Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 312 (2003) 139–141
BBRC
www.elsevier.com/locate/ybbrc

My time with Gunsalus in the 1950s

Roberts A. Smith*

Department of Chemistry & Biochemistry, University of California at Los Angeles, Los Angeles, CA, USA

Received 25 September 2003

"Gunsalus" is a name I knew from my first scientific awakenings in the School of Agriculture at the University of British Columbia in 1947. Professor J.J.R. Campbell, with whom I worked there, had taken his Ph.D. in microbiology with Gunny at Cornell in 1944. Thus, as it happened, Gunny was in a sense my scientific grandfather and, as it turned out later, my scientific father and in many ways my mentor.

I grew up in an agricultural/logging community on Vancouver Island, where, having missed conscription into military service by a few months, I remained at the end of World War II a high school student and a part of the Pacific Coast Militia Rangers, the group intended to protect us in the event we were invaded by the Japanese. We may not actually have been much help, as we were poorly trained and our rifles were hand-me-downs from the 1890s South African war and not always operative. We did have a good band, though. I never learned to aim my gun at the desired target but did play a saxophone in the band, and that skill later helped to finance some of my university career.

Also while in high school I worked in the dairy industry and became very interested in dairying as a career. I had the simple idea that I should go to university to learn about dairying's underlying science, with the ultimate aim of one day operating my own dairy farm. Toward that goal, I spent a year after high school working in the dairy industry to pay for my first year, and the money earned during vacation times working in dairying and playing the saxophone in dance bands financed my whole career at the University of British Columbia.

One of the first advisors I met at UBC was Dr. J.J.R. Campbell, who happened to be the professor of dairying and a former student of Gunny's. When I eventually began research with Professor Campbell, he was exploring metabolic pathways in the strictly aerobic microorganism *Pseudomonas aeruginosa*. Among the main problems he outlined for me was the question of how this organism could grow vigorously on acetate as sole carbon source, and this is what I selected to work on. He suggested, after I had shown that cell preparations could oxidize all the intermediates of the citric acid cycle, that I might begin looking for α-keto acids as an indicator of normal citric acid cycle intermediates in cell-free extracts of *P. aeruginosa* grown on acetate alone. The α-keto acids could be easily separated and identified using paper chromatography of their 2,4-dinitrophenylhydrazone derivatives. I certainly found the expected keto acids, α-ketoglutarate and pyruvate, but there was an additional prominent keto acid which I eventually proved was glyoxylate [1]. Glyoxylate was not available commercially but I made a small amount from oxalic acid by magnesium reduction. Cell-free extracts of *P. aeruginosa* clearly used either citrate or *cis*-aconitate as substrate and produced glyoxylate and succinate [1]. Biochemical supply houses did not have isocitrate in their catalogues in 1953, and the sample we acquired did not give rise to glyoxylate and succinate when incubated with our cell-free extracts.

While I was working with Professor Campbell, the name Gunsalus was often in the conversation, so when I decided to continue my studies in bacteriology it seemed natural that I should apply to work with Gunny at the University of Illinois. I finished my studies with Professor Campbell in the summer of 1953 and was married in August of that year. I arrived at Gunny's laboratory in September and my wife joined me 6 weeks later. What a contrast the midwest turned out to be compared to British Columbia! To me, Urbana was the epitome of scientific sophistication. In the Bacteriology Department at that time, Gunsalus, Luria, and Spiegleman provided the most stimulating and exciting academic environment imaginable. It was an exciting time in science: Todd and his colleagues at Cambridge had just proven that RNA

* Fax: 1-310-458-9182.
E-mail address: robertss@ucla.edu.

doi:10.1016/j.bbrc.2003.09.222

was a repeating 3′–5′ phosphodiester structure in 1952, and Watson and Crick published their double helical structure of DNA in 1953. I soon learned that every major biochemist/microbiologist sooner or later made his or her way to Urbana, and Gunny made a point of having time for each to interact with the students and postdoctorals. Thus I had a chance to hear directly from most of the notables of the time about their work. Gunny took on my education in other areas, as well, and I became acquainted with wines for the first time in my life.

