enzyme, heat sensitive and stable for more than one year when stored as the acetone powder at 4° over calcium chloride. Up to 80% enzyme inactivation was obtained by prolonged dialysis at 0–4°. Addition of 0.01 *M* Mn^{++} or Mg^{++} restored 92 and 70% of the lost activity, respectively. The optimum *p*H was 7.5.

Ketopentose was determined by the cysteine–carbazole method,[5] by the use of $FeCl_3$–orcinol spectra, and by paper chromatography in several solvent systems. An equilibrium of 16% D-xylulose and 84% D-xylose was reached in 3 hr. at 27° under the conditions used. However, this equilibrium was shifted to 81.5% D-xylulose and 18.5% D-xylose by the addition of borate (0.062 *M*) to trap the ketopentose. The isomerase reaction did not require phosphate and could be carried out aerobically or anaerobically. The enzyme was not inhibited by iodoacetate, fluoride, azide or β-methyl-D-xyloside. A typical example of the isomerase reaction showing the rate of ketopentose formation anaerobically (without added borate) is given in Table I.

TABLE I

RATE OF CONVERSION OF D-XYLOSE TO D-XYLULOSE BY XYLOSE ISOMERASE

Reaction components per 3-ml. aliquot were: acetone powder 30 mg., D-xylose 80 mg., NaF 0.02 *M*, $MgCl_2$ 0.0033 *M*, $NaHCO_3$ 0.02 *M* in 95% N_2 + 5% CO_2 at 27°. Proteins and nucleic acids were precipitated with trichloroacetic acid and protamine sulfate. Supernatant containing the reaction product was treated with cysteine-carbazole and after 1 hr. at room temperature the resulting colors were read spectrophotometrically.

Enzymatic reaction time, min.	% D-xylose converted to D-xylulose
0	0.00
10	4.25
30	9.00
60	12.00
90	14.85
120	15.50
180	16.25
240	16.00

Paper chromatograms run with phenol–water (4:1),[6] benzyl alcohol–acetic acid–water (3:1:3)[7] and toluene–dioxane–water (4.17:12.5:1) all resulted in good separations of control D-xylulose,[8] D-ribulose,[9] D-xylose and D-ribose. With each solvent system, the product of the enzymatic reaction, freed from D-xylose by bromine oxidation followed by removal of the resultant xylonic acid with Amberlite IRA 400 resin, had an R_f value identical with authentic D-xylulose. The reaction product gave no ribulose spot and did not exhibit the characteristic spectrophotometric peak for ribulose at 540 mμ in the $FeCl_3$–orcinol reaction.

Positive identification of the product of the isomerase reaction as D-xylulose was effected as follows: the reaction product was freed of protein,[10] adjusted to *p*H 7.5, concentrated *in vacuo* (at 30–40°)[11] to a small volume which was then transferred to a cellulose column[12,13] and the components separated using water-saturated butanol as the mobile phase. A modified cysteine–carbazole spot test was used to identify the effluent ketopentose. When combined, the ketopentose fractions consisted of pure sirupy D-xylulose having $[\alpha]^{25}D$ −32.2° (*c* 2.7% in H_2O)[14] and gave a crystalline *p*-bromophenylhydrazone, melting at 128–129° (uncor.) which was unchanged on admixture with an authentic sample.[14]

In view of our previous report[4] on the enzymatic phosphorylation of D-xylose, the existence of xylose isomerase raises the question whether the substrate in the phosphorylation is D-xylose, D-xylulose or both sugars. Conclusive evidence is not yet available and this phase of the problem is under investigation.

