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CHAOS IN THE WORLD OF ENZMYES - HOW VALID IS FUNCTIONAL CHARACTERIZATION WITHOUT METHODOLOGICAL EXPERIMENTAL DATA?

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ABSTRACT

Functional characterization of enzymes plays an essential role in one of the major aims of proteomics research: the modelling of sections of the cellular metabolism with a view to being able to model the whole cellular metabolism and the interaction of cells within tissues and organs. With these purposes in mind, the scientific community established a new branch within the life sciences, called systems biology. However, meaningful modelling by necessity requires comparable and reliable data from standardized enzyme characterizations. From a short, but detailed, investigation of the BRENDA database, it is shown here that the quality of experimental data of enzymes is insufficient for the needs of theoretical biology.

The first step to remedy the situation is to ensure that measurements carried out on enzymes are done so under standard conditions and that all the important information is recorded. With the aim of arriving at an acceptable set of recommendations for experimental conditions, the Beilstein Institut has initiated broad discussions within the scientific community and is further willing to organize and present them as long as appropriate and there is sufficient support.

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QUO VADIS, SYSTEMS BIOLOGY?

Continuous advances and improvements have enabled proteome analyses to proceed with increased depth and efficiency. However, whilst the large international genome sequencing projects elicited considerable public attention with the creation of huge sequence databases, it has become increasingly apparent that functional data for the gene products, in particular for enzymes, has either limited accessibility or is not available. The problem is twofold; on the one hand, deriving data from experimental work is expensive and very time consuming, being a non-trivial process. On the other hand, it is inherently very difficult to collect, interpret and standardize published data since they are widely distributed among journals covering a number of fields, and the data itself is often dependent on the experimental conditions. For these reasons a systematic and standardized collection of functional enzyme data is essential for the interpretation of the genome information.

The investigation of metabolic networks, the regulation of developing and developed cells, cell and tissue specification and further highly complex cellular processes are the central aims of systems biology. Researchers in this discipline use the rapidly grown biological databases to create biological models and simulations as well to develop further powerful algorithms which have made it possible to explore many cellular physiological functions. Systems biology intends to reconstruct the cellular metabolism as a series of overlapping mathematical models. By using all the theoretical and experimental achievements of the various genome and proteomes projects, the data can then be analysed by computational, mathematical and engineering methods in order that predictive models of single biochemical processes, cells, tissues or even entire organs can be generated [1]. Furthermore, a model that works well will be useful for designing initial or further experiments that will verify or refute both working hypothesis of physiological functions of certain modelled systems and the predictions previously carried out.

The integration of experimental and mathematical methods in biology also requires that enzymologists make a conceptual shift; enzyme systems do not reach equilibrium even when they seem stationary - the system is in constant flux. Traditionally, functional characterization of enzymes includes the determination of V_{max} (maximum velocity of an enzyme reaction) and K_m to help define the kinetic behaviour of a given enzyme. However, these parameters are often either unknown or extrapolated from different organisms or species.

Furthermore, traditional enzymology uses enzymatic assays under steady state conditions, so that researchers record the turnover of large amounts of substrate molecules by multiple enzyme molecules. Technological advances now allow enzymologists to capture the kinetics of single enzyme molecules by using a variety and combination of methods and tools such as classical biochemical and biophysical techniques, i.e. fluorescence microscopy, FRET, electrophysiology etc.. However, using these disjunct sets of information, often measured under different conditions, it is not possible to reach the goal of understanding complete pathways. Thus, enzymology should move from the relatively simplistic determination of single enzyme kinetics towards non-equilibrium thermodynamics of populations of enzymes in metabolic contexts. In addition, systems biology can only work successfully if the theoretical part is continuously fed with a large quantity of highly qualitative experimental data.