Jack Campbell was remarkably generous and had allowed me to take the glyoxylate problem with me to Gunny's laboratory in Urbana. It seemed clear that isocitrate was involved somehow, and one day on returning from a visit to California Gunny showed up with a sack of what we called *Sedum spectabile,* from which I was to isolate and crystallize Ls(−)dimethyl isocitric lactone, which was easily converted to isocitrate by saponification [2]. To my surprise, it worked well and we had a lifetime supply of Ls(+)isocitrate. It was simple to grow large quantities of *P. aeruginosa* in the Urbana fermenter and to convert these to cell-free extracts that could be fractionated to remove aconitase. This yielded a preparation incapable of producing glyoxylate from citrate or *cis*-aconitate but clearly catalyzed the aldol cleavage of isocitrate to glyoxylate and succinate [3]. We showed that isocitric lyase required a divalent metal (Mg^{2+} was best) and was activated by a sulfhydryl compound. Clearly, coenzyme A was not involved [3].

I began to look for glyoxylate formation in extracts of other organisms under study in Gunny's lab and grown under a variety of conditions. It appeared that isocitrate lyase occurred principally in aerobically grown organisms utilizing organic acids or acetate as carbon source but not when grown on carbohydrates as sole carbon source [4]. Nor did it occur in facultative organisms grown anaerobically.

According to our organic chemistry friends, all aldol reactions are reversible, so we began to think about the reversibility of isocitrate lyase. All of our attempts to reverse isocitric lyase by starting with glyoxylate and succinate coupling the fractionated enzyme (aconitase-free) with NADP-linked isocitric dehydrogenase at first failed, but when the order of substrate addition was changed, we were successful. Glyoxylate somehow blocked binding by succinate and no reaction could be measured, but when the order of addition of substrates was changed and the reaction started with the addition of glyoxylate we could easily follow the reduction of NADP and thus the concomitant formation of Ls(+) isocitrate [5]. Thus

$$\text{Glyoxylate} + \text{succinate} \rightarrow \text{Ls(+)isocitrate} \quad (1)$$

$$\text{Ls(+)isocitrate} + \text{NADP+} \rightarrow \text{NADPH} + \text{oxalosuccinate} \quad (2)$$

The reversibility of the isocitric lyase reaction was also studied using the exchange of radioactive succinate into isocitrate in the presence of unlabeled glyoxylate and eventually we measured the equilibrium constant for both isocitrate lyase (1) as 28.4 and for citrate lyase (3) as 1.56 [6]:

$$\text{Citrate} \rightarrow \text{acetate} + \text{oxaloacetate}. \quad (3)$$

The citrate cleavage enzyme or citrate lyase had earlier been described by Gillespie and Gunsalus [7] in anaerobically grown S. *faecalis* and by Grunberg-Manago and Gunsalus [8] in anaerobically grown *E. coli*. It differs from the essentially irreversible condensing enzyme reaction in that acetyl coenzyme A is not involved. Thus, both reactions (1) and (3) are quite reversible and have equilibrium constants slightly in the direction of the tricarboxylic acid synthesis.

There are today over 2100 references in Chemical Abstracts to work with isocitric lyase and its role in replenishing C4 dicarboxylic acids via the glyoxylate bypass [9] so prominent in organic acid grown organisms (fungi as well as bacteria) and germinating seeds. Recent publications, principally from the laboratory of Henry C. Reeves [10], have shown that isocitric lyase may be regulated by phosphorylation of one or more histidine residues and if the phosphoryl group is removed catalytic activity is lost.

In 1955, Gunny moved from the Bacteriology Department to head the Division of Biochemistry at the University of Illinois and I changed my major from bacteriology to biochemistry. The folks in biochemistry were golfers and Gunny decided to pursue the game. Fortunately, in those days there was a small nine-hole course close to campus and in the spring and summer Gunny would often pick me up early in the morning as we both tried to improve our games and we could be back in the laboratory by 9 a.m. Later, when I left for UCLA, Gunny gave me the "Gunsmith" trophy, which still sits on my desk today. I think it was an even match: neither of us was going to make a living on the links.

During my time in Gunny's lab there were many excellent colleagues and friends with whom I could interact and from whom I learned a great deal. Many have remained lifelong friends and, despite the competitiveness, it was a happy place in which to work.

