β-METHYL-δ-KETO-$\triangle^{\alpha\beta}$-HEXENOIC ACID AND MESITYL OXIDE AS ACETYL DONORS IN THE ENZYMIC SYNTHESIS OF ACETYLCHOLINE

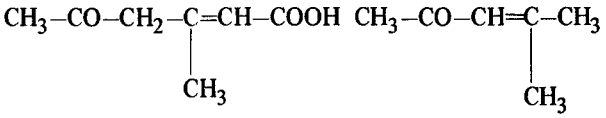
I.ALKONYI and A.SÁNDOR

Pécsi Orvostudományi Egyetem, Biokémiai Intézet, Pécs, Hungary

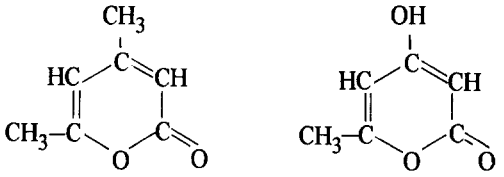
Received 10 November 1969

1. Introduction

We have previously reported [1,2] that the ketoacid* (I), its decarboxylation product, mesityl oxide (II), and its lactone, mesitene lactone (III) as well as TAL (IV) are able to acetylate sulphanilamide in pigeon liver extract in the presence of both ATP and CoA. It was shown that the two lactones III and IV are not hydrolysed by pigeon liver extract and it was suggested that the acetylation is due to the ATP-dependent formation of acetyl-CoA.



(I) (II)



(III) (IV)

It has long been known that brain homogenates are able to synthesize acetylcholine from choline and acetate in the presence of ATP and CoA [3], and rat and guinea-pig brain homogenates can synthesize acetylcholine using acetylcarnitine as acetyl donor [4]. In this paper we show that the ketoacid (I) and mesityl oxide (II), but not the lactones (III) and (IV), are able to act as acetyl donors in rat and guinea-pig brain homogenates in the presence of both ATP and CoA. Further is shown that mesityl oxide is not first hydrolysed to acetate before acting as an acetyl donor.

* Abbreviations: see p. 340.

2. Materials and methods

2.1. *Chemicals*

L-Cysteine-HCl, ATP-Na, mesityl oxide, and choline chloride were obtained from Reanal, Budapest. Glutathione was purchased from Serva, Heidelberg; acethylcholine from Berlin-Chemie and coenzyme A, 75% lyophilized Li salt, from NBC, Ohio. The inorganic chemicals and the other organic chemicals used were commercial preparations of reagent grade.

Mesitene lactone was prepared as already described [5] and the ketoacid by the alkaline hydrolysis of its lactone [1]. TAL was prepared by Collie's method [6].

2.2. *Enzyme preparation*

Wistar albino rats and guinea-pigs were used. The animals were killed by decapitation and the brains removed as quickly as possible, weighed, and rinsed with 0.16 M NaCl to remove blood. The tissue was ground in 100 volumes of acetone in a china mortar at −15°C. After filtration the solid material was dried in a vacuum exsiccator over P_2O_5 for 24 hr. The dry

North-Holland Publishing Company – Amsterdam

Table 1
Acetylation of choline by rat and guinea-pig brain extracts using various acetyl donors

Acetyl donor	Rat brain		Guinea-pig brain	
	μg acetyl-choline formed/g acetone powder	μg extra acetylcholine formed over control	μg acetyl-choline formed/g acetone powder	μg extra acetylcholine formed over control
None	400	0	409	0
Sodium acetate	904	504	1305	896
Ketoacid (I)	920	520	1219	810
Mesityl-oxide (II)	568	168	1077	668
Mesitene lactone (III)	390	–10	398	–11
TAL (IV)	450	50	455	46

Each tube contained, in a final volume of 2.6 ml, 12 mg protein (equivalent to 50 mg acetone powder) and the following (in μ-mole): KCl, 161; NaF, 72; glutathione, 38.2; choline chloride, 5.15; $MgCl_2$, 11.8; physostigmine salicylate, 0.73; sodium phosphate buffer pH 7.0, 20; CoA, 0.11; ATP, 20; and, when indicated, acetyl donor, 20. In the absence of either CoA and/or ATP no acetylation of choline was detected with any of these acetyl donors. Incubation at 37°C for 1 hr. The reaction was stopped by addition of 0.1 ml N HCl and the tubes heated in boiling water for 3 min. The solution was neutralized by addition of 0.1 ml N NaOH and filtered. Acetylcholine was determined in 0.1 ml of filtrate.

