Creatine Phosphokinase

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Lohmann[1] was the first to show that skeletal muscle extracts catalyse the phosphorylation of creatine by adenosine triphosphate (ATP). The reversibility of the reaction has been demonstrated[2]. *Banga*[3] achieved a partial purification of the enzyme and then *Kuby*, *Noda* and *Lardy*[4] succeeded in crystallizing the creatine phosphokinase*) from rabbit muscle. The properties of the enzyme from cerebral cortex have been described[5]. The activity of the enzyme in homogenate supernatants from various rat tissues decreases in the following order[6]: calf muscle, diaphragm, brain, heart, large intestine, small intestine, lung, kidney. No activity is found in normal human serum. *Dreyfuss* and *Schapira*[7-9] found increased activity in human serum in myocardial infarction and myopathies, while *Ebashi* and *Toyokura*[10] reported raised values in serum in progressive muscular dystrophy. For other work, see[11-14]. The activity of the enzyme can be measured with either creatine or creatine phosphate as substrate. The assay with creatine phosphate is more sensitive and less liable to interference[15], but the very high cost of the substrate is against its use in routine laboratories.

According to[14] the activity of the enzyme in human tissues decreases in the following order: skeletal muscle, cerebral cortex, tongue, heart muscle (left ventricle), diaphragm, cerebral medulla, heart muscle (right ventricle), auricle, cerebellum (medulla), smooth muscle, kidney, spleen and liver.

Principle

Creatine phosphokinase (CPK) catalyses the reaction:

$$(1) \qquad \text{Creatine} + \text{ATP} \rightleftharpoons \text{creatine phosphate} + \text{ADP}$$

The activity of the enzyme is measured by the increase of ADP with time with creatine as substrate. The ADP is phosphorylated by phosphoenolpyruvate (PEP) in the auxiliary reaction catalysed by pyruvate kinase (PK):

$$(2) \qquad \text{ADP} + \text{phosphoenolpyruvate} \longrightarrow \text{ATP} + \text{pyruvate}$$

The pyruvate formed is determined with the indicator reaction catalysed by lactic dehydrogenase (LDH):

$$(3) \qquad \text{Pyruvate} + \text{DPNH} + \text{H}^+ \longrightarrow \text{lactate} + \text{DPN}^+$$

The rate of oxidation of DPNH is a measure of the CPK activity.

The following method is essentially that of *Tanzer* and *Gilvarg*[6].

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*) Synonyms: creatine kinase, ATP-creatine transphosphorylase.

1) *K. Lohmann*, Biochem. Z. *271*, 264 [1934].
2) *H. Lehmann*, Biochem. Z. *281*, 271 [1935].
3) *I. Banga*, Stud. Inst. med. Chem. Univ. Szeged *3*, 59 [1943].
4) *S. A. Kuby*, *L. Noda* and *H. A. Lardy*, J. biol. Chemistry *209*, 191 [1954].
5) *A. Narayanaswami*, Biochem. J. *52*, 295 [1952].
6) *M. L. Tanzer* and *C. Gilvarg*, J. biol. Chemistry *234*, 3201 [1959].
7) *J. C. Dreyfuss* and *G. Schapira*, Rev. franc. Etud. clin. biol. *5*, 384 [1960].
8) *J. C. Dreyfuss* and *G. Schapira*, Arch. Mal. coeur *2*, 187 [1960].
9) *G. Schapira* and *J. C. Dreyfuss*, Rev. franc. Etud. clin. biol. *5*, 990 [1960].
10) *S. Ebashi* and *Y. Toyokura*, J. Biochemistry [Tokyo] *46*, 103 [1959].
11) *S. Okinaka* and *H. Sugita*, 84th Ann. Meeting Amer. Neur. Ass., Atlantic City, 1959.
12) *G. Forster* and *J. Escher*, Helv. med. Acta *28*, 513 [1961].
13) *W. Stich* and *A. Tsirimbas*, Der Internist *2*, 592 [1961].
14) *J. P. Colombo*, *R. Richterich* and *E. Rossi*, Klin. Wschr. *40*, 37 [1962].
15) *P. Stein* and *W. Lamprecht*, Klin. Wschr. *40*, 177 [1962].

