The oxidation of propionic acid by a marine bacterium

During the course of an investigation of the metabolism of bacteria of marine origin, one of them, designated B16 and tentatively identified as a species of *Mycoplana*[1], was found to exhibit unusual behaviour in its oxidation of propionic acid. In view of the several reports which have appeared recently regarding the metabolism of propionate in mammalian tissues[2,3,4,5,6], the present observations, although of a preliminary nature, appeared to be worth recording.

In Table I are presented data, based on oxygen uptake determined by the use of conventional Warburg respirometer techniques, showing the rate and extent of oxidation of several compounds by resting cell suspensions of B16 grown in various media. The endogenous oxygen uptake has been subtracted from that observed in the presence of added substrates in calculating the values recorded. While this procedure cannot be completely justified on the basis of the data available, a consideration of the course of oxygen uptake by resting cells with several different quantities of compounds such as acetate, pyruvate, propionate, or succinate as substrates, lead to the conclusion that it was probably the correct one in this instance, as the oxygen uptakes observed were closely proportional to substrate available only when the endogenous uptake was subtracted. In any event, in all instances where oxygen uptake was less than that expected for complete oxidation of the substrate, with the exception of the oxidation of acetate and butyrate by cells grown in either nutrient broth-yeast extract or succinate medium, all rates of oxygen uptake had decreased to that of the endogenous control before oxygen equivalent to the theoretical amount needed for complete oxidation of substrates to carbon dioxide and water had been consumed, whether the endogenous uptake was subtracted or not. This decrease in rate of oxygen uptake to that of the endogenous control was not due to enzyme inactivation but apparently to substrate exhaustion, as the addition of fresh substrate resulted in the immediate resumption of oxygen uptake at an increased rate. It is thus evident that while cells grown in any of the media oxidize acetate, pyruvate, *dl-iso*citrate, succinate, and butyrate with the uptake of less than the theoretical amount of oxygen necessary for their complete oxidation, and cells grown in nutrient broth-yeast extract medium oxidize propionate to a similar extent, cells grown in synthetic media with either succinate or acetate as sole carbon source oxidize propionate with the uptake of the theoretical amount of oxygen for its complete oxidation. The R.Q. for this apparent complete oxidation was determined and found to be 0.85 (calculated value, 0.857). Although not shown in the table, cells grown in synthetic medium with propionate as sole carbon source resembled those grown in nutrient broth-yeast extract medium in their oxidative behaviour toward the compounds considered; while cells grown in a medium with citrate as carbon source resembled those grown in acetate medium.

TABLE I

COMPARISON OF THE RATE AND EXTENT OF OXIDATION OF VARIOUS COMPOUNDS BY WHOLE CELLS OF THE MARINE BACTERIUM B16 HARVESTED FROM THREE DIFFERENT GROWTH MEDIA

Each Warburg cup contained 0.133 m*M* phosphate buffer, pH 6.7, 5 μM substrate (10 μM *dl-iso*citrate), and washed cells equivalent to 1 mg cell nitrogen in a total vol of 2.8 ml of artificial sea water[1]. 0.2 ml 20% KOH in center well. Gas phase air. Temperature 25°. Substrate added after 15 min temperature equilibrium.

*Growth medium**	*Nutrient broth-yeast extract*			*Succinate*			*Acetate*		
Substrate	$Q_{O_2}{}^{N}$**	% *oxidation***	*Time*§ *min*	$Q_{O_2}{}^{N}$	% *oxidation*	*Time min*	$Q_{O_2}{}^{N}$	% *oxidation*	*Time min*
Acetate	220	77	55	134	76	75	820	65	20
Pyruvate	312	37	40	225	40	50	820	50	29
*dl-iso*Citrate	272	36	50	480	46	50	210	40	100
Succinate	272	47	50	576	62	50	182	48	100
Propionate	404	66	55	252	100	160	247	100	158
Butyrate	300	80	90	97	85	320			

* The medium consisted of an inorganic salt solution plus the organic material indicated.

** $Q_{O_2}{}^{N}$ is expressed in μl O_2/hour/mg cell N, and is based on the most rapid rate of oxygen uptake observed.

*** Calculated on basis of theoretical oxygen uptake required for complete oxidation of 5 μM of substrate to CO_2 and H_2O.

