of the enzyme appears similar to that observed at temperatures below the critical point at which the break of the Arrhenius plot occurs.

The physical significance of the entropy of activation is difficult to assess and there is a tremendous gap going from the entropy of activation to molecular mechanism. However, we suggest as a working hypothesis that activation of adenylate cyclase is paralleled by the elimination of diffusion-linked hindrance to the enzymatic function caused by the domain surrounding the enzyme at temperatures above 25°.

DISCUSSION†

In the absence of activators, hepatic adenylate cyclase

1 The abbreviations used are: Eₐ, energy of activation; Gpp(NH)p, guanyl-5'-yl imidodiphosphate.
2 The experimental procedure, the results, and the references are presented in a miniprint supplement in a miniprint format immediately following this paper. For the convenience of those who prefer to obtain the supplementary material in the form of 11 pages of full size photocopies, it is available as JBC Document orders Journal. Order should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, Md. 20014, and must be accompanied by a remittance to the order of the journal in the amount of $1.65 per set of photocopies.
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The extrapolated value of energy of activation, at maximally from 0.8 to 24 kcal/mol. Glucagon had a more complex effect of the plot was not achieved. maximally effective glucagon concentration, full linearization unaltered and corresponded to an differently. In the presence of increasing concentrations of NaF, the slope of the Arrhenius plot above the break point, but also shifted this point toward the higher temperatures as a function of its concentration. At a maximally effective glucagon concentration, full linearization of the plot was not achieved.

The low activation energy of basal adenylate cyclase activity at temperatures above 25° has already been reported (6-8) and several authors also observed that stimulated activities are characterized by higher energy of activation (21-26). In particular, Harwood and Rodbell showed that activation of adipose tissue adenylate cyclase by fluoride was minimal below 25° (26). Rodbell et al. also reported that basal hepatic cyclase activity was relatively unaffected by temperature whereas Gpp(NH)p-stimulated activity displayed marked increase when the temperature was raised from 30 to 37°. This was interpreted as a seemingly selective effect of temperature on stimulation by Gpp(NH)p (1). Orly and Schramm further assumed that the temperature-sensitive reaction of the adenylate cyclase in turkey erythrocytes involved the regulatory GTP site. They suggested that the break in the Arrhenius plot reflected the repeated functional introduction of GTP by the hormone into the regulatory site (3). More recently, Houslay et al. reported that adenylate cyclase in rat liver plasma membranes stimulated by fluoride or Gpp(NH)p yielded linear Arrhenius plots whereas activation by glucagon resulted in a biphasic Arrhenius plot with a well defined break at 28.5 ± 1° (4, 27). Finally, Engelhard et al. reported that the basal adenylate cyclase activity of purified plasma membrane from fibroblasts (LM-cells) exhibited striking triphasic temperature dependence. Incubation in the presence of prostaglandin FGE 1 eliminated the break at 25° and linearized the Arrhenius plot (8). Our data are in good agreement with the results described above. However, all these data have an apparently paradoxical consequence, namely, that adenylate cyclase activation by any stimulating agent is paralleled by an unexpected increase in the energy of activation of the reaction. In our system, adenylate cyclase activation at temperatures above 25° is accompanied by an increase in the $E_a$ from 0.8 kcal/mol to 18, 24, and 12, in the presence of maximal concentrations of fluoride, Gpp(NH)p, and glucagon, respectively (Figs. 2, 5, 6).

It should be noted that the energy of activation comprises two terms, one related to the reaction rate constant, the other involving the entropy of activation. Both terms may vary independently, so that if the entropy term increases, a rise in $E_a$ does not necessarily mean a drop in the reaction rate; according to Arrhenius, the empirical relationship between the rate constant and the $E_a$ is rendered by the following equation:

$$\ln k = -\frac{E_a}{RT} + \ln A$$

where $k$ is the rate constant, $R$ the gas constant, $T$ the absolute temperature, and $A$ the velocity constant. However, $A$ involves an entropy term, which appears in the following equation:

$$\ln k = \ln \frac{RT}{h} - \frac{\Delta H^\ddagger}{RT} - \frac{\Delta S^\ddagger}{R}$$

where $K$ is Boltzmann's constant, $h$ Planck's constant, $\Delta S^\ddagger$ the entropy of activation and $\Delta H^\ddagger$ the enthalpy of activation, related to the experimental energy of activation as follows: $\Delta H^\ddagger = E_a - RT$. Equation 2 explains that a drop in $E_a$ (or in $\Delta H^\ddagger$) does not necessarily coincide with an increase in the reaction rate ($k$) if this drop is associated with a change in the entropy term ($\Delta S^\ddagger$). This equation further shows that an increase in $E_a$, associated with an increase in the reaction rate necessarily indicates a rise in entropy of activation.

When applied to our data, these considerations indicate that the various activators (fluoride, Gpp(NH)p, glucagon) which increase enzyme velocity at all temperatures, would do...
so concomittantly with an increase of entropy of activation above 25°C, which would account for the observed rise in $E_a$. In addition, when the incubation temperature was increased from low temperatures (<25°C) to temperatures higher than 25°C, we observed a drop in energy of activation (maximal in the basal state, and reduced dose-dependently in the presence of each different activator). It is simple to show that the change from low to high temperature (involving a drop in $E_a$) is related to a reduction in the entropy of activation; the demonstration takes into account the continuity in the reaction rate at the temperature where the change in $\Delta H^\ddagger$ and $\Delta S^\ddagger$ occurs. At this break point ($T_b$), the velocity of the reaction is the same for both states. Let the suffix L indicate the thermodynamic constants of the reaction at the temperature below the break, and the suffix H, the constants at the temperature above the break. We can write that:

$$\ln k_L = \ln k_H$$

that is from Equation 2:

$$\frac{\Delta S_L^\ddagger}{R} - \frac{\Delta H_L^\ddagger}{RT_L} = \frac{\Delta S_H^\ddagger}{R} - \frac{\Delta H_H^\ddagger}{RT_H}$$

or

$$\Delta H_L^\ddagger - \Delta H_H^\ddagger = T_b(\Delta S_L^\ddagger - \Delta S_H^\ddagger)$$

The drop in $E_a$ occurring when the temperature is raised is connected with a decrease in entropy of activation:

$$\Delta S_L^\ddagger - \Delta S_H^\ddagger = \frac{E_{al} - E_{all}}{T_b}$$

This pattern may fit in with the idea that raising the temperature above the 25°C break point coincides with the appearance of an ordered process during the reaction. To the contrary, the activators, or a drop in temperature, would eliminate this additional process. It is tempting to assume that the latter is linked to diffusion; temperature, as well as effectors of the enzyme, would influence the conformation of the enzyme in such a way as to increase its maximal velocity and also modify its relationship to the surrounding environment. In fact, we wish to emphasize that adenylate cyclase, and probably most membrane enzymes possess kinetic behavior similar to that of immobilized enzyme systems which are known to be controlled by diffusional effects (28). For these immobilized enzyme systems also, at sufficiently low temperature, in the domain of kinetic control of the reaction, the true energy of activation is observed. At sufficiently high temperature, when the reaction become bulk diffusion controlled, the observed rate is essentially independent of temperature and the apparent energy of activation is close to zero (28).

**REFERENCES**

References 1 to 28 are found on p. 841.
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