Meeting report

REACTION MECHANISMS AND CONTROL PROPERTIES OF PHOSPHOTRANSFERASES

A report of a Joint Biochemical Symposium of the All-Union Biochemical Society of the USSR and the Biochemical Society of the GDR, Schloss Reinhardsbrunn, GDR, May 3–7, 1971

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1. Introduction

The symposium was organized by the Biochemical Societies of the USSR and the GDR. It was attended by biochemists from the USSR, the GDR and a number of other countries. Professor Karl Lohmann (GDR), discoverer of ATP and creatine kinase, the first known phosphotransferring enzyme, was Honorary President.

In their introductory addresses, the chairmen of both societies, Academician S.E. Severin (USSR) and E. Hofmann (GDR) emphasized the endeavours of Soviet and German scientists in the classical era of biochemistry especially in the field of phosphate and other group transfer reactions, the world-famous names of Meyerhof, Lohmann, Engelhardt, Warburg, Belitser and Braunstein being mentioned .

2. Enzymatic hydrolysis of inorganic phosphates

S.M. Avaeva (Moscow) presented a review of her studies of yeast pyrophosphatase. She suggested that the enzyme has a m.w. of about 60,000 and contains two pairs of subunits of similar size, the structure of the whole enzyme being represented by A_2B_2. The enzyme binds pyrophosphate and MgPP. During catalysis a phosphorylation of the active site of the enzyme occurs. The phosphoprotein is stable at pH 7 but is dephosphorylated at acidic pH. At pH 7 rapid dephosphorylation is effected by hydroxylamine and sodium cyanide. P. Heitmann and coworkers (Berlin) also reported their results on the subunit structure of yeast pyrophosphatase. When not chemically modified as it was in Avaeva's work, they found it to contain only two subunits of about 35,000 daltons.

I.S. Kulaev and his collaborators (Moscow, Poustchino) described polyphosphatases and polyphosphate phosphotransferases in microorganisms and their significance in inorganic polyphosphate metabolism. Phosphotransferases which synthesize and metabolize polyphosphates without involving ATP are active systems in phylogenetically ancient groups of microorganisms. A comparative study of phosphotransferases participating both in ATP and polyphosphate metabolism allows one to suggest that polyphosphates had in primitive protobionts the same function as ATP in most other living systems.

3. Phosphotransferases of the glycolytic chain

A comprehensive introduction was given by A. Sols (Madrid). He discussed the allosteric effects of primary products and substrates on several glycolytic enzymes. Three glycolytic phosphotransferases are strongly inhibited by substrates or products. Two of these enzymes are allosterically inhibited by their primary products, animal hexokinase by G6P,

L-pyruvate kinase by ATP, while PFK is allosterically inhibited by one of its substrates, ATP.

J. Ricard (Marseille) presented his investigations of the mechanism of action of yeast hexokinase. One molecule of the enzyme (M.W. 49,000) binds one molecule of glucose in the absence of ATP, with an affinity constant of 1×10^4 M^{-1}. There is no binding of MgATP to the enzyme in absence of hexose. These results are in good agreement with kinetic experiments which showed that yeast hexokinase follows an ordered Bi Bi mechanism or an ordered Bi random Bi mechanism. The French group isolated four isohexokinases from wheat germ, two different L-forms (M.W. 55,000) and two different H-forms (M.W. 110,000). In contrast to yeast hexokinase no reversible aggregation–dissociation between these forms could be observed.

According to S.V. Sokolova (Moscow) hexokinase from leaf petioles of sugar-beet is localized in different compartments, 15–20% in the mitochondrial and 10% in the microsomal fraction, the rest in the soluble fraction.These various hexokinase forms have been studied in respect to substrate specificity, as well as to their activation and inhibition properties.

H.W. Hofer (Konstanz) presented experiments on the reversible association–dissociation behaviour of rabbit muscle PFK. The enzyme, having a M.W. of 374,000, dissociates reversibly into two enzymatically inactive halves. These molecular species associate, to an extent dependent on the protein concentration, to oligomers with molecular weights which are multiples of 190,000. This association is accompanied by changes in the maximal specific activity as well as in the saturation function for F6P. From the distribution of PFK in white (10^{-5} M) and red (10^{-6} M) muscle, it can be concluded that *in vivo* the enzyme is highly aggregated.

E.F. Afting (Freiburg) described the interconversion of ATP-sensitive and ATP-insensitive forms of yeast PFK in the presence of ADP, F6P, NH_4^+ and MgF_2. This "physical modification" represents a time-dependent conformational change in the enzyme, evidently playing an important role in metabolic regulation.

E. Hofmann (Leipzig) discussed some problems of yeast PFK, such as the necessary stereospecificity of the phosphate accepting molecule, the number of binding sites and their cooperative interactions, the order of reaction and subunit structure of the enzyme.