Optimum Conditions for Measurements

The optimum pH for the phosphorylation of creatine is around pH 9 at 30°C[16]. At this pH the following Michaelis constants were measured for the crystalline enzyme[16]: for ATP 5×10^{-4} M; for creatine 1.6×10^{-2} M. The CPK activity depends on the ratio of ATP to Mg^{2+}; according to[11] the optimum is a molar ratio of 1:1 with about 4×10^{-3} to 2.4×10^{-2} M creatine in 0.1 M glycine buffer. No studies have been made on the optimum conditions for measurements of CPK activity in human serum. In this method the measurements are made at 25°C and pH 9, and with the following concentrations: 4×10^{-3} M ATP; 4×10^{-3} M $MgSO_4$; 3.15×10^{-2} M creatine; 3.45×10^{-1} M glycine. The high concentration of buffer is necessary to overcome the buffering capacity of the serum. In the auxiliary and indicator reactions 4.4×10^{-4} M PEP, 3×10^{-4} M DPNH and 100 µg. PK and LDH are used for each assay.

Reagents*)

1. Creatine
2. Glycine, A. R.
3. Magnesium sulphate, $MgSO_4 \cdot 7\ H_2O$
4. Sodium carbonate, A. R., anhydrous
5. Phosphoenolpyruvate, PEP
   cyclohexylammonium salt; commercial preparation, see p. 1024.
6. Reduced diphosphopyridine nucleotide, DPNH
   disodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
7. Adenosine-5′-triphosphate, ATP
   sodium salt, ATP-$Na_2H_2 \cdot 3\ H_2O$; commercial preparation, see p. 1006.
8. Lactic dehydrogenase, LDH
   crystalline, from skeletal muscle, suspension in 2.2 M ammonium sulphate solution; commercial preparation, see p. 986.
9. Pyruvate kinase, PK
   crystalline, from skeletal muscle, suspension in 2.1 M ammonium sulphate solution; commercial preparation, see p. 997.

Purity of the reagents

The LDH and PK preparations must be free from phosphatases, CPK and ATPase. The specific activity of the LDH should be at least 270 units**)/mg. and that of the PK at least 73 units **)/mg. To avoid too large a consumption of DPNH the ATP and PEP should be as free as possible of ADP and pyruvate respectively.

Preparation of Solutions (for *ca.* 10 determinations)

I. Buffer-coenzyme mixture (2 M glycine buffer, pH 9.0; 1.3×10^{-3} M β-DPNH; 2×10^{-2} M ATP; 2×10^{-3} M PEP; 2×10^{-2} M $MgSO_4$):
   Dissolve 2.25 g. glycine, 0.67 g. Na_2CO_3, 17 mg. DPNH-Na_2, 180 mg. ATP-$Na_2H_2 \cdot$ $3\ H_2O$, 15 mg. PEP (cyclohexylammonium salt) and 40 mg. $MgSO_4 \cdot 7\ H_2O$ in doubly distilled water and make up to 15 ml.

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*) Complete reagent kits are available commercially, see p. 1036.

**) A unit according to *Racker* is the amount of enzyme which converts 1 µmole of substrate in 1 min.

[16] *S. A. Kuby*, *L. Noda* and *H. A. Lardy*, J. biol. Chemistry *210*, 65 [1954].

II. Lactic dehydrogenase-pyruvate kinase, LDH-PK (2 mg. of each protein/ml.):
If necessary, dilute the stock suspensions with 2.2 M ammonium sulphate solution to 4 mg. protein/ml. and mix equal parts of the suspensions.

III. Creatine-glycine buffer (6.3×10^{-2} M creatine in 10^{-1} M glycine buffer; pH 9.0):
Dissolve 350 mg. creatine, 300 mg. glycine and 90 mg. Na_2CO_3 in doubly distilled water and make up to 40 ml.

IV. Glycine buffer (10^{-1} M; pH 9.0):
Dissolve 300 mg. glycine and 90 mg. Na_2CO_3 in doubly distilled water and make up to 40 ml.