INTEGRATION OF EXPERIMENTAL AND COMPUTATIONAL BIOLOGY

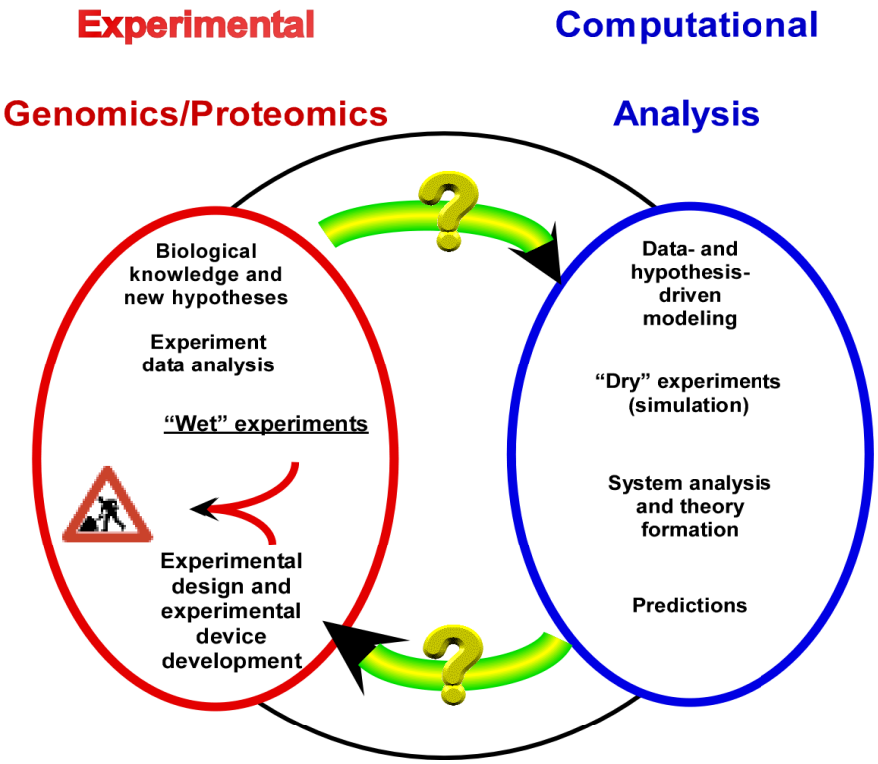


Figure 1. Hypothesis-driven research in modern biology whose one representative discipline is systems biology (adapted from Kitano, H. (2002). *Science* **295**:1662-1664).

The research cycle in modern biology is illustrated in Fig. 1. The cycle can commence either with data- or hypothesis-driven modelling, or with experimental design related to a specific scientific problem.

The models are determined by a computable set of assumptions and hypotheses, which must be tested or confirmed experimentally. The validity of assumptions and hypotheses embedded in each model is proved by "dry" experiments, such as simulations. Inadequate models that show inconsistencies with established experimental facts will be rejected or modified. Those models that pass this test undergo system analysis resulting in a number of predictions which themselves undergo test procedures in "wet" experiments. Data from successful experiments either verify or show the inadequacies of the computational models and enter into the pool of biological knowledge that is source for new hypotheses. By contrast, analysed data from "wet" experiments pass also into this pool of biological knowledge and can be subjected to modelling and simulation. Successful modelling and computational "dry" experiments require strictly comparable data of high quality.

THE COMPREHENSIVE VIEW ON ENZYMES

There is no doubt that proteins and, especially, enzymes, are the most important elements of biochemical processes. Thus, comprehensive characterization of enzymes provides a series of requirements for unambiguous description:

a) proper identification of the protein as a product of a single or several different genes,

b) proper determination of the structure,

c) proper description of the enzymatic reaction and classification of the enzyme according to the Enzyme Nomenclature (see also the articles by Richard Cammack, by Kirill Degtyarenko, and by Keith Tipton et al. in this book), and

d) identification of the catalysing mechanisms from a consideration of the physicochemical properties of the biochemically active sites in the enzyme.

These active sites include both those catalysing binding pocket(s) for the metabolic reaction and binding pockets for inhibitors and/or activators. The results of this research lead to a comprehensive picture of the function-structure relationships of an enzyme. Numerical data about these relationships for a whole series of catalysing enzymes within a physiological pathway would provide systems biology with the opportunity to model, simulate or calculate this pathway under different known physiological and non-physiological conditions.

Functional data of enzymes include measurements of the catalysing behaviour which is dependent upon pH, temperature, ionic strength, inhibiting and activating compounds, substrate specificity, etc.. These are usually numerical data. They are required to describe, for example, the kinetics of a given enzyme and, subsequently, of entire pathways.

