# CREATINE KINASE : A BIOCHEMICAL APPROACH

*Kreatin Kinaz : Biyokimyasal Yaklaşım*

Nihat MERT*

*Özet :* Bu derlemede kreatin kinaz biyokimyasal yaklaşım ile incelenmiştir.

*Summary :* In this review creatin kinase was overviwed from the biochemical point of view.

## *Introduction*

Creatine kinase (CK) is one of the transferring hposphorus group of enzymes, also known as Adenosine 5'-triphosphate - creatine phospho transferase. According to acceptor group CK is a phospho - transferase with a nitrogenous as acceptor. In the enzyme classification the place of CK is 2. 7. 3. 2. (3, 8).

CK catalysis the reaction

$$Mg\ ATP^{-2} + Creatine \longrightarrow Mg\ ADP^{-1} + Phosphocreatine^{-2} + H^{+}$$

with the reaction proceeding from left to right begin arbittarily designated to forward direction.

The enzime has a wide tissue distribution and can generally be associated with a physiological role of ATP regenaration in conjuction with contractile or transport systems. It was first crystallized from rabbit skeleton muscle, later is also crystallized from different animals, such as ox muscle, human, monkey, frog, turtle, etc. All enzymes are considered to occur in the cell cytoplasma. It has been found associated with the mitocondria. It may represent 25 - 50 % of total enzyme (8).

## *Structure*

In vertebrata CK occurs in three forms readly distinguishable by their electrophoretic mobility. These were fast, slow and intermediate mobility.

(*) Yrd. Doç. Dr., Yüzüncü Yıl Üniversitesi Veteriner Fakültesi, Biyokimya Bilim Dalı, Van.

It was shown later that the enzyme existed as a dimer. These are called; muscle type (MM), brain type (BB) and hybrid (MB) isoenzymes. Another type CK is CK - AT (atypical CK) and its mobility is between MM and BB (4).

During development, the BB type isoenzyme appeared first in all tissues studied. Atrophy of white muscle fibers whether caused by denervation, vitamin E deficiency or hereditary distrophy, is accompanied by loss of MM type isoenzyme and reapperance of brain from the so called reversion to the fetal state.

The original investigation of the physical properties of CK yielded a molecular wieght of 81.000 and until a detailed reinvestigation produced a new figure 82.600 daltons. But experimentally determined velues for other purified CK fall within the range 78.500 - 85.100 (2, 8).

The enzyme contains two catalytic sites, and two reactive thiol groups per dimer of MW (Molecular weight) is 82.600. In denaturating media-8M urea-CK dissociates into two subunits each consisting of a single polypeptide chain containing no disulfite bond. Detailed studies of tryptic peptides point strongly to an identity of the two polypeptide choin.. The two reactive thiol groups were shown that they both formed part of unique 25 residues tryptic peptide sequence.

Amino acid analysis shows that BB form of enzyme contains significantly less of basic amino acid than the MM form.

Optical rotatory studies indicate a compact globular structure containing 25 - 30 % - helix and less than 15 % B - pleated sheot.

Immunological evidence is in accord with that from studies of the primary structure in indicating that the tertiary organization of the same isoenzyme from different species is more similar than that of the MM and BB isoenzymes of a single species (8).

*Specific Activity*

For forward reaction (Phosphocreatine, PCr aynthesis) a specific activity of 75 unite yield on appearent second order velocity constant (k') equal to 1 ml/μmcl/min.

k, - kE in the reaction dx/dt - kE (ATP) 2 (Creatine)°

the condition are such that the reaction is appearently second order with respect to ATP and the appearent second order rate constant is proportional to enzyme concentration. There are not $V_{max}$ condition.

The pH activity curve shows a broad optimum between 7,5 and 9,5 for the forward direction. For the back reaction shows a lower optimum as might be expected since a proton is taken up at physiological pH the enzyme is only 20 % active.

The fish CK has low specific activity 1/3 to 1/5 that of rabbit. Hybrid MB has similar specific activity to those of parental forme (MM, MB) (8).

*Stability*

After prolonged dialysis against several changes of nonvolatile (giycine) low ionic strength (0.001 - 0.005 I) buffer pH 9.0 rabbit muscle CK shows a remarkable stability. It also had good stability at low temperature (— 18 C and 0 - 4 C).