St. Liebe (Leipzig) showed that yeast PFK exists in different oligomeric forms dependent on pH, effectors and age of the enzyme preparation. An oligomeric model, based on a protomer of 180,000 daltons which associates to form the dimer, trimer and tetramer, was proposed. According to Diezel and Kopperschläger (Leipzig) this enzyme partially dissociates under alkaline conditions to fragments of 60,000 daltons. In SDS electrophoresis, however, the unmodified as well as the carboxymethylated or performic acid oxidized enzymes show a subunit M.W. of about 90,000.

G. Zimmermann, W. Wenzel and J. Gauer (Leipzig presented gel filtration experiments using crystalline human erythrocyte PFK, which gave enzymes of 240,000, 360,000, 480,000 and 720,000 daltons, composed of 120,000 daltons subunits which were also seen in SDS electrophoresis.

B. Kühn (Berlin) discussed the importance of the PFK reaction in the regulation of glycolysis in red cells. Another erythrocyte enzyme, glyceraldehyde phosphate dehydrogenase (M.W. 144,000) had been crystallized and kinetically characterized by D. Maretzki (Berlin).

G.F. Domagk (Leuven) dealt with the crystallization and characterization of FDP aldolase from beef muscle. Its M.W. of 156,000 and amino acid composition were found to be similar to rabbit muscle aldolase. However, there are differences in the methionine contents, the ORD spectra, and immunological specificities.

K. Brand (Dortmund) reported that crystalline transaldolase from *Candida utilis* has a M.W. of 66,000 and is composed of two subunits. The aldol cleavage of F6P is a two-step reaction, formation of a Schiff's base between the carbonyl group of the substrate and the ϵ-amino group of a lysine residue and base catalysis by histidine. One proton protects two histidine residues in photooxidation experiments suggesting that both groups are closely linked in the protein. Despite the existence of two subunits, transaldolase apparently contains only a single active site.

In an introductory lecture B. Hess (Dortmund) reviewed our knowledge of pyruvate kinase. The liver enzyme can be separated by electrofocusing into an acid form (IP pH 5.3), which is able to bind FDP and an alkaline form (IP pH 6.1) which is unable to bind this allosteric activator. The speaker con-

cluded that these two forms evidently represent the R- and T-states of the enzyme. FDP shifts the equilibrium between them to the more active R-state.

H.-J. Wieker (Dortmund) concluded that H^+-ions also shift this equilibrium to the R-state. In this transition the protonation of a group having a pK_a of 5.35 occurs.

H. Holzer (Freiburg) presented a thorough discussion of the regulation of enzymatic activity by enzymatic modification of the enzyme protein, illustrated by several well selected examples. He dealt especially with the regulatory significance of enzymatic phosphorylation–dephosphorylation and adenylylation–deadenylylation reactions.

R. Wohlhueter (Freiburg) discussed the thermodynamics of the adenylylation of glutamine synthetase and showed that the adenylyl-*O*-tyrosine bond of the modified enzyme represents a new type of "high-energy phosphate bond" ($\Delta G^0 = -9.6$ kcal/mole).

C. Gancedo (Madrid) described studies on the regulation of FDPase in living yeast. Glucose causes a decrease of this enzymatic activity. The author postulated a specific activation process of a selective proteolytic activity acting on FDPase.

4. Enzymatic synthesis and splitting of RNA

Among the highlights of the meeting were the lectures and discussions on the control mechanisms in RNA synthesis.

E. Bautz (Heidelberg) focussed on transcriptional control after infection of bacteria by DNA phages, which in one case have been shown to involve the production of an entirely new RNA polymerase. Infection of bacteria by DNA phages results in the transcription of only a fraction of their genes, namely of those that are expressed immediately after infection. Transcription of the rest of the viral genome appears to be under the control of a phage specific protein, which could either be a new σ-factor, redirecting the core enzyme to recognize another set of promoters, as discussed by W. Zillig (Munich), or a modification protein which could alter the specificity of the host polymerase. A third alternative, found recently for phages T7 and T3, is that the virus produces a new RNA polymerase which specifically recognizes a DNA sequence which is unique to that phage. The T 7 as well as the T 3 RNA polymerase have a much less complicated structure than the native *E. coli* enzyme and therefore should become extremely useful tools with which to study the mechanism of RNA synthesis. W. Zillig (Munich) reported on fragmentation and reconstitution of *E. coli* RNA polymerase. The specific function of β' in the binding of enzyme to the template was discussed as well as the absolute requirement of α for enzymatic activity, the role of β for binding rifampicin and for binding α and initiation factor σ.

K. Sebesta, K. Horska and A. Holy (Prague) presented inhibition studies on RNA polymerase with ATP-analogues to determine the properties of the ATP-specific binding site. Inhibition by L-ATP is not counteracted by excess ATP. Both the association of the enzyme with the template and the elongation are affected by this analogue.

Modified substrates were also used by A. Holy (Prague) for studying the specificity of several nucleolytic enzymes. On the basis of their interaction with L-nucleotide derivatives, the nucleolytic enzymes can be separated into two groups, The difference lies in a different role of the nucleoside moiety in the formation of the enzyme-substrate complex. In addition, enzyme interaction studies with anomers of natural nucleotides, with modified heterocyclic bases, altered sugar moieties and modifications of the phosphoric acid ester residue were presented.