However, if a number of enzymes in a given pathway is investigated under (at least) comparable experimental conditions, this data will also be comparable and will be suitable to feed further steps of analysis. In general, further analysis means in silico, i.e. computer-based, investigation of the kinetics of branches or entire pathways. Results of these analyses reflect the metabolism of biological compounds under "normal" and stressing physiological conditions. These analyses are certainly core topics of systems biology. However, researchers encounter increasing numbers of collections of data on enzyme characterizations, all of which should be used cautiously. If functional data lacks proper comparability, sound numerical analysis of pathways, cells, tissues or even entire organs, will not be possible.

Apart from metabolic networks mentioned above, systems biology also attempts to understand cellular networks of molecular interactions. When such a network has been established together with gene expression profiles, it is possible to explain and predict both interactions (and also generate hypotheses which have to be proven) that regulate the observable expression dynamics and why and when a gene is turned on and off in response to the state of this network [2]. It should then be possible for systems biology to not only depict the cellular metabolic pathways, such as those in the well-known Boehringer poster, but to do this in three dimensions with a higher level of information than for example the KEGG pathway map. The application of these digitized maps may be found in the understanding and simulation of the treatment of diseases such as diabetes. The results can be used for the development of new "intelligent" drugs [3].

FIRST STEPS TOWARDS MODERN BIOLOGY

It may be of interest to note here that a series of projects to simulate entire cells, such as the e-cell project led by Masaru Tomita [4], are currently in their initial phases. The Escherichia coli cell simulation used by this group represents a hybrid model and includes both quantitative kinetic and qualitative stoichiometric data. The last data type had to be used due to the lack of available kinetic data. Another group led by Jacky Snoep and Hans Westerhof [5] has established The Silicon Cell project to model parts of metabolic pathways.

Part of which allows the simulation of some of the common metabolic pathways via the Internet with a Java-based program (see also Jacky Snoep's article in this book). In this project, the researchers were hindered through the lack of kinetic data. The group is now having to re-determine the required enzymatic reaction data under internal standardized experimental conditions. Some other functional data are retrievable from KEGG [6] or EcoCyc [7].

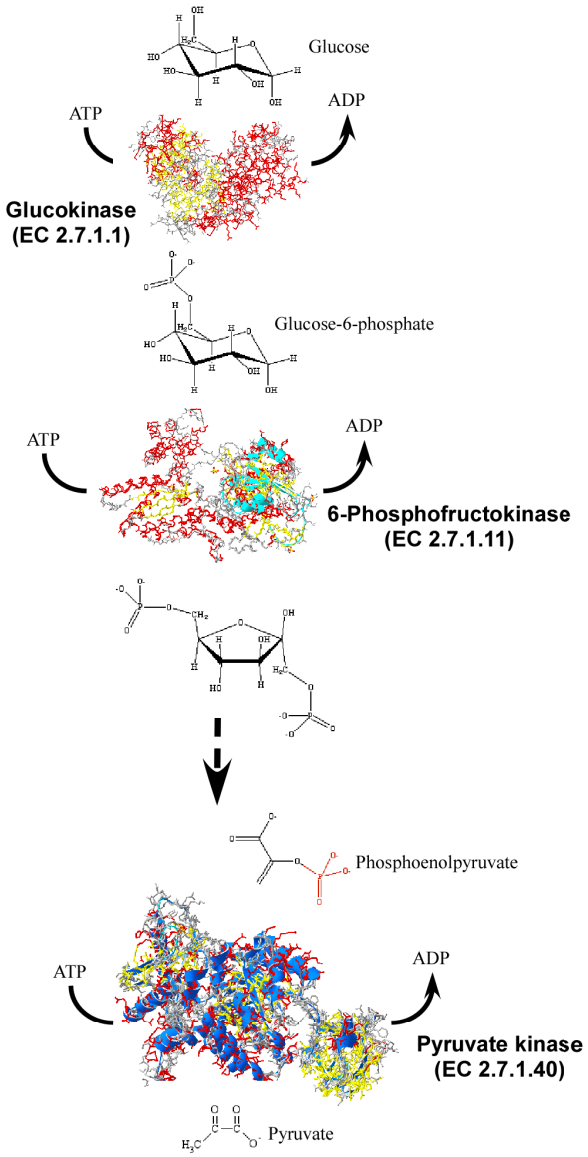
The intention here is to show the necessity for the standardization of experimental conditions on the basis of one example only. We present some enzymological and methodological data for the key enzyme involved in the glucose degradation pathway, called glycolysis, also known as the Fructose-1,6-bisphosphate pathway, or Embden-Meyerhof-Parnas pathway.