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DIVISION OF APPLIED BIOLOGY
NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA
ROLF M. HOCHSTER
R. WADE WATSON

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ON THE ENZYMATIC MECHANISM OF COENZYME A ACETYLATION WITH ADENOSINE TRIPHOSPHATE AND ACETATE[1]

Sir:

The activation of acetate was recently partially clarified when it was found that this reaction involved a pyrophosphate split of ATP, indicating the over-all reaction

$$ATP + HS\cdot CoA + acetate \rightleftharpoons AMP + PP + acetyl\sim S\cdot CoA \quad (1)$$

As intermediary, an S-phosphoryl derivative had been suggested as initial product of a reaction ATP and CoA.[2,3] More recent work in our laboratories, however, made such a mechanism more and more unlikely, suggesting, rather, mechanisms involving enzyme-bound intermediaries.[4,5] A considerable clarification of a mechanism of this type appeared to be possible by the use of isotopes. Through testing for exchange with labelled pyrophosphate as well as labelled acetate, it appeared possible to obtain a rather precise information on the intermediate course of the reaction.

Radioactive pyrophosphate was prepared by

(1) The following abbreviations are used in this communication: ATP = adenosine triphosphate; HS·CoA = sulfhydryl-coenzyme A; CoA = coenzyme A; AMP = adenosine monophosphate; PP = pyrophosphate; ex = enzyme, whereby "x" may represent the group which takes part in the chemical reactions; and Ad = adenosine. This investigation was supported in part by research grants from the U. S. Public Health Service, from the Rockefeller Foundation, and from the Atomic Energy Commission.
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heating to red heat radioactive K_2HPO_4; purified yeast enzyme was used for the exchange experiments. As shown in Table I, pyrophosphate exchanged with the pyrophosphoryl group in ATP very rapidly and, significantly, in the absence of CoA. This observation excluded immediately a CoA pyrophosphate as an intermediary. Instead, it indicated an initial reaction between ATP and enzyme, resulting in an AMP∼enzyme link with liberation of inorganic pyrophosphate.

TABLE I

PYROPHOSPHATE EXCHANGE BETWEEN RADIOACTIVE INORGANIC PYROPHOSPHATE AND ATP WITH YEAST ENZYME

Enzyme, units	CoA, μM	Pyrophosphate cts./min./ml.	Pyrophosphate cts./min./μM	ATP cts./min./ml.	ATP cts./min./μM	Exchange, %
...	..	97,100	29,400	140	60	0.3
10	..	68,600	21,100	28,270	13,400	74.3
10	1	79,700	23,100	16,350	7,680	44.7
10	2	80,700	24,700	13,920	6,360	36.8

The vessels were incubated at 37° for 20 minutes. Each vessel contained in 1 ml.: 3.4 μM P^{32}-potassium pyrophosphate buffered at *p*H 7.5; 2.2 μM ATP; 50 μM KF; 10 μM $MgCl_2$; 20 μM H_2S; 200 μM tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 in addition to the enzyme and CoA as noted above. The enzyme fraction used was purified 11-fold over the original extracts from quick-frozen baker's yeast. The separation of ATP and pyrophosphate was carried out by charcoal adsorption of ATP in the manner described by Crane and Lipmann.[6]

As shown in the last two lines of Table I, CoA inhibits the ATP ⇄ PP exchange proportional to concentration. This indicates that AMP∼enzyme subsequently exchanges AMP for CoA. Therefore, the presence of CoA decreases the concentration of AMP∼enzyme and thereby slows down the rate of exchange with pyrophosphate. The correctness of this mechanism is further suggested by the results of the exchange of isotopic acetate with acetyl CoA. The data of Table II demonstrate the last step of the sequence to be an exchange of enzyme∼S·CoA with acetate to form acetyl ∼ S·COA and free enzyme. The combined results prompt us to propose the following sequence of reactions:

$$\text{Ex} + \text{Ad-P}\sim\text{PP} \rightleftharpoons \text{Ex}\sim\text{P-Ad} + \text{PP} \quad (2)$$

$$\text{Ex}\sim\text{P-Ad} + \text{HSCoA} \rightleftharpoons \text{Ex}\sim\text{SCoA} + \text{Ad—P} \quad (3)$$

$$\text{Ex}\sim\text{SCoA} + \text{CH}_3\text{COOH} \rightleftharpoons \text{Ex} + \text{CH}_3\text{CO}\sim\text{ScOA} \quad (4)$$

