A CYCLIC AMP - STIMULATED PROTEIN KINASE IN ADIPOSE TISSUE

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Summary

A protein kinase that is stimulated four- to six-fold by adenosine 3',5'-monophosphate has been partially purified from rat epididymal adipose tissue and isolated fat cells. The enzyme catalyzes the phosphorylation of histone, casein, and phosphorylase kinase. It is postulated that the effect of cyclic AMP on this enzyme is related to its effect on lipolysis and/or other processes in adipose tissue.

Introduction

The stimulation of lipolysis in adipose tissue by adenosine 3',5'-monophosphate (cyclic AMP) is well documented (1,2), but the mechanism of action of this nucleotide at the enzyme level is not clear. An ATP requirement for cyclic AMP stimulation of an adipose tissue lipase has been reported (3), which suggests that a phosphorylation reaction(s) is involved. Furthermore, the likelihood that protein phosphorylation reactions lie behind the various metabolic manifestations of cyclic AMP has been established in studies on the mechanism of activation of phosphorylase in muscle (4,5,6). Experiments in this latter area culminated recently (7) in the discovery of a cyclic AMP-dependent protein kinase involved in activation of phosphorylase kinase. It was of interest, therefore, to determine whether adipose tissue contained a cyclic AMP-stimulated protein kinase.

Materials and Methods

A modification of the previously described method was used for the measurement of protein kinase activity (7). Assay reaction mixtures at pH 6.5

contained: potassium phosphate, 1 μmole; γ^{32}P-ATP, 0.12 μmole; magnesium acetate, 0.36 μmole; cyclic AMP or other nucleotides (where added), 0.2 mμmoles; protein substrates in varying amounts; and enzyme (10-100 μg protein) in a final volume of 0.1 ml. The incubations were carried out at 30°C. Reactions were initiated by addition of a solution of γ^{32}P-ATP and magnesium acetate and terminated by addition of 0.2 ml of bovine serum albumin (6.25 mg/ml) and 1.5 ml of 6.6% trichloracetic acid in rapid succession. The determination of ^{32}P incorporation into protein was carried out essentially as described previously (6). In the absence of added substrates a low level of phosphate uptake took place involving component(s) of the enzyme preparation itself; the extent of this phosphorylation was insignificant with respect to the degree of protein phosphorylation that occurred in the presence of added substrate (<5%) and it was unnecessary to apply corrections for it.

Partial purification of the protein kinase was carried out by the following procedure. Rat epididymal fat pads were removed from 150-200 gm rats and placed in a tared beaker containing 0.15 M KCl at 25°C. A thirty-three percent homogenate (W/V) in 0.15 M KCl was prepared in a hand-glass homogenizer and the homogenate was centrifuged at 50,000 x g at 4°C for 30 minutes. After centrifugation, the fat cake, which contained little protein kinase activity, was discarded. The infranatant fraction was removed and fractionated by the addition of solid ammonium sulfate. The fraction that precipitated between 30 and 45% saturation contained most of the enzyme activity. The enzyme was redissolved in 0.05 M phosphate buffer, pH 6.5, and dialyzed against three changes of the same buffer for twelve hours. The specific activity (μmoles of ^{32}P incorporated/mg protein/min.) of the final enzyme was about ten-fold greater than that of the original homogenate. The enzyme preparation contained no detectable phosphorylase kinase activity.

Isolated fat cells were prepared essentially as described by Rodbell (8). After washing the cells, they were homogenized and the enzyme was purified in the same manner as described for fat pads. Phosphorylase kinase was prepared

according to the method of DeLange et al (6). The casein used was the vitamin-free preparation from Nutritional Biochemicals Corporation and the histone was calf thymus Type II-A from Sigma Chemical Company. Cyclic nucleotides were a gift from Boehringer Mannheim Corporation.