The isolectric point of CK is 6.1. Physical measuremente at low pH indicate rather complex changes in structure.

Ox brain CK at 35 C inactivation begins at pH 8.0 even in the presence of thiol. At 35 C the range of maximum stability is limited to pH 6,3 - 7,5 and even here 50 % of activity is lost in 30 minutes.

*Substrate Specifiuy*

Of the seven known naturally cocuring substrates for guanidine phosphotransferases only giyoccyaminKe will also act as a substrate for rabbit muscle CK. Othor phosphoryl group acceptor was N-ethyl glucosamine, phosphoarginine, and phosphotaurocymine bind to enzyme as inhibitor but are not substrate. Interaction of the methyl group with the enzyme plays a specific role in the catalytic process (1).

*Nucleotide Substrate*

Although the true subtrate for the CK is the metal-nuclectide complex the limited evindence available on the effecte of $Mg^{+2}$, $Mn^{+2}$ or no metal ion suggests that while the metal ion may effect the extent of binding. It doistn't alter specifity for the nucleotide substrate.

Nucleotide substrate has base, sugar and phosphate chain. Nucleotide triphosphate is essential for the forward reaction and the diphosphate for the back reaction, However, AMP, ATP, pyrophosphate and orthophosphate all bind to the enzyme (8).

*Enzyme Kinetic and Mechanism*

*Effect of metal ions*

For the rabbit muscle CK $Mg^{+2}$, $Mn^{+2}$, $Ca^{+2}$, $Co^{+2}$ have been found to act as activator while $Ba^{+2}$, $Sr^{+2}$, $Ni^{+2}$, $Cd^{+2}$ and $Zn^{+2}$ were either inactive or inhibitory (8). Low levels activity $Sr^{+2}$, $Ba^{+2}$ and $Fe^{+2}$ was also found to activate CK in cerebrospinal fluid (6).

Adding up to 10 times $Fe^{+2}$, $Cu^{+2}$, $Zn^{+2}$ the amount present 0,5 ml. of cerebrospinal fluid, there was no inhibition of CK activity in the case of $Fe^{+2}$, $Cu^{+2}$ and only a silght inhibition in the case of $ZN^{+2}$.

Increasing Mg concertration in the reaction mixture increases the CK activity of cerebrospinal fluid. Addition of EDTA stimulates CK activity of serum by % 10 at 37 C. The activity of CK - BB and CK - MM dissolved imidazole buffer, decreases with increase of Ca, an effect more pronounced for isozyme BB than for MM. In the presence of EDTA the CK activity also decreases gradually with increasing concentration of Ca. (6).

Its well known than Ca act as activator and may replace Mg in the reaction :

Creatine phosphate + Mg - ADP ———→ Creatine + Mg - ATP

The Ca - ADP complex binds more strongly to CK, but gives only about an eight the activity obtained by using Mg - ADP as substrate (6).

The competitive inhibition by free nucleotide with respect to the metalnucleotide substrate has been demonstrated with CK and studies on it have shows that higher concentration of $MgCl_2$ cause noncompetitive inhibition with respect to both MgADP and phoshocreatine (3).

The role of metal ion in the phosphocreatine (PCr) - E - MeADP complex was to polarize the N - P bond of PCr in the transphosphorylation reaction. It was also recognized that binding PCr to the enzyme coulld alter the conformation of the metal - Nucleotide binding site and, consequently the binding of a particular metal - nucleotide complex. (8).

A reinvestigation of the dissociation constant for ATP and the Mg ATP complexes gave pKa values of 3,93 and 6,97, and $pK_{M2}$ volule of 4,88. This means the scheme shows the interrelationship of the possible complexes between ÖTP. $Mg^{+2}$, and $H^{+}$. The important ATP species are Mg - $ATP^{2+}$, $ATP^{-4}$, and $HATP^{-3}$. (Scheme. I) (8).

*Substrate binding*

Under some conditions rabbit muscle CK appears to show a very simple pattern of substrate binding with both nucleotide and guanidine substrate binding to the enzyme in a manner that is independert of the

second substrate. The observed Michaelis constant decreases as the concentration of the second (fixed) substrate is raised giving a family of Lineweaver Burk plots that intersect above the abscissa and to the left the ordinate for all four substrates (shown in the scheme 2) (Table I).