H. Witzel (Münster) described his studies of the catalytic mechanisms in RNases. Analysis of kinetic data with modified substrates and inhibitors in combination with UV, CD and NMR measurements gave detailed information about substrate binding and the catalytic processes. The author demonstrated that apparently RNAses A, T_1 and T_2 use different catalytic mechanisms for lowering activation energy, even of identical steps.

5. Adenosine triphosphatase

The field of ATPases was introduced in a lecture by A.D. Vinogradov (Moscow) on the soluble ATPase from nitochondria. Kinetic studies revealed that $MgATP^{2-}$ is the true substrate, while free ATP and MG are inhibitory. In crude preparations of mito-

chondria an ATPase inhibitor could be detected, which only reacts with the active state of the enzyme.

Yu. N. Leikyn (Moscow) reported on the specific ionic form of phosphate which accompanies active Ca^{2+} uptake in rat liver mitochondria. Apparently only the monoanion of phosphate is transported through the membrane ($K_m = 7 \times 10^{-5}$ M).

A.A. Boldyrev (Moscow) dealt with properties of cation transfer ATPase of muscle membranes and discussed the effects of some biologically active compounds (histidine, dipeptides, caffeine, acetylcholine) on its activity and on the ratio of cation transferred to ATP hydrolysed. Similar investigations were made by N.P. Meshkova (Moscow) on the inhibition of myosin ATPase by phosphorylated carnosine and imidazole derivates.

V.I. Deshcherevsky (Poustchino) reported on the mechanism of activation of myosin ATPase by actin and M.V. Georgadze (Poustchino) presented his studies on the effect of NH_4^+ on the ATPase activity of myosin and the polymerization of actin.

S.L. Bonting (Nijmegen) gave a review of Na-K-activated ATPase and active cation transport, a field in which the author made a series of very important discoveries. The Na-K-ATPase cation pump is present in three types of systems, single cell cystems, excitatory systems and secretory systems. Three Na^+ ions are transported per molecule of ATP hydrolyzed.

D. Dettmer (Leipzig) presented work on the interaction of monosaccharides with intestinal Na-K-ATPase. Glucose and other so-called actively transported monosaccharides inhibit Na-K-ATPase in a manner dependent on the Na and K concentrations and change the Na-activation curve to a sigmoidal shape. These effects can be localized at the phosphatase step. From his results Dettmer constructed a model of Na-K-ATPase, in which a conformational state dependent on an intermediary thioacyl bond is suggested as the possible reaction site of glucose.

H. Remke (Leipzig) discussed monosaccharide-dependent Na-influxes across the membrane of intestinal microvilli. He proposed a direct involvement of Na-K-ATPase in active sugar transport.

K.R.H. Repke (Berlin-Buch) presented a model of Na-K-ATPase, according to which the enzyme exists in two different conformational states, a Na^+-binding and a K^+-binding form. The first transports Na^+, at the outer membrane face it changes to the K^+-binding form and vice versa.

6. Pyridoxalphosphate-containing enzymes

This topic was introduced by E. Helmreich (Würzburg) who reported on the function of pyridoxal-5′-phosphate in glycogen phosphorylase. Reconstitution of apophosphorylase with analogues of PLP, especially those in which the 5′-phosphate group has been esterified yields an enzyme whose conformation is very similar to that of active PLP phosphorylase, which is however, enzymatically inactive. The author proposed from this and other most interesting findings that the phosphate group of PLP in glycogen phosphorylase participates apparently in a general acid-base catalysis with nucleophilic substitution and retention of configuration.

D. Palm (Würzburg) described investigations of the conformational changes upon binding of PLP and PLP-analogues to the apoenzyme of phosphorylase *b*. It is assumed that PLP is enclosed in a hydrophobic region which is formed during reconstitution. In the presence of 5′-AMP access of the solvent to this apolar region becomes more restricted.

N.B. Livanova (Moscow) studied the action of various ligands on the initial rate of the decrease of fluorescence intensity during the tryptic digestion of phosphorylase *b*. An observed decrease of enzyme fluorescence upon 5′-AMP binding is the result of this dimer-tetramer transition. H.Will and E.G. Krause (Berlin-Buch) reported on the kinetic behaviour of the isoenzymes of pig myocardium and muscle glycogen phosphorylase *b*. Using the computer program developed by J. Reich the enzymes were found to represent a mixed V,K-type. J. Reich et al. (Berlin-Buch) presented a general computer strategy for the analysis of kinetic and binding curves, and Cumme (Jena) discussed theoretical aspects of enzymes with metal complexes as substrates.

7. Protein–protein interactions

Under the chairmanship of H. Hanson (GDR) a round table discussion of general problems of protein–protein interactions took place. An introductory lecture was by T. Keleti (Budapest) on the regulation of enzymic activity by intra- and intersubunit interactions, with special reference to the regulatory aspects of D-glyceraldehyde-3-phosphate

dehydrogenase activity. J. Ovadi (Budapest) showed that ATP causes an inactivation and a dissociation of tetrameric glyceraldehyde-3-phosphate dehydrogenase into dimers.