Results

The phosphorylation of histone by the adipose tissue protein kinase in the presence and absence of cyclic AMP is shown in Figure 1. Although some phosphorylation of histone occurred in the absence of cyclic AMP, the

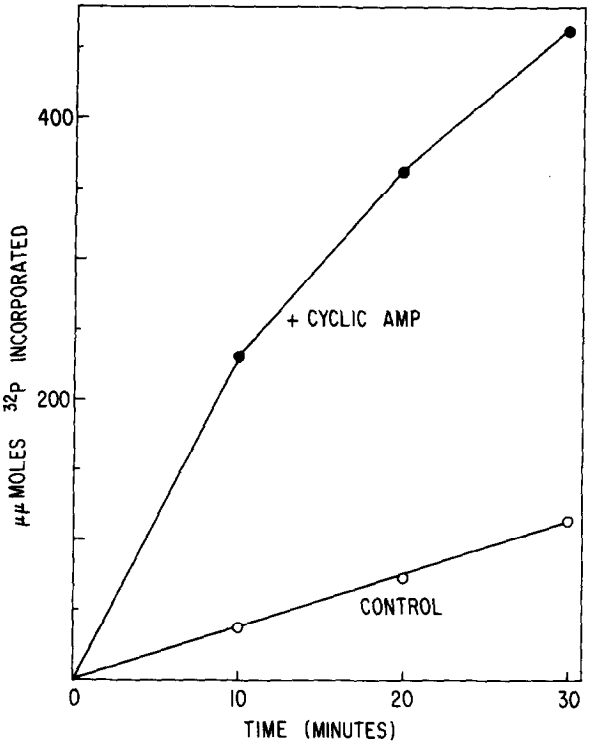

Figure 1 -- The effect of incubation time on cyclic AMP stimulation of adipose tissue protein kinase activity. Conditions and reaction mixtures as described in the text. The substrate was histone, 6 mg/ml. The undiluted 50,000 x g supernatant fraction (See Materials and Methods) was the source of the enzyme. The cyclic AMP concentration was 2×10^{-6}M.

nucleotide had a five-fold stimulatory effect on the reaction. The cyclic AMP concentration employed in this experiment was comparatively high (2×10^{-6}M),

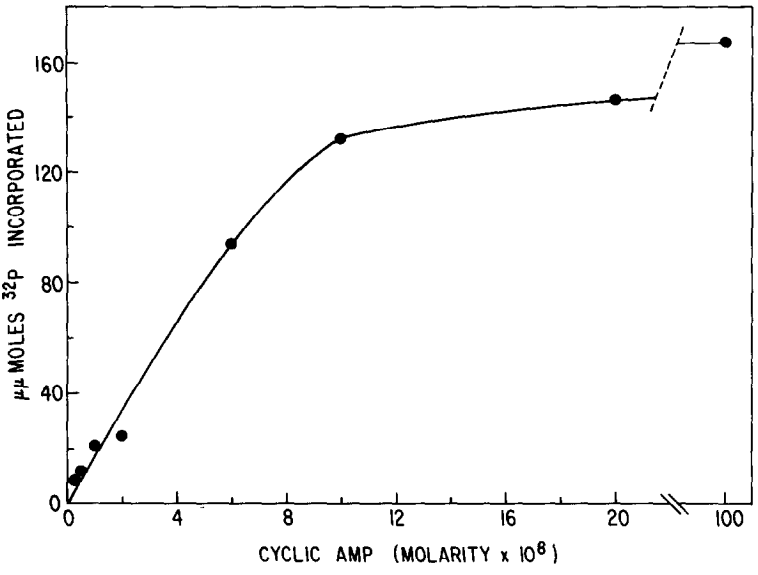

Figure 2 -- The effect of cyclic AMP concentration on adipose tissue protein kinase activity. Incubation time was ten minutes. Other conditions were as described in Figure 1.

but it has been observed that the protein kinase is very sensitive to this nucleotide, half maximal stimulation being obtained at a concentration of about 5×10^{-8}M (Figure 2).

The stimulation of the protein kinase by cyclic AMP showed a high degree of specificity. One other nucleotide, inosine 3',5'-monophosphate (cyclic IMP), did, however, stimulate the enzyme significantly. For example, at a concentration of 2×10^{-6}M, cyclic IMP was 80% as effective as cyclic AMP at this concentration. Half maximum stimulation by cyclic IMP occurred at approximately 4×10^{-7}M. Chromatographic examination of the cyclic IMP sample ruled out the possibility that its effect could be due to contamination with cyclic AMP. N^6-2'O-dibutyryl adenosine 3',5'-monophosphate, guanosine 3',5'-monophosphate, and uridine 3',5'-monophosphate had some stimulatory effects but the possibility of slight contamination of these substances with cyclic AMP could not be ruled out as an explanation of the stimulation observed. Nucleotides that showed little or no activity at 2×10^{-6}M were thymidine

