order process; in either direction, at high reactant concentration, the rate tends to approach a limiting value, indicating that under these conditions a monomolecular process may become rate-limiting. The data might be consistent with a general reaction scheme of the type

$$\text{Cu}^{2+} + \text{Fe}^{2+} = (C_1) \Rightarrow (C_2) \Rightarrow \text{Cu}^{2+} + \text{Fe}^{3+}$$

suggested by the rapid formation of one or more complexes (C) between the two proteins within which electron transfer takes place. Attempts to detect directly the presence of a thermodynamically stable complex between the various forms of the two proteins are presently being carried out.

An explanation of the mechanism of electron transfer between azurin and cytochrome 551, in analogy with lines developed for simple systems (1), would require knowledge of rates of electron exchange between oxidized and reduced forms of each protein. Such rates are not available at present, but experiments are being devised to estimate them.

A more detailed analysis of the kinetics of the system, hopefully including this additional independent information, will be presented in a forthcoming paper.

REFERENCES

Activation of Adipose Tissue Lipase by Skeletal Muscle Cyclic Adenosine 3',5'-Monophosphate-stimulated Protein Kinase*

J. D. Corbin,‡ E. M. Reimann,§ D. A. Walsh, AND Edwin G. Krebs

From the Department of Biological Chemistry, University of California School of Medicine, Davis, California 95616

SUMMARY

A purified rabbit skeletal muscle adenosine 3',5'-monophosphate (cyclic AMP)-stimulated protein kinase enhanced lipolytic activity in adipose tissue homogenates. This effect required the presence of cyclic AMP and ATP and was completely blocked by a protein inhibitor of cyclic AMP-stimulated protein kinases. It is inferred that this effect is due to the phosphorylation and activation of a lipase in a system analogous to that involved in the activation of muscle glycogen phosphorylase.

It is well documented that cyclic AMP* mediates the lipolytic effects of many hormones in adipose tissue (1, 2); however, evidence for the mechanism of action of this nucleotide in stimul-
FIG. 1. The activation of muscle phosphorylase kinase by muscle and adipose tissue cyclic AMP-stimulated protein kinases. Purified phosphorylase kinase (6), 0.1 mg, was incubated in reaction mixtures containing ATP, 0.018 pmole; magnesium acetate, 0.66 pmole; cyclic AMP, 0.1 mpmole; EDTA, 0.05 pmole; sodium glycerol-P, pH 6.8, 0.2 pmole; P-mercaptoethanol, 0.9 *mole; and the protein kinase2 in a total volume of 0.11 ml. The reactions were terminated by diluting the mixtures in cold 0.01 M glycerol-P buffer, pH 6.8, containing 0.045 M β-mercaptoethanol. Phosphorylase kinase activity at pH 6.8 was determined as described previously (5). In Curves A and B, 12 histone units3 of the cyclic AMP-stimulated protein kinase from rabbit skeletal muscle or adipose tissue, respectively, were added. In Curves C and D, 6 histone units of each enzyme were added (same order). Curve E was a control with no added protein kinase.

FIG. 2 (left). Lipolytic activity in adipose tissue homogenates with varying concentrations of rabbit skeletal muscle cyclic AMP-stimulated protein kinase. Reaction mixtures contained potassium phosphate, pH 6.7, 4 pmoles; FFA-free bovine serum albumin (Sigma), 12 mg; cyclic AMP, 5 mpmoles; magnesium chloride, 1.25 mmoles; ATP, 0.6 μmole; 0.1 ml of adipose tissue homogenate; and 1 μg of a purified protein inhibitor of cyclic AMP-stimulated protein kinases (3, 10-12) in a total volume of 0.445 ml. The reactions were initiated by the addition of the homogenates and terminated by pipetting 0.2-ml aliquots of the reaction mixtures into 6 ml of chloroform plus 1 ml of 0.1 M potassium phosphate buffer, pH 6.8, for extraction of FFA and assay by the method of Duncombe (13).

FIG. 3 (right). Effect of excess protein inhibitor on the stimulation of homogenate lipolytic activity by muscle protein kinase. The protein kinase (5.4 × 107 histone units) and 20 μg of protein inhibitor were added where indicated. This amount of protein inhibitor was in addition to the 1 μg routinely added to all reaction mixtures (see text). Other conditions were as described in Fig. 2. The length of the bars represents the range of duplicate determinations.

Fig. 4. Nucleotide specificity of the protein kinase in the stimulation of lipase and the effect of heat denaturation of the enzyme. Heat denaturation of the protein kinase was brought about by placing the enzyme in an oven at 125° for 10 min. The length of the bars represents the range of duplicates in one experiment. Where indicated, 1.9 × 106 histone units of protein kinase were added. Cyclic AMP and 5'-AMP (where added) were 1.5 × 10^-6 M in the reaction mixtures. Other conditions were as in Fig. 2.

