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Historical Paper

Reminiscences of My Association with Professor Vladimir Prelog

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I carried out my PhD work from September 1960 until May 1, 1963 at the Eidgenössische Technische Hochschule under the guidance of Professor V. Prelog. The topology of the active sites of the oxidoreductase from *Curvularia falcata* and from horse liver alcohol dehydrogenase was explored using a number of enantiomerically pure decalones and cyclohexanones as substrates. Our results prompted Professor Prelog to develop the so-called diamond lattice theory.

After I finished my diploma as »Ingenieur-Chemiker ETH« in the summer 1960 I had to make a difficult decision. I had the choice between two or three prominent professors of ETH Zürich who offered me research problems for a PhD work. I have known my academic teachers from lecture or laboratory courses as well as from examinations. When I decided to start PhD work with Professor Prelog I did not know how fortunate my step was. The main reason for my preference to work with Professor Prelog was my interest in biochemical aspects of organic chemistry. I heard from a fellow-student that the so-called »microbiology subgroup« of the Prelog research team was involved with an interesting mixture of stereo- and biochemistry. Former members of the microbiology subgroup investigated the reduction of a number of decalones by whole cells of *Curvularia falcata*. These reductions were strictly stereospecific and obeyed the rules formulated by Prelog (Figure 1).

The idea behind this work was to use molecules of rigid and well-defined shape for »palpating« the active site of the corresponding enzymes. At that time no crystal structure of an enzyme was known, so this was the only possibility to learn about the shape of the active sites. When I started my PhD

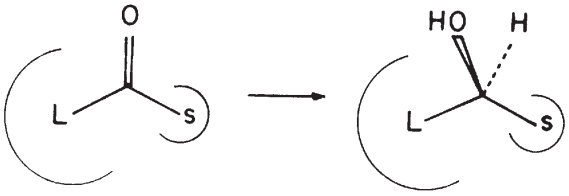

Figure 1. Prelog rule for the stereospecific reduction of ketons by the oxidoreductase from *Curvularia falcata* (L = large group, s = small group).

work in September 1960 two postdoctoral fellows Werner Acklin and Hans Dutler just started to isolate the oxidoreductase from *Curvularia falcata* responsible for the stereospecific reductions *in vivo*. They established a minimum of equipment for the enzyme isolation but for centrifuges we had to run over to the biochemistry department located in the next building. After a short introduction I was charged with the enzyme isolation. 35 years ago it was not a routine job. There were no filled chromatography columns available nor sophisticated ion exchange and molecular sieve materials. We had to prepare our hydroxylapatite and fill our columns with it ourselves. Instead of using computers we had to be present for hours and hours in the cold room and watch our columns while applying onto them our protein solution. Later Professor Prelog bought for us an LKB fraction collector, the first one at ETH and the only one for a long time. For the chromatography of organic compounds in the normal laboratories we had to change the fractions by hand. I remember that I supervised an undergraduate student separating decalones on a large chromatography column. The separation lasted all night and we slept alternately on an old fur-lined cloak which we used otherwise in the cold room.

Professor Prelog, leading a large research group, had no time to do experiments at the bench himself. But once when I had to make sodium amalgam he asked me to prepare in the hood a larger mortar and a pestle as well as one or two kilos of mercury and some sodium under petrol.

Then he came in a white lab coat – he wore this all day even in the office or giving lecture courses – and asked me to cut clean pieces of the sodium and throw them with a pincette into the mortar where the mercury was placed. He stirred the mixture with the pestle vigourously unimpressed by the spectacular fireworks. He said the mixing must be very fast in order to bring as much sodium into the amalgam as possible before it solidifies. I used this amalgam to prepare 1-deuteroglucose by reducing gluconolactone in deuterium oxide. Then I wanted to crystallize the syrupy material from methanol but had no success. After a few days of cooling it in the fridge or in an ice bath I felt quite desperate and told Professor Prelog about my

problem. He advised me to warm the glucose solution on a water bath to about 50 °C. This was just the opposite of what I practised in my laboratory courses but nevertheless I did what he told me. And suddenly – to my surprise – the whole solution crystallized!

After two years of hard work I succeeded to isolate the oxidoreductase from *Curvularia falcata* and also prepared a number of enantiomerically pure decalones. By the use of stereospecifically deuterated NADPH I had also shown that the enzyme transferred deuterium specifically from the B(*Si*)-side of the reduced coenzyme to the substrate.

My kinetic measurements with a great number of decalone derivatives confirmed and brought former *in vivo* results to a quantitative basis. Then I found a literature reference about the reduction of cyclohexanone by horse liver alcohol dehydrogenase. This prompted me to try my decalones with this commercially available enzyme and presented my results at the weekly meeting of the microbiology subgroup in Professor Prelog's office. This was just before Christmas 1962. PG (this was his nick-name when he was absent) listened to me and talked about a »Maria-Theresia-Orden« which used to be an award for officers of the K & K monarchy, who carried out a risky endeavour which was without or against the order of their commanders. If they succeeded they were awarded, if not they were executed! After the Christmas holiday (PG used to spend it by skiing in Arosa) at our first meeting, he presented the so-called »diamond lattice theory«. This was an ingenious combination of the stereochemical and kinetic results with his former experiences with adamantane. Underlying this theory is the idea that the decalone or decalol derivatives can be put together to a diamond lattice. Good substrates of an enzyme occupy only allowed positions at the lattice, whereas poor or no substrates occupy at least one forbidden position. Thus each enzyme has a characteristic cut of the diamond lattice which can be accomodated at its active site. This is illustrated in Figures 2–4. The cuts of the diamond lattice characteristic for the oxidoreductase from *Curvularia falcata* and for alcoholdehydrogenase from horse liver are depicted in Figures 3 and 4, respectively.