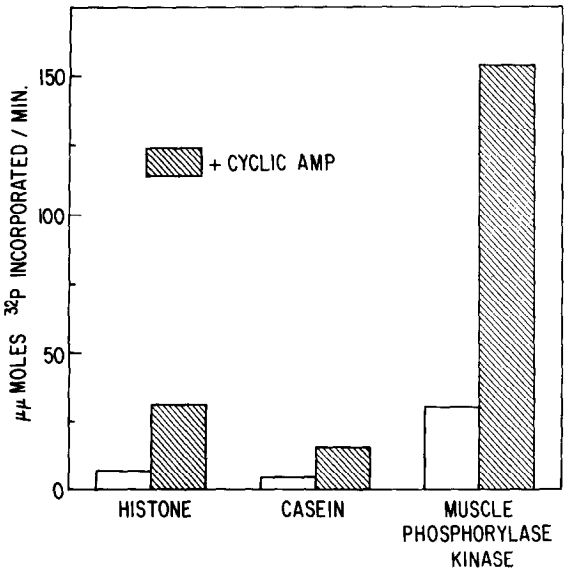

Figure 3 -- A comparison of substrates for adipose tissue protein kinase. The substrate concentrations were 0.92 mg/ml. Incubation time was one minute. Other conditions were as described in Figure 1. Phosphorylation of phosphorylase kinase was appreciable in the absence of added adipose tissue protein kinase and cyclic AMP had a stimulatory effect; however, the numbers in the figure have been corrected for the endogenous rates of phosphorylation.

3',5'-monophosphate, 2'-deoxyadenosine 3',5'-monophosphate, cytidine 3',5'-monophosphate, adenosine 3'-monophosphate, inosine 5'-monophosphate, uridine 5'-monophosphate, and adenosine 5'-monophosphate.

In addition to histone, the adipose tissue protein kinase was active toward casein and phosphorylase kinase. Figure 3 shows the rate of phosphorylation of these proteins in the presence and absence of cyclic AMP under identical conditions of protein concentration (mg/ml). The most rapidly phosphorylated substrate was phosphorylase kinase, and, indeed, its initial rate of phosphorylation may be even faster than that shown since in this instance the reaction was decreasing with time, even at the one minute point. The stimulatory effect of cyclic AMP was three- to five-fold with all three substrates tested.

The effect of agents on rat epididymal fat pads may be qualitatively

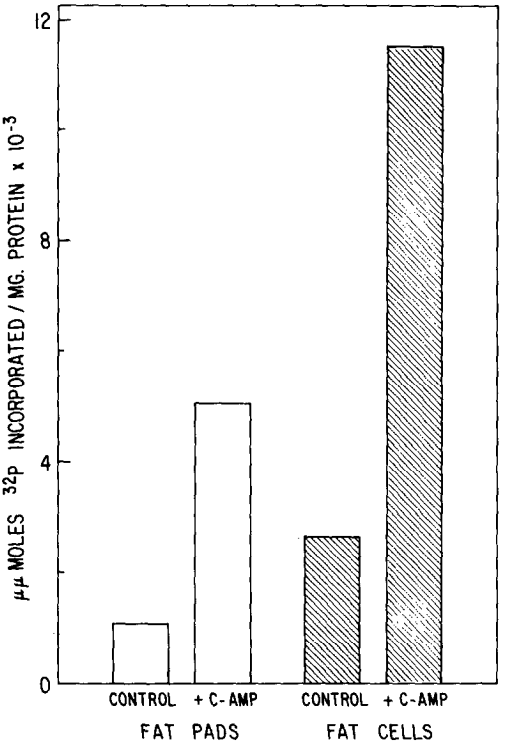

Figure 4 -- A comparison of protein kinase activity in epididymal fat pads and isolated fat cells. Incubation time was ten minutes. Both enzymes were the supernatant fractions from 50,000 x g centrifugation of the homogenates (See Materials and Methods). Other conditions were as described in Figure 1.

different from effects in isolated fat cell preparations (9). This may be due to the fact that fat cells comprise only about one-third of all cell types in the fat pads (10). In order to establish that the protein kinase activity existed in the relatively pure isolated fat cells, a comparison of enzyme activity of partially purified preparations from fat pads and isolated fat cells was made (Figure 4). In the presence or absence of cyclic AMP the specific activity of the enzyme was three-fold higher in the preparation from isolated fat cells, indicating that most of the protein kinase activity in fat pads is probably localized in the fat cells themselves.