THE WAY INTO THE LABYRINTH OF SYSTEMATIC DISORDER

The Cologne BRENDA database was chosen as the preferred data repository for the investigation of enzymological data and associated experimental conditions. BRENDA is the acronym for "Braunschweig Enzyme Database". This database was developed at, and is maintained by, Dietmar Schomburg's group at the University of Cologne and offers an exceptional collection of functional enzyme data [8] (see also Dietmar Schomburg's contribution in this book). In contrast to the databases SwissProt [9], PIR [10] or PDB [11] which provide predominantly information on protein sequences and structures, this database covers a wide range of functional enzyme characteristics. It contains approximately half a million entries in more than 50 fields that can be searched in various combinations for information on about 22,000 enzymes with some 3500 "different" enzymes. The data on enzyme function for this database are extracted directly from the primary literature by graduate biologists and chemists.

This enzyme database was used to evaluate the quality and quantity of the functional enzyme data of glycolysis (Embden-Meyerhof-Pathway)(Fig.2). This pathway was selected for our evaluation because it is almost certainly one of the best-understood metabolic pathways within the cell where it plays a key role in the degradation of sugar and the subsequent provision of chemical energy for the cellular catabolism. Furthermore, it was expected that for glycolysis the best information about the enzymes involved with respect to their functional characteristics in most organisms would be available. To avoid biochemically less interesting enzymes and obtaining poor data, three key enzymes from the glycolysis were selected: glucokinase (GK, EC 2.7.1.1), 6-phosphofructokinase (6-PF, EC 2.7.1.11) and pyruvate kinase (PK, EC 2.7.1.40),

from baker's yeast *Saccharomyces cerevisiae* and *Escherichia coli* as well as f*rom Bacillus stearothermophilus* and the slime fungus *Dictyostelium discoideum*.



In our case, the main criteria for the functional description of these enzymes were data on the turnover kinetics such as activity and Michaelis constant (K_m), information about activating and/or inhibiting compounds and molecules such as cofactors, allosterically acting compounds as well as ions. Additionally, we were interested in temperature and pH profiles which give information on the maximum activity of the enzyme at a given temperature or pH.

After this information had been collated for all three enzymes and all four organisms, the experimental conditions for some of this information were examined using the original literature, firstly to study the reasons for the differences in the data set within one descriptive criterion and secondly, to see if the data was comparable and suitable for modelling and simulation.

Figure 2. Glycolysis: key enzymes and their substrates [19, 20].

It should be noted that, since this investigation was carried out in mid 2003, BRENDA has continued to increase in both content and in data structure, it is therefore possible that there will be some inconsistencies between our results and the current information available from BRENDA.

IN THE MIDDLE OF THE LABYRINTH

The functional data of the three key enzymes of the glycolysis of the four different organisms, two prokaryotes and two eukaryotes, were reviewed. Examples of the experimental methods and conditions which led to the functional characteristics of the enzymes, as described in the literature cited in BRENDA, have also been collected.

The investigation of the functional characteristics of the three key enzymes of the glycolysis gave the results shown in Table 1. The study on the functional enzyme data was commenced with queries for the yeast Saccharomyces cerevisiae and the coliform bacterium E. coli. But since the retrieval results from BRENDA were zero for glucokinase and low for pyruvate kinase, it was necessary to expand the investigations to two further biochemically well-known organisms: Bacillus stearothermophilus and the slime fungus Dictyostelium discoideum.

As can be seen from Table 1, even the results for yeast and E. coli were surprising since these two organisms appear to have been well studied by both biochemical and molecular biological methods. Yeast plays an important economic role, its genome was completely sequenced eight years ago and since then functional and structural proteomics has made great strides [12-15]. On the other hand, E. coli is the main organism in expression studies and acts as the main transformation vector [16-18]. Thus, comprehensive data on their fundamental metabolic pathway was expected. The best investigated enzyme of both organisms seems to be 6-phosphofructokinase (6-PF).