It is noteworthy that, as shown in the last two lines of Table II, addition of AMP does not appreciably influence the rate of acetyl∼CoA ⇄ acetate exchange. It therefore seems that the bond between enzyme and CoA is less energy-rich than the AMP∼enzyme bond. This is confirmed further through the inhibition by CoA of the ATP ⇄ PP exchange which indicates that CoA favorably competes with AMP for the enzyme. In other words, a fall in free energy occurs in the direction from AMP∼enzyme to CoA·S∼enzyme. The enzyme used in these experiments was practically free of Mg. Through exchange experiments with and without magnesium we were able to show that magnesium is involved, in the ATP-enzyme reaction, but is not involved in the enzyme∼CoA-acetate reaction.

Although no definite suggestions can be made at the present time with regard to the grouping on the enzyme which binds AMP and CoA·SH, it is attractive to presume the group is enzyme-bound phosphate. In such a case, the primary reaction between enzyme and ATP would closely resemble the type of reaction described by Kornberg,[7] namely, a pyrophosphate split of ATP with simultaneous formation of a pyrophosphate bridge from the residual AMP to another molecule. Furthermore, the interchange of AMP and sulfhydryl-CoA then would lead to enzyme-phosphoryl∼S·CoA. It seems rather attractive, on the other hand, to speculate that the here-observed formation of an enzyme-mononucleotide may well foreshadow this as a rather general biosynthetic mechanism involved, for instance, possibly in nucleic acid synthesis.

TABLE II

EXCHANGE OF ACETATE BETWEEN $CH_3C^{14}OOH$ AND ACETYL CoA WITH YEAST ENZYME

Enzyme, units	AMP μM	Acetate cts./min./ml.	Acetate cts./min./μM	Acetyl CoA cts./min./ml.	Acetyl CoA cts./min./μM	Exchange, %
...	..	77,900	39,000	108	0.6	0.003
20	..	58,100	26,800	16,830	10,650	53.3
20	5	56,400	26,000	16,900	10,700	54.6

The vessels were incubated at 37° for 40 minutes. Each vessel contained in 1 ml.: 1.58 μM acetyl CoA; 2.17 μM C^{14}-acetate; 10 μM $MgCl_2$; 200 μM tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5 in addition to the enzyme and AMP as noted above. The enzyme fraction used was the same as in Table I. *Note that, compared with the experiment of Table I, twice as much enzyme and double the incubation time was used here.* Reaction 4, therefore, is considerably slower than reaction 2. The separation of acetyl CoA and acetate was carried out by charcoal adsorption of acetyl CoA in a manner similar to that used for separation of ATP and pyrophosphate.[6] Acetate activity was determined in the supernatant of the charcoal adsorbate. Acetyl CoA was decomposed on the charcoal with hydroxylamine and the hydroxamic acid activity measured in the supernatant.

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BIOCHEMICAL RESEARCH LABORATORY, MARY ELLEN JONES[8]
MASSACHUSETTS GENERAL HOSPITAL AND THE
DEPARTMENT OF BIOLOGICAL CHEMISTRY FRITZ LIPMANN
HARVARD MEDICAL SCHOOL
BOSTON, MASSACHUSETTS

CHEMISCHES LABORATORIUM DER UNIVERSITÄT MÜNCHEN
BIOCHEMISCHE ABTEILUNG HELMUT HILZ
GERMANY FEODOR LYNEN

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(8) Postdoctoral fellow of the Atomic Energy Commission.

A THEORY OF A CERTAIN TYPE OF IRREVERSIBLE POLAROGRAPHIC WAVE

Sir:

The present study was undertaken in an attempt to elucidate polarographic processes in which, regardless of the fact that the electron transfer reaction is faster than the diffusion rates, "irreversible" slopes of the plot of log $(i_d - i)/i$ against the potential[1] are obtained. This type of polarographic process belongs to a group in which the product of

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