set the result equal to zero, giving $dR/R = \cot i\ di$. From the *American Ephemeris* (*8*) we may obtain *i*, *di*, *R*, and *dR* tabulated for various dates. Then by substituting we obtain the time when any shadow, as seen from Earth, subtends its maximum angle. We find that this occurs on February 25.3 (Universal Time). A second such occurrence, after opposition, is on June 11.1.

Around the two periods of optimum visibility there occur intervals of "good" visibility, defined by $r > 0.7\ r$ (second maximum). For the coming opposition these intervals are from February 1.7 to March 15.0 and from May 9.2 to September 3.0, 1967.

Up to this point we have considered only the Earth-Mars geometry as a criterion for visibility of Martian relief. However, there are certain complicating factors that should also be considered. Among these are light scattering and contrast vitiation by the Martian atmosphere. The scattering and contrast vitiation will be proportional to the Martian air mass along the line of sight, or sec β, where β is the zenith distance of Earth as seen from the top of the prominence. For β we may substitute $90°-(i + \gamma)$. Then atmospheric contrast vitiation will be proportional to sec $[90°-(i + \gamma)]$, which is csc $(i + \gamma)$. The subtended angle of the shadow α is also proportional to csc γ. Therefore, for any value of *i* the reduced contrast with decreasing γ just balances the increase in visibility due to shadow length. The decreasing contrast with decreasing *i*, however, makes it clear that observations made near opposition are not likely to succeed.

Another complicating factor is the frequent presence of morning haze along the sunrise terminator and the less-frequent evening haze along the sunset terminator. Both the morning and evening haze can be avoided by observing well away from opposition. Also the greater frequency of morning haze favors observations made before opposition.

With all these factors considered, probably the best times for observing Martian relief during the coming opposition will be between 1 February and 1 March and between 1 June and 15 August 1967.

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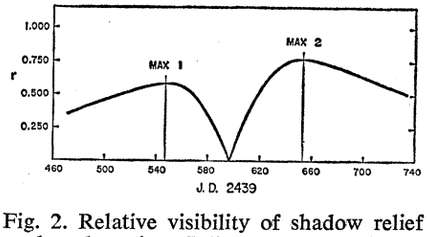

Fig. 2. Relative visibility of shadow relief *r* plotted against Julian Date. Maximum relative visibility before opposition occurs at J. D. 2,439,546.8 which is February 25.3, 1967. Similarly, maximum relative visibility occurs at J. D. 2,439,652.6, June 11.1.

References and Notes

1. J. E. Mellish, private communication.
2. ———, *Sky and Telescope* **31**, 339 (1966).
3. A. Dollfus, *Ann. Astrophys.* (Paris) **28**, 722 (1965); ———, in *Planets and Satellites*, G. P. Kuiper and B. M. Middlehurst, Eds. (Univ. of Chicago Press, Chicago, 1961), pp. 534–571; J. H. Focas, *Ann. Astrophys.* **24**, 309 (1966).
4. W. K. Hartmann, *Icarus* **5**, 565 (1966).
5. G. N. Katterfeld, *Izv. Vses. Geogr. Obshch.* (*Izvestiya All Union Geogr. Soc.*) **91**, 272 (1959).
6. A. B. Binder, *Science* **152**, 1053 (1966).
7. I thank E. A. Whitaker for assistance in checking the above calculations.
8. *American Ephemeris and Nautical Almanac, 1967* (U.S. Government Printing Office, Washington, D.C., 1965).

17 January 1967

Aldolase Reaction with Sugar Diphosphates

Abstract. *Xylulose-, fructose-, and octulose-diphosphates are substrates for rabbit muscle aldolase with essentially identical* K_m *values, but they are cleaved at different rates. After treatment with carboxypeptidase, chymotrypsin, or subtilisin, aldolase cleaves all of these substrates at the same (deceased) rate; the modified aldolase preparations are also equally impaired in their ability to catalyze the detritiation of specifically labeled dihydroxyacetone phosphate. These results suggest that aldolase exhibits "induced fit," in which the rate of cleavage is determined by the distance between the sites on the protein to which the two phosphate groups of a substrate are bound. The activity of the modified aldolases is limited by a step involving making or breaking a carbon-hydrogen bond.*

Rabbit muscle aldolase cleaves fructose-1,6-diphosphate (FDP) or several analogs reversibly to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate or corresponding aldehydes (*1*). In the absence of an aldehyde, aldolase catalyzes a stereospecific exchange of one hydrogen atom of DHAP with the hydrogen of water (*2*). Drechsler *et al.* (*3*) showed that the cleavage of FDP is reduced to about 5 percent of the original rate when aldolase is treated with carboxypeptidase, and Rutter *et al.* (*4*) showed a simultaneous decrease in the rate of liberation of tritium from labeled DHAP to about 0.1 percent. These effects of carboxypeptidase treatment were interpreted by Rose *et al.* (*5*) to be caused by an alteration in the ability of aldolase to catalyze the partial reaction in which the carbon-hydrogen bond of DHAP is made or broken; this step becomes rate-limiting after carboxypeptidase treatment.

Among the many aldehydes that can replace glyceraldehyde-3-phosphate in the condensation reaction are glycolaldehyde phosphate and ribose-5-phosphate, which produce xylulose-1, 5-diphosphate (XDP) and an octulose-1,8-diphosphate (ODP), respectively. Xylulose-diphosphate and ODP were synthesized by aldolase with either FDP (plus triose phosphate isomerase) or DHAP with glycolaldehyde phosphate and ribose-5-phosphate. The condensation products were purified by chromatography on columns of Dowex-1 formate according to the procedure of Bartlett (*6*). The fractions containing substances that gave a positive reaction for a substrate in the spectrophotometric assay with aldolase, glycerophosphate dehydrogenase, and reduced diphosphopyridine nucleotide (DPNH) were pooled and freed from the ammonium formate buffer by passage through a column of Dowex-50-H^+ followed by extraction with ether. The sugar components were identified as pentose by the reaction with orcinol (*7*) or octulose by the reaction with cysteine and H_2SO_4 (*8*). Purity of the sugar diphosphate was established by the presence of two organic phosphate groups per potential DHAP in the spectrophotometric assay.

