The Effect of Fluoride on the State of Aggregation of Adenylate Cyclase in Rat Liver Plasma Membranes

B. Richard MARTIN, Janet M. STEIN, Edwina L. KENNEDY and Christine A. DOBERSKA
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

(Received 15 October 1979)

Irradiation inactivation was used to monitor changes in the state of adenylate cyclase in rat liver plasma membranes in the presence of F^- . F^- caused a decrease in the target size from 328 000 to 237 000 at 0°C and from 329 000 to 219 000 at 30°C. Adenylate cyclase was activated by F^- at both 0°C and 30°C. The effect of F^- was biphasic, activating up to a concentration of 10 mM and inhibiting at higher concentrations. If adenylate cyclase was maximally activated with glucagon and p[NH]ppG ([β -imido]GTP) all concentrations of F^- were inhibitory. The implications of the results with respect to the mechanism of activation of adenylate cyclase are discussed.

In a recent study (Martin *et al.*, 1979) we employed the irradiation-inactivation method of Kepner & Macey (1968) to examine the alterations in the state of aggregation of rat liver plasma-membrane adenylate cyclase during the activation of the enzyme by glucagon and guanine nucleotides.

We found that the basal or ground state of the enzyme had a target size of about 300 000. Treatment with GTP at 30°C resulted in the appearance of a species of lower molecular weight, and this effect was potentiated by glucagon, giving a single homogeneous species with a target size of about 200 000. The decrease in target size could also be achieved by treatment with the non-hydrolysable GTP analogue p[NH]ppG ([β -imido]GTP). As a result of these observations we suggested that the activation of adenylate cyclase by guanine nucleotides requires the dissociation of a regulatory subunit of about 100 000 mol.wt., leaving a free active catalytic subunit of about 200 000 mol.wt. The aim of the present study was to establish whether a similar dissociation takes place during activation by F^- .

Experimental

Materials

ATP, cyclic AMP, p[NH]ppG and creatine kinase were obtained from Boehringer (London), Lewes, East Sussex, U.K. Phosphocreatine was obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Glucagon was a gift from Dr. W. Bromer, Eli Lilly, Indianapolis, IN, U.S.A. [32 P]P_i and cyclic [3 H]AMP were obtained from The Radiochemical Centre, Amersham, Bucks. U.K. [α - 32 P]ATP was prepared by

the method of Martin & Voorheis (1977). All other chemicals were of the highest purity available.

Methods

Assay of adenylate cyclase activity. Adenylate cyclase activity was determined by the method of Salomon *et al.* (1974). Incubations were in 0.1 ml containing 25 mM-Tris/HCl buffer, pH 7.4, 0.5 mM-ATP, 0.1 mM-cyclic AMP, 10 mM-MgCl₂, 1 mM-dithiothreitol, 5 mM-phosphocreatine and 5 units of creatine kinase.

Irradiation inactivation. The procedure for irradiation-inactivation analysis was as described by Martin *et al.* (1979).

Rat liver plasma membranes. Plasma membranes were prepared, by the method of Pilkis *et al.* (1974), from female Wistar rats (body wt. 130–150 g).

Results and Discussion

Irradiation-inactivation analysis

Fig. 1 shows the effect of preincubation of liver plasma membranes with F^- at 0°C and 30°C on the target size of adenylate cyclase. The composition of the preincubation medium was the same as that used for the determination of adenylate cyclase activity, except that dithiothreitol was omitted since thiols cause increased loss of activity on irradiation. Preincubation with F^- at 0°C caused a decrease in the target size from 328 000 to 237 000, and preincubation with F^- at 30°C caused a decrease from 329 000 to 219 000. Thus the effect of F^- is similar to the effect of glucagon together with GTP, which caused a decrease in target size from 301 000 to 226 000, and p[NH]ppG, which

Fig. 1. Effect of F^- on the irradiation inactivation of rat liver plasma-membrane adenylate cyclase

Rat liver plasma membranes (0.1 mg of protein) were incubated at 0°C (a) or 30°C (b) in 0.2 ml of medium containing 25 mM Tris/HCl buffer, pH 7.4, 0.1 mM-ATP, 0.1 mM-cyclic AMP, 10 mM-MgCl₂, 5 mM-phosphocreatine and 5 units of creatine kinase with no further additions (O) or with 10 mM-NaF (●). After 10 min the incubation was stopped by rapidly freezing the mixture by immersing the tubes in liquid N₂. The frozen incubation mixtures were freeze-dried, and the tubes were gassed with O₂-free N₂ and sealed. The samples were then irradiated in an electron beam by using the 15 MeV linear accelerator at Addenbrooke's Hospital, Department of Radiotherapeutics, University of Cambridge, Cambridge, U.K. After irradiation the samples were resuspended in 0.2 ml of water, and the adenylate cyclase activity was determined in the presence of 10 mM-NaF as described in the Experimental section. A 20 min incubation at 30°C was employed.

caused a decrease in target size from 315 000 to 223 000 (Martin *et al.*, 1979). It therefore seems likely that the activation of adenylate cyclase by F^- involves the dissociation of the same regulatory subunit that is released from the catalytic subunit during activation by guanine nucleotides.