Stability of the solutions

Store all solutions, stoppered, in a refrigerator at 0—4°C. Prepare the buffer-coenzyme mixture freshly each week and the creatine-glycine buffer and glycine buffer freshly every month. Deterioration of the solutions is usually due to the growth of micro-organisms. The enzyme suspensions are stable for about a year.

Procedure

Preferably use fresh serum. Slight haemolysis does not interfere as no CPK could be detected in erythrocytes[17].

Spectrophotometric measurements

Wavelength: 340 or 366 mμ; light path: 1 cm.; final volume: 3.50 ml.; temperature: 25°C (should be constant, preferably use a constant temperature cuvette holder). Measure against the control (to exclude DPNH oxidase and phosphatases hydrolysing PEP, which are frequently contained in pathological sera[18]).

Pipette successively into the cuvettes (experimental and control):

1.00 ml. serum
0.70 ml. buffer-coenzyme mixture (solution I)
0.05 ml. LDH-PK suspension (II).

Mix with a small glass or plastic rod flattened at one end and allow to stand for about 5 min. The pyruvate and ADP contained in the PEP and ATP respectively react with the DPNH.

Mix into the cuvettes:

Experimental	*Control*
1.75 ml. creatine-glycine buffer (solution III)	1.75 ml. glycine buffer (solution IV).

The CPK reaction in the experimental cuvette now proceeds. Adjust the spectrophotometer so that when the control cuvette is in the light path the optical density is 0.300 and then read the optical density of the experimental cuvette (E_1). Readjust the optical density of the control cuvette to 0.300 and after exactly 10 min. read the optical density of the experimental cuvette (E_2). The difference in optical density $E_1 - E_2 = \Delta E$ is used for the calculations. ΔE measured at 366 mμ should not be greater than 0.300/10 min.; otherwise dilute the serum with 0.9% NaCl solution and repeat the measurements.

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[17] *E. Bernt*, unpublished.
[18] *F. Fischer* and *G. Siebert*, Klin. Wschr. *39*, 202 [1961].

With normal sera the CPK activity is so low that the accuracy of the measurements is limited by the stability of the instrument. The obtaining of negative values for ΔE cannot be excluded.

Calculations

According to the American literature the usual definition[19] of a unit is the amount of enzyme in 1 ml. of sample, which changes the optical density of DPNH at 340 mμ by 0.001 in 1 min., in a 3 ml. assay mixture at 25°C.

Therefore with a 1.0 ml. sample in 3.5 ml. assay mixture and with a 10 min. incubation period:

at 340 mμ: $(\Delta E_{340}/10 \text{ min.}) \times 116$ = units/ml. sample

at 366 mμ: $(\Delta E_{366}/10 \text{ min.}) \times 116 \times 1.89 = (\Delta E_{366}/10 \text{ min.}) \times 220$ = units/ml. sample.

Example

1.00 ml. serum (myocardial infarction) was analysed and the following optical densities were measured at 366 mμ:

$$E_1 = 0.328$$
$$E_2 = 0.273$$
$$\Delta E = 0.055$$

$0.055 \times 220 = 12.1$ units/ml. serum.

Conversion to other units

See page 33.

Normal Values

No CPK activity is detectable in normal serum. Values below 1.5 units/ml. serum are considered normal.

Stability of the Enzyme in the Serum Sample

The enzyme isolated from skeletal muscle is relatively stable[11]. Although the enzyme which occurs in serum in pathological conditions originates from muscle a strict analogy cannot be drawn. The stability of the enzyme in serum has not been studied.

Sources of Error

Alkaline phosphatase (produces pyruvate from PEP), ATPase and myokinase (produce ADP from ATP) and DPNH oxidase (oxidizes DPNH) interfere with the determination. Such interference is eliminated by the control cuvette. So far as is known, there is no interference from serum enzymes with the reaction in the form described here.

Details for Measurements in Tissues

No studies have been made of the intracellular distribution of the enzyme. The most appropriate method is to measure the activity in whole homogenates (as[20]) or (after thorough disintegration of the cell particles) in the supernatant obtained on high speed centrifugation (as[6]).

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[19] *W. E. C. Wacker, D. D. Ulmer* and *B. L. Vallee*, New England J. Med. *255*, 449 [1956].

[20] *I. T. Oliver*, Biochem. J. *61*, 116 [1955].