Functional data for 6-PF can be obtained from several publications, it being the most important enzyme in glycolysis. However, there are many missing data for pyruvate kinase for both organisms: for example, data on the inhibitors, temperature and pH range are missing for *E. coli* as well as for *S. cerevisiae*. Kinetic data, such as K_m value and specific activity, for *E. coli* were completely absent. The best data for all three enzymes were found for *B. stearothermophilus* which is a prominent member of extremophile organisms. All criteria for the functional description of the glycolytic key enzymes seem to be fulfilled. Finally, the slime fungus *D. discoideum* provided the least amount of data; there are only a few functional data available for the first step of the glucose degradation carried out by the enzyme glucokinase.

Table 1. Comparison of the functional characteristics of the glycolytic key enzymes from four different organisms.

Parameter	Enzyme / EC-Nr. Glucokinas				6-Phosphofructokinase 2.7.1.11				Pyruvatkinase 2.7.1.40			
	Bacillus spec.	*S. c.*	*E.c.*	*Dictyostelium d.*	*Bacillus spec.*	*S. cerevisiae*	*E. coli*	*D. d.*	*Bacillus spec.*	*S. cerevisiae*	*E. coli*	*D. d.*
K_M-Value [mM]	0.15; 0.06 (ATP), 0.52 (gluc)			0.12 (gluc), 1.1 (ATP)	0.0392 (F6P), 0.055 (ATP)				0.2 (PEP), 0.7 (ADP)	0.049 (IDP), 0.16 (ADP)		
Substrates	ATP + D-glucose			ATP + D-glucose	ATP + D-Fruc 6-P	ATP + D-Fruc 6-P (Glu-1-P, SedHep-7-P)	ATP + D-Fruc 6-P		ADP + PEP	ADP + PEP	ADP + PEP	
Products	ADP + D-gluc 6-P			ADP + D-gluc 6-P	ADP + D-Fruc 1,6 BisP	ADP + D-Fruc 1,6 BisP	ADP + D-Fruc 1,6 BisP		ATP + Pyruvat	ATP + Pyruvat	ATP + Pyruvat	
Cofactors						ADP, AMP	GDP		AMP (allosteric), GMP (activation), CMP (activation), GDP (activation)		AMP (PKII)	
Activating Compound						Fruc 2,6-BisP			Gluc-6-P, Rib-5-P, 3-P-Glyc		Fruc 1,6 DiP (PKI), Gluc 6-P, Rib 5-P (PKII)	
Inhibitors	N-Acetyl-alpha-D-glucosamine, D-Maltose, $AgNO_3$, PCMB, $HgCl_2$, $Pb(NO_3)_2$, 1,10-Phenanthroline, Iodoacetamide, N-Ethylmaleimide, p-Hydroxymercuri-benzoate, Glucose 6-phosphate			Glucose 6-phosphate, ADP	Citrate, Diphosphate, Phosphoenolpyruvate (most potent inhibitor), F6P, $MgATP^{2-}$, F1,6BisP	Citrate, ATP, $MgATP^{2-}$	Citrate, PFK2 enzyme, PEP		Mg^{2+}, ADP, PO_4^{3-}, Ca^{2+}, Cu^{2+}, Ni^{2+}, Sr^{2+}		ATP, Succinyl-CoA (PKI)	
MW [Da]	87.000/67.000				135.000	835.000	142.000		242.000	209.400	190.000 (PKII), 225.000 (PKI)	
Metals/Ions	Mg2+ (required); Mn2+ (45% of the activity with Mg2+); Co2+(68% of the activity with Mg2+)			Mg^{2+} (completely dependent on presence of Mg^{2+})	Mg^{2+}, K^+, NH_4^+, Li^+	Mg^{2+}, K^+, NH_4^+, PO_4^{3-}	Mg^{2+}, MgATP, Mn^{2+}, $MnATP^{2-}$, K^+, NH_4^+		Mn^{2+}, Mg^{2+}, Co^{2+}, K^+, NH_4^+, Na^+, Cu^{2+}, Ni^{2+}, Sr^{2+}	Mg^{2+}, K^+		
Specific Activity [µM/min/mg]	334/304			0.51	160	148	215, 263, PFK1: 190; PFK2: 205		210, 333	340, 250, 219		
Temperature Optimum [°C]	30/37 (assay at), 60			20 (assay at)	30 (assay at)	25 (assay at)	27		30 (assay at)	30 (assay at)	25 (assay at)	
Temperature Stability [°C]	60/70 (10% loss)				30 (reverses inhibition by Mg^{2+}, ATP and PEP), 30 (assay at)				25 (in 25 mM Tris pH 7.5); 65 (rapid inactivation);			
pH Optimum	9			7	8,2 (8,7)	7	(PFK1): 6.5 with two optima at 6.5 + 8.5; (PFK2): 8.5 with two		6.8 (60°C), 7,2 (30°C)			
pH Range	6, 10.5 (max)			6, 9 (max)					5.5, 7.4 (max) at 60°C; 6.4, 8.2 (max) at 30°C			
Literature	Goward et al. 1968, Biochem. J. Hengartner et al., 1973, FEBS Lett.			Baumann, 1969, Biochemistry	Marschke et al., 1982, Methods Enzymol. Shirakihara et al., 1988, J. Mol. Biol. Byrnes et al., 1994, Biochemistry	Hofmann et al., 1982, Methods Enzymol. Stellwagen, 1975, Methods Enzymol.	Kotlarz et al., 1982, Methods Enzymol. Kemerer et al., 1975, Methods Enzymol. Kruger et al., 1988, Arch. Biochem. Biophys.		Sakai et al., 1986, J. Biochem. Tuominen & Bernlohr, 1975. Methods Enzymol.	Aust et al., 1975, Methods Enzymol. Hunsley & Suelter, 1969, J. biol. Chem.	Malcovati & Valentini,1982, Methods Enzymol	

