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CHRONICLES

Fiftieth Anniversary of the Discovery of Adenylate Kinase

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Until to the middle of the XXth century, it remained unclear how the second labile phosphate group of the ATP molecule could be involved in ATP-dependent reactions. Using a number of purified enzymes (hexokinase, myosin ATPase, etc.), only a single labile phosphate group was shown to take part in these reactions, which resulted in ADP formation. However, in crude systems, both labile phosphate groups were used. The problem was solved for muscle by the works of Colowick and Kalckar [1, 2] with the discovery of an enzyme catalyzing the reversible reaction:

$$2\ \text{ADP} \rightleftarrows \text{ATP} + \text{AMP}.$$

This enzyme was named myokinase. It appeared to possess a unique thermostability. Investigating extracts from other animal tissues in terms of their thermostability, the authors demonstrated that the enzyme was completely absent in liver, kidneys, and other animal tissues; only slight activity was found in heart and brain. Thus the enzyme was named myokinase.

I found another approach to the problem. The wartime year of 1943 year passed, the change in the course of the war was already obvious, and repatriation was gradually beginning. Having come back to Moscow, in 1943 I defended my Ph.D. thesis, which I had prepared before the war. I decided to leave teaching at the Biochemistry Department of the First Moscow Medical Institute, where I had worked for 5 years, and to devote myself to research work.

By then, the world-famous biochemist Ya. O. Parnas had come to Moscow, being brought from Lvov by the Soviet army. In Moscow, he was elected Academician of the Academy of Sciences of the USSR and named a director of two scientific institutes, the All-Union Institute of Experimental Medicine (VIEM) and the Laboratory of Physiological Chemistry of the Academy of Sciences of the USSR. There were vacant positions, and after a conversation with Parnas, I was accepted into a VIEM laboratory as a researcher.

During the war, any research work was impossible. Parnas organized and chaired an information center: biochemists from all over Moscow (and not only Moscow) got together at his "Thursdays" to listen to reports on various scientific subjects. Those materials were published.

After the war was over, it became possible to restart research. Parnas was interested in the processes of glycogenolysis and adenine nucleotide exchange.

By that time, Szent-Gyorgyi had published a report on the discovery of an "ADP isomer" and an enzyme "ADP isomerase" [3, 4]. Parnas suggested that I test those results.

There were no chemicals available in the postwar time, and we had to isolate and purified enzymes and substrates ourselves. So, we isolated ATP from rabbit muscles, and then obtained ADP using ATP and myosin. Detailed investigation of the results of Banga [3] using the ADP isomerase isolated as described in the work showed that the results could be attributed to the action of myokinase discovered by Colowick and Kalckar [1, 2]. My results were published [5], and the problem of ADP isomer and ADP isomerase was closed.

The aim of my study was to find how the labile phosphate of ADP was used in animal tissues aside from muscles (for example, in liver containing significant amounts of ATP and ADP). Using two reactions of ATP hydrolysis by myosin and hexokinase, unlike Colowick and Kalckar, I discovered an enzyme, which was analogous to myokinase, but differed greatly in its properties: it was unstable towards heating in neutral medium. At Parnas' suggestion, the enzyme was named ADP phosphomutase [6]. Later, however, according to the international rules, it was named adenylate kinase.

The myosin test is based on the ability of purified myosin to hydrolyze ATP yielding ADP, which is used by adenylate kinase to synthesize ATP. The reaction proceeds until ADP is completely exhausted. The hexokinase reaction in the presence of glucose and ATP yields glucose-6-phosphate and ADP, which is used again in the adenylate kinase reaction to produce ATP.

Adenylate kinase from liver extracts was stable towards heating in acid medium, but it was completely inactivated on boiling of the extract in neutral medium. The failure of Colowick and Kalckar [1, 2] was due to the fact that they searched for an enzyme with the same properties as those of myokinase (thermostability) in other extracts. However, the enzyme properties appeared to be different from those of myokinase, although the catalytic reaction was the same.

Furthermore, the same approach allowed me to reveal adenylate kinase in a number of animal tissues—heart, kidneys, and erythrocytes [7]. Adenylate kinase

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of all animal tissues investigated maintained activity under short-term boiling in acid medium, while in neutral medium the adenylate kinase from liver and erythrocytes was almost completely inactivated and the adenylate kinase from heart and kidney retained little activity.

To determine the cause of such a peculiar stability of muscle adenylate kinase towards heating in neutral medium, a method was developed for muscle adenylate kinase isolation providing higher purification extent than the method suggested by Kalckar [2]. It was demonstrated that the purified muscle adenylate kinase did not exhibit the thermostability in neutral medium, like adenylate kinase from other animal tissues [8]. It was concluded that the thermostability of muscle adenylate kinase depended on the presence of a specific stabilizer of unknown nature.

Thus, the adenylate kinase mechanism of ADP conversion appeared to be the same in various animal tissues, especially in tissues with intensive metabolism (muscles, heart, liver, erythrocytes).

My works on adenylate kinase interested leading bioenergeticists because they were closely connected with the use of ATP and oxidative phosphorylation. A few years after the publication of my works in 1948-1949, data of other leading groups supporting my results were published [9-14].

Besides the investigation of adenylate kinase activity in animal tissues, I also showed the presence of adenylate kinase in yeast [15], this suggesting widespread existence of the adenylate kinase mechanism in the exchange of adenine nucleotides, and, consequently, its universal role in bioenergetic processes.

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