This result indicates that the reaction mechanism is sequential, thus, both, substrates react with the enzyme before either product diss ociates but may be either of the ordered. Theorel - Chance or the rapid equilibrium random type of mechanism, in that scheme K is kinetic constant and Ki is dissociation constant of each substrate from the free enzyme.

A rapid equilibrium random mechanism with two dead - end complexes Cr - E - MgADP and PCr - E - MgATP, would show a pattern of product inhibition in which, for the forward reaction, MgADP would be competitive with MgATP, noncompatitive with creatine and noncompetitive with MgATP. A similar situation would hold for the back reaction (8).

*Effect of temperature*

The effect of temperature on the kinetic parameters of CK is poorly documented. Temperature has only a small effect on Km for both creatine and MgATP over the normal physiological range (8).

*Anion effect*

$SO_4^{2-}$, and $PO_4^{3-}$ ions all acted as competitive inhibitors of phosp hocreatine and noncompetitive inhibitors of MgADP. Hence interaction of the anion with a site on the enzyme that normally bound the transferable phosphoryl group of the substrate was inferred. NaCl is a noncompetitive inhibitor of the reaction. has very little effect in enhancing inhibition by the products (PCr or MgATP) andshas only a slight effect on the Km for ony substrate, and the Ki valus for the varied in the double reciprocal plot.

Electron paramagnetic ressonance measurements showed that $NO^-_3$, $NO^-_2$ and $SCN^-$ had dissociation constant of less than 10mM with $NO^-_3$ having a dissociation constant of less than 1 mM and being much more effective than the other two.

*Equilibrium of reaction :*

Equilibrium constant expresses in terms of the total concentration of each substrate in the reaction mixture, that is :

$$K_{eq} = \frac{(PCr)\ (ADP)}{(ATP)\ (Cr)}$$

$K_{eq}$ can change with pH and $Mg^{2+}$ ar $Mn^{2+}$ ion concentration at constant temperature (8).

Structural features of the purine binding site of CK were explored by $^1H$ NMsR speectrocopy at 360 MHz. Using the measurement of «truncated driven muclear Overhauser effect» (TOE). Irradiation of the adenin C - 2 and C - 8 proton resonances in the CK - ADP complex by this technique resulted in the negative enhancement of a number of ressonances of the protein (intramilecular $NO^E$'s). Independent evidence for an interaction between pruner moity of the coenzyme and aromatic amino acid chromophores comes also from quenching studies of protein flourencence (7).

There is Flow - quench cryoenzymic studies on CK. They studied both pure water and 40 % ethylene glycole (+20°C to O°C). Activation energy was same for both solvent, but 40 % ethylene glycole slow down reaction last two enzymes have break at low temperature but CK does not have any break in the Arrhenius plot (1).

The Michealis constant determination for creatine and ATP were determined at - 15 C using time delay flow-quench apparatus. The values are for creatine 7,5 mM and for ATP 0,2 mM. These are very to values at 4 in pure water (1).

Studies on transient states show three phases :

1 — A transient lag phase
2 — A transient burst phase
3 — Steady state

from this result they assumed three intermediatis, like following :

$$E\text{-}ATP + Cr \underset{\leftarrow}{\overset{KdR}{\longrightarrow}} E.ATP.Cr \underset{K\text{-}2}{\overset{K_2}{\longrightarrow}} E.ATP.Cr \underset{K\text{-}3}{\overset{K_3}{\longrightarrow}} E\ ADP.PCr \xrightarrow{K_4}$$

$$E\text{-}ADP + PCr\ (1, 5).$$

*Thiol group :*

The role of the single reactive cysteine group per subunit, one part of the catalytic site, play stabilization role. Thiol group existens in a par-

tially ionized state that does not change significantly over the whole range of pH in which the enzyme is structurally stable (8).

In the mechanism for the catalytic reaction, a role was suggusted for the essential thich in which it withdrew a proton from the creatine guanidino group and initiated a circular flow of electrons from the guanidino group via the and - phosphorly groups of ATP back to the thiol; thus, transfer of phosphoryl group from ATP to creatine was greatly facilitated (8) (Scheme 3).

*Mechanism of Transphosphorylation*

1 — Each subunit contains one catalytic site.

2 — Each catalytic site contains seperate substrate binding site.

3 — Both substrates bind simultaneously to the enzyme.