What might the reasons be for this lack of data?

Data available from the SwissProt, KEGG, PDB and PIR databases indicate that the listed enzymes of the four organisms mentioned above have been well investigated with respect to their structures and sequences. Queries within these databases give a comprehensive collection of data on protein identification, subunit composition and stoichiometry as well as crystallographic data, information about isolation and storage of purified proteins. But comments on the function of these proteins are extremely short. Furthermore, the functional enzyme data found in special enzyme databases such as BRENDA are fragmentary and some enzymes lack any functional information. This was a surprising and alarming result of our short study. On the basis of such poor data availability, it is hard to imagine that metabolic simulation and modelling could be carried out successfully.

The next step in our evaluation was to obtain information from the original research literature on the experimental conditions. An investigation of the material & methods sections of the appropriate publications which describe among others, the functional characterization of a given enzyme led to the analysis shown in Table 2.

At first glance, the functional data of each enzyme of all the organisms appear to have been obtained by comparable methods: the coupled optical test and/or the pH stat assay. The first noted method was carried out by means of different sets of auxiliary enzymes depending on the glycolytic enzyme studied to record the rate of NADH oxidation. The second method records the flow of protons by pH-sensitive electrodes. However, the decisive differences within the applied methods are the basic experimental conditions. Measurements were performed under different temperatures (ranging from 25°C to 37°C), also apparently different wavelengths to record NADH oxidation (which might be less critical) were used, and, finally, the assay buffers had different compositions. In particular, for the assay buffers, there is a wide range of simple compositions (e.g., for pyruvate kinase of *E. coli*) and rather "complicated" compositions (e.g., for pyruvate kinase of *S. cerevisiae*) with respect to the number and types of compounds. Investigation of enzyme function is supposed to be carried out under the simplest conditions to avoid side effects that would lead to changes in enzyme reactions which are hard to interpret.

In conclusion, from the standpoint of single methodological aspects, such as temperature or pH, it is generally conspicuous that the laboratory experimental conditions chosen to determine kinetic key parameters such as activity or K_m lead to values that are hardly comparable for all three enzymes.

Michaelis-Menten kinetics and specific activity both strongly depend on these basic parameters mentioned above.

The above noted fundamental differences in the application of obviously commonly used methods confirmed the necessity to standardize experimental conditions for enzyme characterizations.

Table 2. Analysis of methods and experimental conditions, obtained from literature.
A) for yeast, i.e. *Saccharomyces cerevisiae*. Differences in the methods of yeast fractionation, protein isolation and purification.