Table 2
Breakdown of mesityl oxide by guinea-pig brain extract in the presence and absence of ATP and CoA

Mesityl oxide	ATP + CoA + glutathione	Absorbance at 242 nm	
		Incubated with brain extract	Incubated separately from brain extract
-	-	0.047	0.049
+	-	0.248	0.248
-	+	0.065	0.058
+	+	0.215	0.258

Each tube contained, in a final volume of 2.6 ml, the following (in μmole): KCl, 161; NaF, 72; choline chloride, 5.15; $MgCl_2$, 11.8; physostigmine salicylate, 0.73; sodium phosphate buffer pH 7.0, 20; and, when indicated, mesityl oxide, 10; CoA 0.11; ATP, 20; glutathione, 38.2. Incubation at 37°C for 1 hr either with or separately from 12 mg protein (equivalent to 50 mg acetone powder). When the protein solution was incubated separately it was added to the other solution at the end of incubation. The reaction was stopped by the addition of 10 ml ethanol at -20°C, the mixture was centrifuged at 4000 rpm at -5°C for 20 min; 2 ml of the supernatant were dissolved in 50 ml doubly distilled water and the absorbance read against an appropriate water-ethanol blank.

powder thus obtained was suspended in 50 ml ice-cold 0.16 M NaCl containing 3 mg/ml cysteine adjusted to pH 7.0. The suspension was stored overnight at −20°C then, after thawing, it was centrifuged at 5000 *g* at 0°C for 10 min. The supernatant was dialysed against 100 volumes of the same suspending fluid at 0°C for 2 hr with stirring. The preparation thus obtained was used immediately. In each incubation the amount of this preparation used corresponded to 50 mg dry brain acetone powder (12 mg protein).

Acetylcholine was determined by the method of Chang and Gaddum [7] and protein by a biuret method [8].

3. Results and discussion

From the results in table 1 it can be seen that in rat and guinea-pig brain homogenate the lactones III and IV are not able to act as acetyl donors in the synthesis of acetyl choline, even in the presence of ATP and CoA. This contrasts with their ability to acetylate sulphanilamide in pigeon liver homogenates [1]. The ketoacid I and mesityl oxide are, however, able to acetylate choline in brain homogenates, but, unlike acetylcarnitine [4], they require both ATP and CoA for this activity.

The data in table 1 show that the ketoacid I brings about approximately the same amount of acetylation of choline as does the same concentration of acetate, in both rat and guinea-pig brain homogenates, while mesityl oxide is always less active as an acetyl donor. This is in contrast to the greater activity of mesityl oxide in acetylating sulphanilamide in pigeon liver homogenates [1].

The greater acetylating activity of guinea-pig brain extract compared with rat brain extract shown in table 1 is in accord with the findings of Thomitzek and Strack [4].

To investigate whether mesityl oxide is first hydrolysed to acetate before acting as an acetyl donor, the experiments shown in table 2 were performed. In these the changes in mesityl oxide concentration were followed by measuring the absorbance at 242 nm, its absorption maximum [9]. The results show that mesityl oxide is not hydrolysed in the incubation medium used in the absence of ATP and CoA.

* Abbreviations: Ketoacid: β-methyl-δ-keto-$\Delta^{\alpha\beta}$-hexenoic acid; mesitene lactone: 4,6-dimethyl-α-pyrone; TAL: triacetic acid lactone; CoA: coenzyme A.

References

[1] I.Alkonyi, A.Sándor, D.Szabó and F.Tárnok, Acta Biochim. Biophys. Acad. Sci. Hung. 4 (1969) 141.
[2] D.Szabó, E.Pálfi and I.Alkonyi, Acta Biochim. Biophys. Acad. Sci. Hung., submitted for publication.
[3] D.Nachmanson and M.Berman, J. Biol. Chem. 165 (1946) 551.
[4] W.D.Thomitzek and E.Strack, Acta Biol. Med. Germ. 13 (1964) 110.
[5] I.Alkonyi, Chem. Ber. 98 (1965) 3099.
[6] N.I.Collie, J. Chem. Soc. 59 (1891) 609.
[7] H.C.Chang and J.H.Gaddum, J. Physiol. 79 (1933) 255.
[8] N.Ditterbrandt, Am. J. Clin. Path. 18 (1948) 439.
[9] G.Scheibe, Ber. Dtsch. chem. Ges. 58 (1925) 586.