4 — The presence of one subtrate on the enzyme enhances the binding of the second substrate, but subrate binding sites are essentially performed. CK is not «induced fit» enzyme.

5 — The metal - nucleotide complex is the true subtrate of the enzyme.

6 — When the nucleotide substrate binds to the enzyme : increases the receptivity of the creatine binding site, and initiation of the catalytic process occur. These are sort of conformational changes.

7 — Enhancement of the binding of the guanidino substrate by the nuclectide requires the 6 - amino group of adenin.

8 — The binding of creatine to the free enzyme produces a conformational changes.

9 — Creatine is bound in the creatine binding site with a planar guanidino group orientated in a highly specific manner.

10 — The three dimensional orientation of the metal - nucleotide substrate in its binding site is also well defined (Scheme 4).

11 — The metal ion is located across the, B phosphoryl groups in the ADP and ATP complexes and is not hydrated in the anion-stabilized dead - and complex.

12 — The nucleotide and guanidino substrates on the catalytic site are oriented in such a way is to allow simple transfer of a phosphorly group from one to another.

13 — In creatine the nitrogen atom of the guanidino group that is Trans to the methyl group acts as the phosphoryl acceptor.

14 — Transphosphorylation involves the conversion of the site that readily binds a tetrahedral anion into one that selectively binds planar anion.

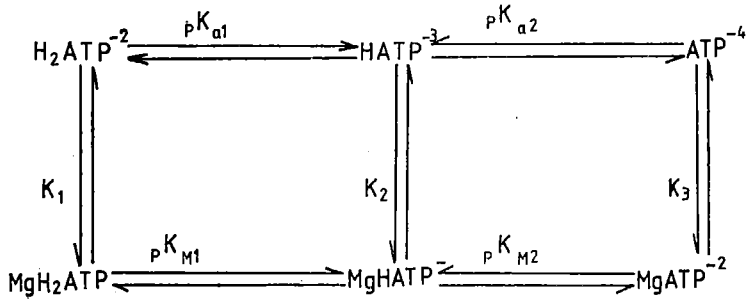
15 — Conformational changes allow the nucletide and guanidine to apprcach other.

16 — The reactivity of the essential thiol groups is closely linked to the conformational changes that accur when both substrates are bound to the enzyme.

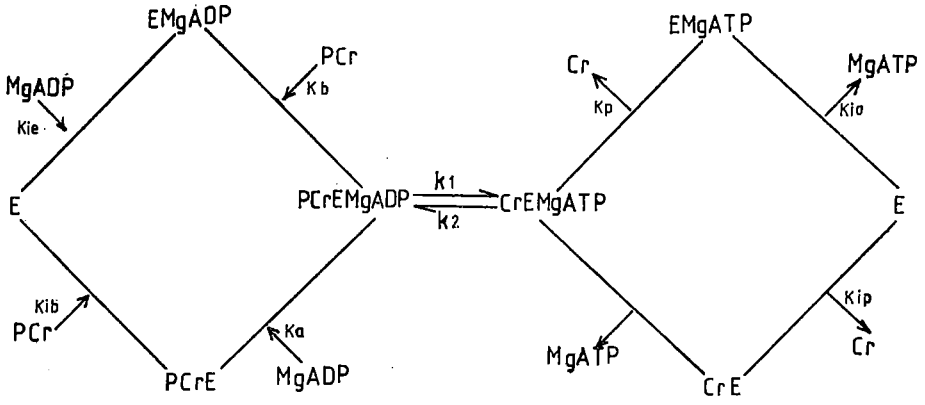
17 — The integrity of a histidine and a lysine side chain are also essential for catalytic activity.

Table I. Kinetic constants for rabbit muscle creatine kinase measured at pH 8.0 and 30 C (8).