Enzyme	**Phosphofructokinase** **(EC 2.7.1.11)**	**Pyruvatekinase** **(EC 2.7.1.40)**
Reaction	Fruc-6-P + ATP --> Fruc-1,6-bis-P + ADP + H^+	PEP + H^+ + ADP --> ATP + Pyruvate
Principle	coupled optical test with aldolase, triose-phosphate isomerase and glycerol-3-phos-phate dehydrogenase (DH)	uptake of protons with a pH stat, decrease in absorption at 230 nm due to loss of PEP or coupled optic test with lactate DH
Product formation	Fruc-1,6-bis-P formation indicated by NADH dependent reduction of dihydroxy-acetone-P to glycerol-3-P	reduction of pyruvate, oxidation of NADH
Optimization	addition of AMP as stron allosteric acitiva-tor	
Reagents	<u>Assay mixture:</u> Imidazole/HCl, 100 mM, pH 7.3, Fruc-6-P, 3 mM, ATP, 0.6 mM, pH 7.2, $MgSO_4$, 5 mM, $(NH_4)_2SO_4$, 5 mM, AMP, 1 mM, NADH, 0.2 mM, <u>Auxiliary enzymes:</u> Imidazole/HCl, 100 mM, pH 7.2, Aldolase, 14 u/ml, Triosephosphate isomerase, 136 u/ml, Glycerol-3-P-DH, 12 u/ml <u>Buffer mixture:</u> Imidazole/HCl, 100 ml, pH 7.2 Fruc-6-P, 1 mM 2-Mercaptoethanol, 5 mM PMSF, 0.5 mM	<u>Assay mixture:</u> $(CH_3)_4N$ cacodylate, 100 µM, pH 6.2, $MgCl_2$, 24 µM, KCl, 100 µM, Tricyclohexylammonium FDP, 1 µM NADH, 0.16 (0.15) µM, <u>Auxiliary enzymes:</u> Lactate DH, 33 µg (3 mg/ml in 200 mM Tris-HCl, pH 7.5)
Measurement	NADH oxidation at 340 nm and 25°C against water	NADH oxidation at 230 (?)(340) nm and 30°C against water
Literature	- Hofmann & Kopperschlaeger (1982). *Methods Enzymol.*	- Aus *et al.* (1975). *Methods Enzymol.* - Hunsley & Suelter (1969). *J. biol. Chem.*

B) for *Escherichia coli*

Enzyme	Phosphofructokinase (EC 2.7.1.11)	Pyruvatekinase (EC 2.7.1.40)
Reaction	Fruc-6-P + ATP --> Fruc-1,6-bis-P + ADP + H^+	PEP + H^+ + ADP --> ATP + Pyruvate
Principle	coupled optical test using aldolase, triose-phosphate iosmerase, glycerol-3-P-DH; uptake of protons with pH stat	direct: proton uptake with pH stat; coupled optical test using lactate DH
Product formation	conversion of Fruc-1,6-bis-P to glycerol-3-P; Oxidation of 2 µM NADH per µM Fruc-1,6-bis-P formed	reduction of pyruvate, oxidation of NADH
Optimization		
Reagents	Assay mixture: Tris-Cl, 80 mM, pH 8.2, $MgCl_2$, 1 mM, Fruc-6-P, 6.7 mM, Auxiliary enzyme mixture: Tris-Cl, 10 mM, EDTA, 2mM, Aldolase, 1.5 mg/ml, Glycerol-3-P-DH, 0.5 mg/ml, NADH, 0.15 mM, ATP, 1 mM, pH 8, pH stat assay: KCl, 80 mM, $MgCl_2$, 10 x [ATP], ATP, variable, Fruc-6-P, variable, pH 8.5	Assay mixture: Tris or HEPES, 10 mM, pH 7.5, $MgCl_2$, 10 mM, KCl, 50 mM, ADP, 2 mM, PEP, 10 mM, Auxiliary enzyme mixture: Lactate-DH, 22 u/ml, NADH, 0.12 mM, PK, 0.03 - 0.1 u/ml
Measurement	oxidation of NADH at 340 nm and 29°C against water; change of [H^+] recorded by calomel and glass electrode at pH 8.5	oxidation of NADH at 340 nm at 25°C against water
Literature	Kemerer et al. (1975). *Methods Enzymol.*	Malcovati & Valentini (1982). *Methods Enzymol.*