After my PhD examination in 1963 I have never lost contact with Professor Prelog and visited him whenever I went to Zürich. In 1972 I settled down in Karlsruhe where I have been teaching biochemistry ever since. Of course I was delighted when in 1975 he shared the Nobel Prize with another friend John (Kappa) W. Cornforth for their contribution to stereochemistry including the stereospecificity of enzymic reactions.

A few years ago when I visited PG in his ETH office he invited me to have lunch with him at the professor's mensa. Before I left he told me whenever I come to Zürich I am his guest for lunch. He kept that in spite of my menace that I travel to Zürich every day to exploit his generosity (I wonder whether he did the same if I lived much closer to Zürich). Unfortunately, I

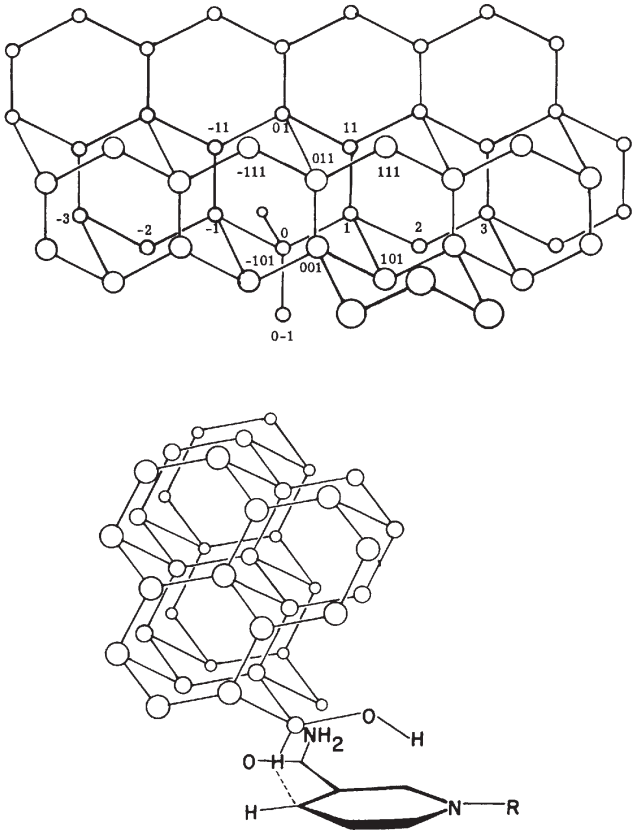

Figure 2. Illustration of the »diamond lattice« theory. Here the alcohol products, showing a numbering system for the positions (above) and the positioning with respect to the nicotinamide ring of NAD.

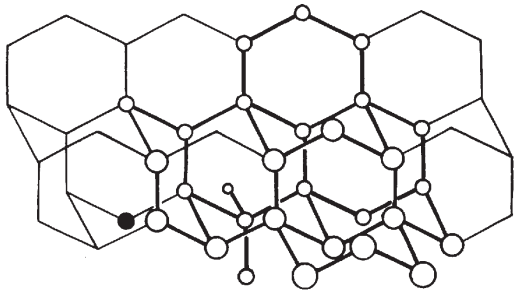

Figure 3. Cut from the diamond lattice characteristic for *Curvularia falcata* oxidoreductase. The filled circle indicates the forbidden position.

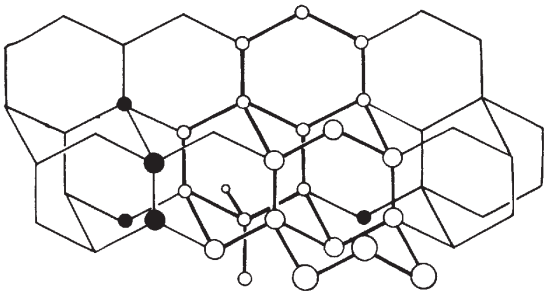

Figure 4. Cut from the diamond lattice characteristic for horse liver alcohol dehydrogenase. The filled circle indicate forbidden positions.

cannot sustain my menace, but I visit him once or twice a year and enjoy talking with him or rather listening to him. He kept his humour and loves to tell stories and anecdotes as ever.

Dear Vlado, I hope to be able to visit you and enjoy your hospitality for many more years. Congratulations to your 90th birthday!

SAŽETAK

Sjećanja na moje druženje s profesorom Vladimirom Prelogom

János Rétey

Svoju sam disertaciju radio od rujna 1960. do 1. svibnja 1963. na Eidgenössische Technische Hochschule pod vodstvom profesora Vladimira Preloga. Primjenom mnogih enantiomerno čistih derivata dekalona i cikloheksanona u njoj je istražena topologija aktivnih mjesta oksidoreduktaze iz *Culvularia falcata* i alkohol-dehidrogenaze konjske jetre. Naši rezultati naveli su Preloga da postavi tzv. teoriju dijamantne rešetke.