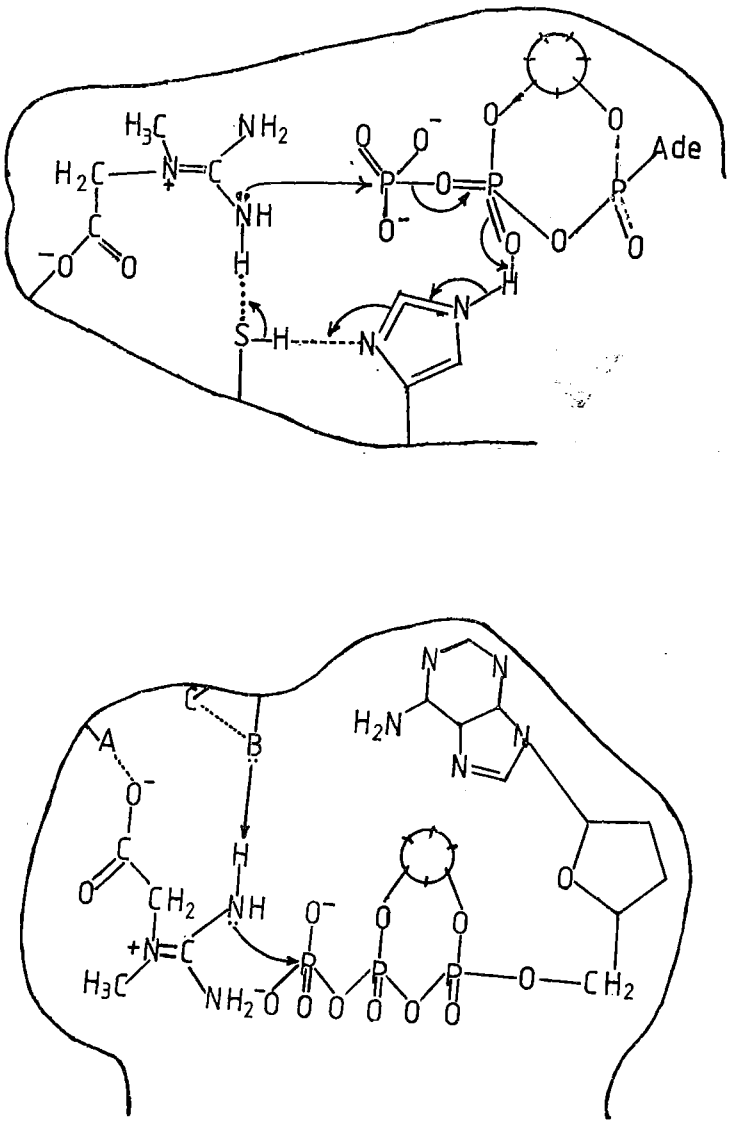
| Kinetic constant | Equilibrium | | Value of constant (mM) |
|---|---|---|---|
| $K_{iq}$ | MgATP + E | EMgATP | 1,2 + 0,3 |
| $K_p$ | Cr + EMgATP | CrEMgATP | 6,1 + 1,0 |
| $K_{ip}$ | Cr + E | CrE | 15,6 + 4,9 |
| $K_q$ | MgATP + CrE | CrEMgATP | 0,48 + 0,10 |
| $K_{ia}$ | MgADP + E | EMgADP | 0,17 + 0,02 |
| $K_b$ | PCr + EMgADP | PCrEMgADP | 2,9 + 0,3 |
| $K_{ib}$ | PCr + E | PCrE | 8,6 + 1,3 |
| $K_a$ | MgADP + PCrE | PCrEMgADP | 0,05 + 0,01 |