Production of FFA from endogenous triglyceride was usually found to be linear with time for at least 1 hour, and the amount of FFA produced in 45 min could be used as a measure of lipase activity in the homogenates. Fig. 2 shows that this activity is increased when purified rabbit skeletal muscle cyclic AMP-stimulated protein kinase is added. In the concentration range tested the enzyme was effective only when cyclic AMP was present in the reaction; this correlates well with the dependency of the enzyme on cyclic AMP in the phosphorylation of casein (8). In the experiment of Fig. 2 and in subsequent experiments the purified protein inhibitor of cyclic AMP-stimulated protein kinases (see above) was routinely included in reaction mixtures in an amount just sufficient to block the activity of any endogenous protein kinase in the fat pad homogenate. This was done with the expectation of being able to accentuate the response that would occur due to addition of the muscle kinase. When the inhibitor was added in excess, it was capable of completely blocking the stimulatory effect of the muscle enzyme on lipolysis (Fig. 3). The effect of the protein kinase on activation of the lipase did not occur when 5'-AMP was substituted for cyclic AMP or when the protein kinase was denatured by heating prior to testing (Fig. 4). In a total of six experiments in which the protein kinase concentration was varied, an average of 1.5 × 106 histone units of protein kinase were sufficient to stimulate the lipolytic activity maximally in 0.1 ml of homogenate. This amount is approxi-
nates are fortified with the cyclic AMP-stimulated protein kinase, a definite enhancement of lipase activity is seen when ATP and cyclic AMP are present. This provides a good basis for further work needed to establish unequivocally the existence of a "lipase b" and a "lipase a" analogous to phosphorylase b and phosphorylase a, respectively. The muscle protein kinase (5.4 X 10^6 histone units) was added where indicated. The length of the bars represents the range of duplicates.

The concept that the hormone-sensitive lipase of adipose tissue may occur in phosphorylated and nonphosphorylated forms has been in existence for a number of years. This was based on evidence that the cyclic AMP effect on lipase activity in homogenates appeared to require ATP (15-17). Conclusive evidence for this point, however, has been difficult to obtain. In the present approach to the problem in which adipose tissue homogenates were fortified with the cyclic AMP-stimulated protein kinase, a definite enhancement of lipase activity is seen when ATP and cyclic AMP are present. This provides a good basis for further work needed to establish unequivocally the existence of a "lipase b" and a "lipase a" analogous to phosphorylase b and phosphorylase a of glycogen metabolism (18).

**REFERENCES**


**Phosphatidylcholine Requirement in the Enzymatic Reduction of Hemoprotein P-450 and in Fatty Acid, Hydrocarbon, and Drug Hydroxylation***

(Received for publication, July 10, 1970)

Henry W. Strobel, Anthony Y. H. Lu, Joanne Heidsma, and Minor J. Coon

From the Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48104

**SUMMARY**

The heat-stable factor required for fatty acid, hydrocarbon, and drug hydroxylation in a reconstituted liver microsomal enzyme system containing hemoprotein P-450 has been identified as phosphatidylcholine. Synthetically prepared dioleoylglycerol-3-phosphorylcholine was fully active when substituted for the microsomal factor, as judged by the rate of laurate, hexane, octane, ethylmorphine, or benzphetamine hydroxylation in the presence of TPNH, oxygen, hemoprotein P-450, and TPNH-hemoprotein P-450 reductase. Various acyl derivatives of glyceryl-3-phosphorylcholine showed, at their optimal concentrations, the following increasing order of activity with benzphetamine as the substrate: distearoyl; a mixture of 1-monopalmitoyl and 1-monostearoyl; dipalmitoyl; dioleoyl; and a mixture of dilauroyl and monolauroyl.

Electron transfer from TPNH to hemoprotein P-450, as determined from the carbon monoxide reduced difference spectrum under anaerobic conditions, was completely dependent upon the presence of microsomal lipid. Stopped flow measurements showed that the rate of electron transfer was biphasic in the presence of the lipid, with a rapid phase largely completed in less than 1 sec and having a first order rate constant of about 100 min^-1, followed by a slow phase having a first order rate constant of about 6 min^-1, but only the slow rate could be detected in the absence of the lipid. Under similar conditions, but with air as the gas phase, the turnover number (moles of benzphetamine hydroxylated per mole of hemoprotein P-450) was 22 min^-1. These results show that the lipid is essential for the enzymatic reaction.

**REFERENCES**

Activation of Adipose Tissue Lipase by Skeletal Muscle Cyclic Adenosine 3',5'-Monophosphate-stimulated Protein Kinase
J. D. Corbin, E. M. Reimann, D. A. Walsh and Edwin G. Krebs


Access the most updated version of this article at http://www.jbc.org/content/245/18/4849

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/245/18/4849.full.html#ref-list-1