My career in Gunny's lab took a different direction after the isocitric lyase work and I began to investigate succinyl CoA synthase [11], a very important component of reactions converting α-ketoglutarate to succinate. Probably my only contribution to the problem was that I learned if I grew *E. coli* on succinate the cell extracts contained many times the level of enzyme found in glucose-grown extracts. It remained for others to considerably purify the enzyme [12] and to show that a phosphorylated enzyme formed during the

reaction [13]. Later, Kreil and Boyer showed that it was a histidine in the enzyme [14] which was phosphorylated.

As I was getting ready to leave Illinois, Gunny asked if I would like to stay and help with the introductory lab class and I gladly accepted, since this would give me a chance to experience faculty life. I even had the opportunity to supervise a Master's student. Emily Sawyer was very interested in δ-aminolevulinate sythesis, particularly as earlier investigators were unsuccessful in showing succinyl CoA as an intermediate in the process in animal tissues. Emily grew *Rhodopseudomonas spheroides* aerobically in visible spectrum light, conditions under which it produced large quantities of porphyrins. She clearly showed succinyl CoA was used by the organism to succinylate glycine in the formation of δ-aminolevulinate [15].

In 1958, I accepted a faculty position at UCLA and spent the next 29 years teaching and carrying on a program largely looking at P–N bonds in proteins and methionine and folic acid metabolism in malignant tissue. Gunny and I remain friends and have visited from time to time over the years. He became a hands-on scientist again and produced a large and diverse variety of papers in several areas, making him clearly one of the more important biologist/biochemists of the latter part of the 20th century. He seemed equally at home in Paris, Urbana, and La Jolla and was always engaging and encouraging when we met.

In 1987, I changed my career and became head of research and development of ICN Pharmaceuticals. There I led the team that developed ribavirin, an antiviral drug now used in combination with interferon in the treatment of hepatitis C.

References

[1] J.J.R. Campbell, R.A. Smith, B.A. Eagles, A deviation from the conventional tricarboxylic acid cycle in *Pseudomonas aeruginosa*, Biochim. Biophys. Acta 11 (1953) 594.
[2] H.B. Vickery, D-Isocitric acid, Biochem. Prep. 3 (1953) 44.
[3] R.A. Smith, I.C. Gunsalus, Isocitritase: a new tricarboxylic acid cleavage system, J. Am. Chem. Soc. 76 (1954) 5002.
[4] R.A. Smith, I.C. Gunsalus, Distribution and formation of isocitritase, Nature 175 (1955) 774.
[5] R.A. Smith, I.C. Gunsalus, Isocitritase: enzyme properties and reaction equilibrium, J. Biol. Chem. 229 (1957) 305.
[6] R.A. Smith, J.R. Stamer, I.C. Gunsalus, Citritase and isocitritase reactions: equilibria—energetics, Biochim. Biophys. Acta 19 (1956) 567.
[7] D.C. Gillespie, I.C. Gunsalus, Bacteriol. Proc. (1955) 80.
[8] M. Grunberg-Manago, I.C. Gunsalus, Bacteriol. Proc. (1953) 73.
[9] H.L. Kornberg, H.A. Krebs, Synthesis of cell constituents from C2 units by a modified tricarboxylic acid cycle, Nature 179 (1957) 988.
[10] E.F. Robertson, H.C. Reeves, Phosphorylation of isocitrate lyase in *Escherichia coli*, Biochimie 71 (1989) 1095.
[11] I.C. Gunsalus, R.A. Smith, Oxidation and energy coupling in keto acid metabolism, in: Proc. Int. Symp. on Enzyme Chemistry, Academic Press, 1958, pp. 77–86.
[12] J. Gibson, C.D. Upper, I.C. Gunsalus, Succinyl CoA synthetase from *Escherichia coli* I: purification and properties, J. Biol. Chem. 242 (1967) 10.
[13] J.A. Grunau, Ernst Knight Jr., E.S. Hart, I.C. Gunsalus, Succinyl CoA synthetase from *Escherichia coli* II: assays for partial reactions, J. Biol. Chem. 242 (1967) 15.
[14] G. Kreil, P.D. Boyer, Detection of bound phosphohistidine in *Escherichia coli* succinate thiokinase, Biochem. Biophys. Res. Commun. 16 (1964) 545.
[15] E. Sawyer, Masters thesis, Univ. of Illinois (1958).