Effect of temperature

F^- was equally effective in decreasing the target size at either 0°C or 30°C (Fig. 1). This is in marked contrast with the guanine nucleotide effect, which was markedly temperature-dependent, and did not occur at 0°C (Martin *et al.*, 1979). If, as we have proposed, the activation of adenylate cyclase results from the dissociation of a regulatory subunit, then F^- should be capable of activating the enzyme at 0°C. The results presented in Table 1 confirm this. In common with many membrane integral enzymes, adenylate cyclase (Houslay & Palmer, 1978) shows a very marked temperature-dependence, since increasing temperature from 0°C to 30°C results in a 31-fold increase in basal activity and a 38-fold increase in activity in the presence of F^- . Similar observations have been made by Houslay *et al.* (1976). However, the degree of stimulation of adenylate cyclase activity by F^- was very similar at

Table 1. Effect of temperature on the activation of rat liver plasma-membrane adenylate cyclase by F^-

Rat liver plasma membranes were incubated in 0.1 ml of assay medium as described in the Experimental section with further additions as given in the Table. Incubations were for 40 min with 0.1 mg of membrane protein at 0°C and for 10 min with 0.04 mg of membrane protein at 30°C. Results are means $\pm$ S.E.M. for three parallel incubations.

Temperature (°C)	Adenylate cyclase activity (nmol/10 min per mg of protein)		Increase in activity (%)
	No addition	10 mM-NaF	
0	0.0119 $\pm$ 0.0004	0.095 $\pm$ 0.0014	800
30	0.365 $\pm$ 0.016	3.587 $\pm$ 0.067	980

both temperatures (Table 1). This is consistent with the observation that dissociation is as effective at 0°C as at 30°C.

Extent of activation

The irradiation-inactivation plots give a straight line in the presence of F^- , implying the presence of a single homogeneous species. It therefore appears

Fig. 2. Effect of F^- on rat liver plasma-membrane adenylate cyclase activity

Rat liver plasma membranes (0.04 mg of protein) were incubated for 20 min at 30°C as described in the Experimental section in the presence of various concentrations of NaF. The s.e.m. values of three parallel incubations are indicated by error bars except when smaller than the symbols used.

Fig. 3. Effect of F^- on the activity of rat liver plasma-membrane adenylate cyclase that had been preactivated with glucagon and p[NH]ppG

Rat liver plasma membranes (0.8 mg of protein) were incubated at 30°C in 1 ml of a medium containing 25 mM-Tris/HCl buffer, pH 7.4, 10 mM-MgCl₂, 1 mM-dithiothreitol, 1 μM-glucagon and 0.1 mM-p[NH]ppG. After 10 min the adenylate cyclase activity was determined in the presence of 1 mg of glucagon and 0.1 mM-p[NH]ppG with various concentrations of NaF as described in the legend to Fig. 2. The s.e.m. values for three parallel incubations are indicated by error bars except where smaller than the symbols used.

that F^- causes complete dissociation of the regulatory subunit from the catalytic subunit. In our previous report (Martin *et al.*, 1979) we suggested that the free fully dissociated catalytic subunit represents the fully activated state of the enzyme. It might therefore be expected that treatment with F^- should be as effective in activating adenylate cyclase as treatment with glucagon in the presence of guanine nucleotides. However, in common with other workers (Birnbaumer *et al.*, 1972; Pohl *et al.*, 1971), we found that the adenylate cyclase activity in the presence of F^- at 30°C was less than the activity in the presence of glucagon and p[NH]ppG (Table 1). It was also found that the effect of F^- on adenylate cyclase was biphasic. Concentrations of F^- up to about 10 mM activated the enzyme, whereas higher concentrations resulted in a decrease of activity compared with this optimum (Fig. 2). When rat liver plasma membranes were pretreated with glucagon in the presence of p[NH]ppG to produce maximal activation and subsequently assayed in the presence of various concentrations of F^- , we found that all concentrations of F^- were inhibitory (Fig. 3).

It therefore seems that F^- has two effects on the adenylate cyclase of mammalian plasma membranes, an activation resulting from the dissociation of regulatory subunit and an inhibitory effect that appears to be independent of the dissociation.

The aim of the present study was to establish whether F^- activates adenylate cyclase by a similar mechanism to the activation of the enzyme by guanine nucleotides. It seems likely that the activation by both F^- and GTP results from the dissociation of a regulatory subunit from the catalytic subunit.

References

- Birnbaumer, L., Pohl, S. L. & Rodbell, M. (1972) *J. Biol. Chem.* **247**, 2038–2043

- Houslay, M. D. & Palmer, R. W. (1978) *Biochem. J.* **174**, 909–919
- Houslay, M. D., Metcalfe, J. C., Warren, G. B., Hesketh, T. R. & Smith, G. A. (1976) *Biochim. Biophys. Acta* **436**, 489–498
- Kepner, G. R. & Macey, R. I. (1968) *Biochim. Biophys. Acta* **165**, 188–203
- Martin, B. R. & Voorheis, H. P. (1977) *Biochem. J.* **161**, 555–559
- Martin, B. R., Stein, J. M., Kennedy, E. L., Doberska, C. A. & Metcalfe, J. C. (1979) *Biochem. J.* **184**, 253–260
- Pilkis, S. J., Exton, J. H., Johnson, R. A. & Park, C. R. (1974) *Biochim. Biophys. Acta* **343**, 250–267
- Pohl, S. L., Birnbaumer, L. & Rodbell, M. (1971) *J. Biol. Chem.* **246**, 1849–1856
- Salomon, Y., Londos, C. D. & Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548