§ The length of time required for the rate of oxygen uptake to decrease to that of the endogenous control following the addition of substrate.

The data imply that cells grown with acetate (or citrate) as sole carbon source do not oxidize propionate solely *via* succinate, either through preliminary condensation of CO_2 with propionate to yield succinate directly[4,5], or *via* the intermediate formation of *iso*succinate[3,4], for the rate of oxygen uptake with propionate as substrate exceeds that with succinate, which should not be possible if the rate of oxygen uptake with propionate as substrate is limited by some oxidative step(s) subsequent to succinate. If , however, the rate of oxidation of succinate when succinate was the substrate was limited at some point prior to the first oxidative step, such as by cell permeability or substrate activation, then the difference in rates of oxygen uptake could occur, but such a limitation cannot account for the result, as succinate is in fact utilized more rapidly than is propionate, even though cells from the acetate medium take up oxygen more rapidly when attacking propionate than succinate.

Whether or not propionate is oxidized *via* pyruvate through the mechanism proposed by MAHLER AND HUENNEKENS[2] in cells grown in synthetic media with succinate, acetate, or citrate as carbon sources cannot be determined from the data available.

The complete oxidation of propionate has been observed in this study only under conditions of greatly reduced rate of utilization of substrate and hence might be due to a slower rate of entry of propionate than of those compounds only partially oxidized into a common enzyme system. That this is unlikely, however, is evident from the fact that butyrate is utilized even more slowly than propionate and yet is only partially oxidized (Table I).

Evidence has been obtained that is consistent with the view that a conventional tricarboxylic acid cycle is present in this organism[7]. The results reported here indicate the possible existence of an alternate system for the complete oxidation of propionate.

The whole cell preparations used in this work oxidize *d*-lactate, *dl*-lactate, glycolate, malonate, oxalate, and formate at an extremely low rate, if at all.

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Quelques considérations préliminaires sur la conversion du chymotrypsinogène en chymotrypsine-α

Le mécanisme chimique de l'activation "lente" du chymotrypsinogène est vraisemblablement assez complexe puisqu'il comporte, d'une part, les protéolyses de l'activation "rapide" donnant naissance à la sérylarginine[1-3] et, d'autre part, certaines protéolyses additionnelles[4,5] responsables de la présence dans la chymotrypsine-α de deux résidus terminaux supplémentaires: la tyrosine C-terminale et l'alanine N-terminale. Ces protéolyses additionnelles ne semblent d'ailleurs pas faire partie du processus d'activation proprement dit. Elles sont néanmoins intéressantes par les lumières qu'elles projettent sur la formation de la plus classique des chymotrypsines, par le nouvel argument qu'elles fournissent en faveur de l'existence chez les chymotrypsines d'un centre actif étroitement limité, et enfin par les exemples suggestifs qu'elles donnent d'une famille d'enzymes issue d'une même protéine-mère grâce à une série de dégradations successives.

Le résidu C-terminal supplémentaire étant la tyrosine, on peut penser que les protéolyses additionnelles de l'activation "lente" sont d'origine chymotrypsique et qu'elles résultent donc, soit de l'attaque préalable du zymogène par les chymotrypsines de l'activation "rapide"[6] (π et δ), soit d'une autolyse ultérieure de ces enzymes. Les chiffres du Tableau I (colonnes 5–8) indiquent que les deux phénomènes ont lieu et qu'ils forment des protéines contenant de la thréonine et de l'alanine en position N-terminale. Ils expliquent donc vraisemblablement l'apparition de ces mêmes résidus pendant l'activation "lente" (colonnes 1–4).

Quelques remarques importantes doivent être faites au sujet des chiffres de ce tableau: a) L'attaque du chymotrypsinogène par la chymotrypsine ne semble provoquer aucune activation notable. Pour être "activante", la protéolyse du zymogène doit donc se produire en un endroit défini de la molécule, lequel paraît être la liaison Arg–Ileu coupée par la trypsine[2]. En outre, on comprend maintenant pourquoi le chymotrypsinogène est beaucoup plus stable que le tryp-