C) for *Bacillus spec.*

Enzyme	Glucokinase (EC 2.7.1.1)	Phosphofructokinase (EC 2.7.1.11)	Pyruvatekinase (EC 2.7.1.40)
Reaction	Glucose + ATP --> Gluc-6-P + ADP	Fruc-6-P + ATP --> Fruc-1,6-bis-P + ADP + H^+	PEP + H^+ + ADP --> ATP + Pyruvate
Principle	coupled optical test using Gluc-6-P-DH	coupled optical test with aldolase, triosephosphate isomerase and glycerol-3-phosphate-DH	coupled optical test using PEP and Lactic-DH
Product formation	Gluc-6-P formation and oxidation of NADPH	Fruc-1,6-bis-P formation indicated by NADH dependent reduction of dihydroxyacetone-P to glycerol-3-P; 1 mol Fruc-1,6-bis-P --> Oxidation of 2 mol NADH	Lactate formation and oxidation of NADH
Reagents	Reaction mixture: Tris/HCl, 50 mM, pH 9, Glucose, 10 mM, ATP, 2 mM, $MgCl_2$, 3 mM, NADP+, 1 mM, Gluc-6-P-DH, 5 µg/ml	Assay mixture (*am*): Imidazole-HCl, 20 mM, pH 6,82, KCl, 100 mM, $MgCl_2$, 3 mM, ß-mercapto-EtOH, 1 mM Auxiliary enyzmes (*ae*): BSA, 1.5 mg, Aldolase, 4 mg, Triosephosphate isomerase, 32 u/ml, Glycerol-3-P-DH, 8 u/ml --> dilution of enzyme solution in 2.5 ml aq.dest.; 0.1 ml of *ae* was used per 1 ml *a*m	Assay mixture: Imidazole-HCl, 25 µM, pH 7.2 (50 µM, pH 7.1), KCl, 25 µM (50 µM), $MgCl_2$, 3.5 µM (7 µM), ADP, 1 µM (NaADP, 4 µM) NADH, 0.06 µM (0.12 µM) Auxiliary enzymes: PEP, 2 µM, Lactic-DH, 10 µM (40µM)
Measurement	NADPH oxidation at 340 nm and 37°C against water	NADH oxidation at 340 nm and 30°C against water	NADH oxidation at 230 (340) nm and 30°C against water
Literature	- Hengartner & Zuber (1973). *FEBS Lett.*	- Marschke & Bernlohr (1973). *Methods Enzymol*.	- Sakai et al. (1986). *J. Biochem.* - Tuominen & Bernlohr (1975). *Methods Enzymol.* - Bücher & Pfleiderer (1955). *Methods Enzymol.*

ESCAPING FROM ENZYMOLOGICAL CONFUSION?

Much of the functional data of enzymes have been generated under non-standardized experimental conditions. Moreover, these data are usually determined by individual laboratory-specific application and implementation of the experimental design. Consequently, the chances of success for systems biology to escape from the verbally overused -omics-sciences are poor, as long as the quality of the input as well as the out-going modelling data cannot be improved. Furthermore, from the point of view of the applications of systems biology, the importance of reliable experimental data is without question.

In conclusion, there is a definite requirement for standardization of the experimental conditions mentioned above. The current situation is approaching chaos in that functional characterization is being carried out without methodological experimental data. Of course, such standardization, firstly needs broad discussions within the scientific community which hopefully will lead to some common acceptance so that each researcher will carry out his/her experiments accordingly. Moreover, it is necessary to improve the integration of mathematical biology with experimental biology to offer experimentalists the opportunity to learn the language of mathematics and dynamical modelling and theorists to learn the language of biology. Both practicians and theorists are invited to participate in the discussions of the requirements needed for the successful development of systems biology.

The Beilstein-Institut will support this process of discussion and standardization process by hosting:

a) the 1st ESCEC symposium,

b) the discussion of suggestions for standardizing laboratory conditions, and

c) the ESCEC commission which will consist of representatives of several diverse directions of enzymology (see also www.strenda.org).

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