To date, the properties of the cyclic AMP-stimulated adipose tissue protein kinase appear to be similar to those of the skeletal muscle protein

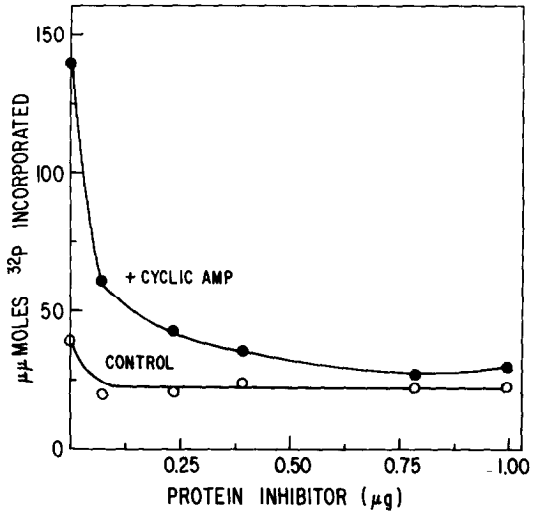

Figure 5 -- The effect of a muscle protein inhibitor of phosphorylase kinase kinase on adipose tissue protein kinase activity. The ten-fold purified preparation (See Materials and Methods) was the source of the enzyme. Incubation time was ten minutes. Other conditions were as described in Figure 1.

kinase described by Walsh et al (7). It was of interest, therefore, to test whether a muscle protein which inhibits the latter kinase would also effect the adipose tissue protein kinase.[1] The results of such an experiment are shown in Figure 5. As can be seen, the muscle protein was an effective inhibitor of the adipose tissue enzyme in the presence of cyclic AMP, and with increasing concentrations depressed its activity to the level seen in the absence of cyclic AMP. The muscle protein inhibitor was also effective when casein was used as the substrate.

The partially purified adipose tissue protein kinase retained its activity if stored in an ice bath for as long as two weeks. It had a pH optimum of 6.5 and was inhibited by increasing ionic strength.

[1]The protein inhibitor (11-13) was kindly supplied by Dr. Donal A. Walsh of this department; it was purified from rabbit muscle through the Sephadex G-75 step of a procedure to be described in a forthcoming paper.

Discussion

Since a major function of adipose tissue is the release of triglyceride fatty acids and glycerol, and since this process has been shown to involve cyclic AMP (1,2), it is of interest to speculate as to whether the cyclic AMP-stimulated protein kinase described in this study has a role in the control of this process. The localization of the kinase in the fat cells themselves and the report of an ATP requirement for the activation of an adipose tissue lipase (3) lend support to this idea. By analogy with the role of cyclic AMP in its effect on glycogenolysis in muscle, phosphorylation of a lipase by a cyclic AMP-dependent kinase would appear to be a reasonable hypothesis, since in the regulation of glycogenolysis a cyclic AMP-dependent protein kinase has been identified as the initial link in a cascading series of protein phosphorylation reactions (7,14).

The adipose tissue kinase has been purified only partially, and the preparation may well contain more than a single protein kinase at this stage. Hence, any studies relative to its specificity must be viewed as preliminary. It is thus too early to state whether a single cyclic AMP-dependent enzyme or several might be involved in the different metabolic control systems that are believed to depend on enzyme phosphorylation in this tissue. The latter include the postulated lipase system, phosphorylase kinase activation, and the conversion of glycogen synthetase from the I to the D form, assuming that the latter two reactions occur in adipose tissue as well as in other tissues where they have been studied (7,15). The fact that muscle phosphorylase kinase was the best of the three protein substrates tested could be interpreted as meaning that phosphorylase kinase in contrast to histone and casein is one of the natural substrates for the enzyme.

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