SCHEME I



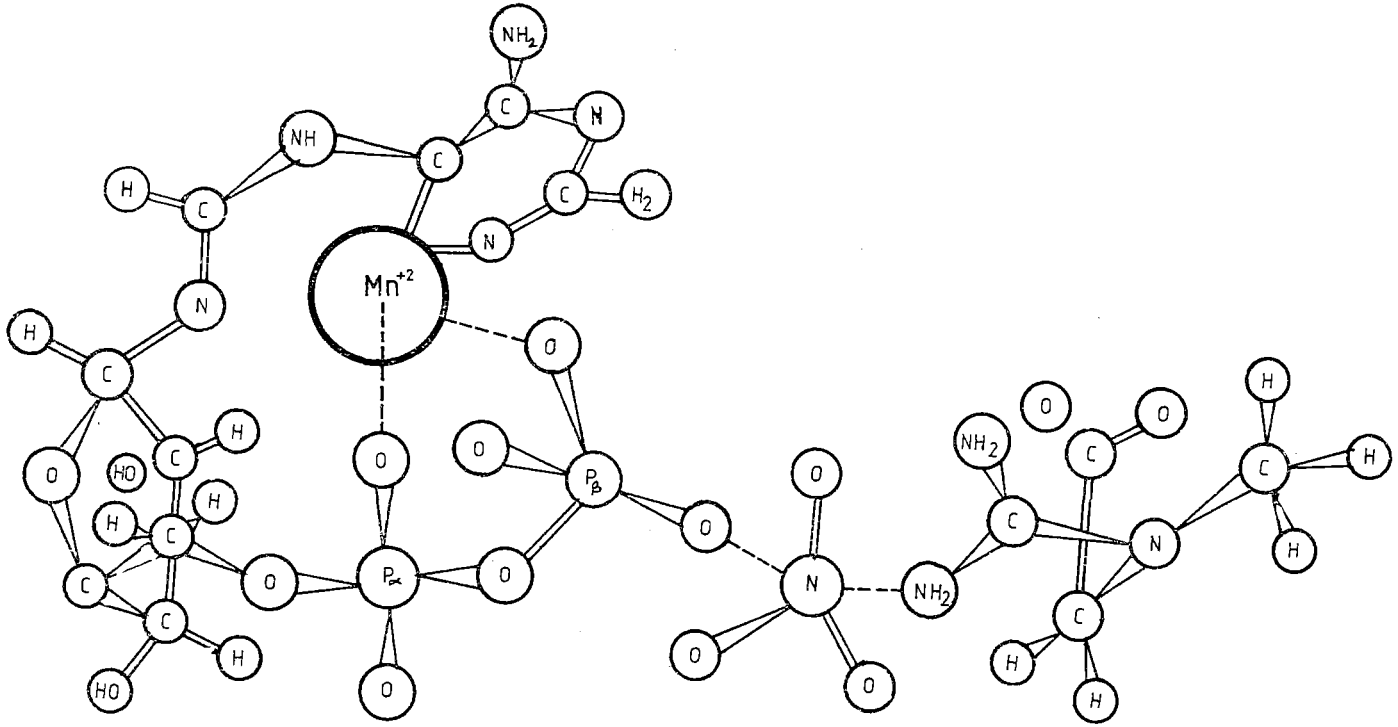

SCHEME II







*SCHEME 3 : Representation of the mechanism of action of creatine kinase incorporating recent findings. Only the initial stage of phosphorylation process is shown in each case since the reaction pathway has not been altered. (Fef. no 8).*




*SCHEME 4 : Perspective drawing of the three - dimensional organisation of the Cr-E-Mn ADP-nitrate complex. (Ref. no 8).*

*REFERENCES*

1 — Barman, T. E., Brun, A., and Traverse F., (1980). A flow - quench apparatus for cryoenzymic studies. Application to the creatine kinase reaction, Eur. J. Biochem., 110, 397 - 403.

2 — Degani, Y., and Degani C., (1979). Subunite - selective chemical modification of creatine kinase. Evidence for asymmetrical association of the subunites. Biochemistry, 18 (26), 5197.

3 — Richterich R., Wiesmann U., and Cuntz, B. (1968). In «Homologous Enzyme and Biochemical Evolution» Eds. N. Van Thoai and J. Roche p. 243, Gordont Breach, New York.

4 — Sax S. M., Moore J. J., Giegel J. L., and Welsh M. (1979). Further observation on the incidence and nature of atypical creatine kinase activity. Clin. Chem., 25/4, 535 - 541.

5 — Traverse F. and Barman E. T. (1980). Cryoenzymic studies on the transition - state analog complex creatine kinase ADP Mg. Nitrate creatine. Eur. J. Biochem. 110, 405 - 412.

6 — Urdal, P., and Stromme, J. H. (1979). Effects of Ca, Mg and EDTA on creatine kinase activity in cerebrospinal fluid. Clin. Chem. 25/1 147 - 150.

7 — Vasak, M., Nagayama, K., Wutnrich, K., Mertens, M. L., and Kagi J. H. R. (1979). Creatine kinase, nuclear magnetic resonance and fluorescence evindence for interaction of adenosine 5' - Diphosphate with aromatic residue (s). Biochemistry, 18, 5050 - 5055.

8 — Watts, D. C. (1973). Creatine Kinase (Adenosine 5' - Triphosphate Creatine Phosphotransferase). In «The Enzymes» Ed. Paul D. Boyer Vol : VIII Part A, 383 - 455.