The longer and shorter analogs were compared with FDP as substrates for aldolase by determination of K_m and V_{max} values (*9*). Contrary to expectation, the data of Table 1 show that the distance between phosphate groups makes no significant difference in the binding of the three substrates

Table 1. Aldolase reaction with sugar diphosphates.

Substrate	Native aldolase		Carboxypeptidase-modified aldolase	
	K_m	V_{max} (μmole min^{-1} mg^{-1})	K_m	V_{max} (μmole min^{-1} mg^{-1})
XDP	$5 \times 10^{-5}M$	1.9	$5 \times 10^{-5}M$	0.38
FDP	$3 \times 10^{-5}M$	6.3	$3 \times 10^{-5}M$	0.31
ODP	$2 \times 10^{-5}M$	0.78	$6 \times 10^{-5}M$	0.375

to the enzyme. Ginsburg and Mehler (*10*) have shown by equilibrium dialysis measurements that aldolase has three pairs of binding sites for phosphate and that each pair binds a molecule of FDP. The K_m determined kinetically is essentially identical to the dissociation constant calculated from binding studies. In contrast to the identity of the binding of the three sugar diphosphates, as shown in the table, the analogs are split at slower rates than FDP.

When carboxypeptidase-modified aldolase was studied with these substrates, the K_m values were found to be essentially unchanged, but the V_{max} values were all reduced to a common value, about 5 percent of the V_{max} for FDP with native aldolase.

These results permit two conclusions to be drawn. First, they confirm the interpretation of Rose *et al.* (*5*) that the rate-limiting reaction with native aldolase precedes the formation of an enzyme • DHAP complex and that C—H bond making or breaking in the formation of DHAP becomes limiting after carboxypeptidase treatment. In the case of FDP cleavage it was shown by Rose *et al.* that the breaking of the C—C bond was not affected by carboxypeptidase treatment, since the rate of exchange of the aldehyde portion with glyceraldehyde-3-phosphate was not changed.

Assuming that all of the substrates react by a common mechanism, it would be expected that the rates of the cleavage step for XDP and ODP would be similarly unchanged, but that the net reactions would be limited by the C—H step, as shown for FDP. The substrates that react at different rates with native aldolase participate in analogous but different rate-limiting reactions; after modification of the enzyme they produce the same enzyme-DHAP complex, which dissociates through the same rate-limiting step.

Second, the data indicate that the induced-fit concept of Koshland (*11*) applies to aldolase. The structures of the three substrates are identical at the first four carbon atoms, which include all of the structures required for reaction. The only differences are in the length of the sugar, which can determine the position of the second phosphate group. Since this group is important in binding the substrate to the enzyme at a specific site, it may be considered that the location of the second binding site relative to the first is not fixed, but is determined by the substrate. The velocity of the rate-limiting step in the reaction of the native aldolase is then determined by the conformation of the enzyme produced by combination with the substrate.

Since substrates that lack a second phosphate group (fructose-1-phosphate, for example, with a K_m about 100 times that of FDP) are split by aldolase, the conformation produced by binding at two sites cannot be considered to be necessary for enzyme activity, but the change in protein structure caused by binding of the second phosphate must bring at least one group into proximity to the substrate to facilitate reaction.

An alternative explanation for data such as those of Table 1 was suggested by Jencks (*12*), who proposed that molecules of different sizes could bind to an enzyme with the same K_m by undergoing different "strains," and that the increased binding energy of the favored substrates is balanced by the energy required to induce strain and thereby reduces the activation energy of the catalytic reaction. While this explanation might apply to the comparison of FDP and ODP, it is difficult to apply it to the case of XDP; XDP has only five carbon atoms, four of which bear substituents essential for reaction with the enzyme and the fifth of which bears the second phosphate group that participates in binding. If aldolase were a rigid molecule with binding sites for phosphate that could be bridged by XDP, the rate of cleavage of XDP would be expected to be at least as fast as that of FDP, which has more flexibility because of its extra carbon.

Another possibility to be considered is the suggestion of Hartman and Barker (*13*) that several anomeric forms of the substrates may exist and, as analyzed by Rose (*14*), if only one of these is a true substrate, the V_{max} measured may not be a true value because of the presence of a form of the substrate that acts as a competitive inhibitor. If such forms indeed exist, they should inhibit the reaction with modified aldolase as well as that with the native enzyme. Also, the forms to be considered as possible alternatives for the sugar diphosphates are open chains and cyclic hemiacetals. Since XDP can exist only as an extended chain, the enzyme can see only one (reactive) form of this substrate, which, however, is split more slowly than FDP, which can exist in a furanose ring. It is concluded, therefore, that the rates of reaction observed with native aldolase represent the rates of cleavage of the C—C bond and that this rate is modified by the conformational changes in aldolase as it adjusts to substrates of different chain lengths. When aldolase is modified by carboxypeptidase, the C—H bond making or breaking becomes rate-limiting and differences in rate caused by C—C splitting are not seen. It is of interest in this connection that the rate of cleavage of sugar diphosphates by the modified aldolase is the same as the rate of cleavage of fructose-1-phosphate by both native and modified enzymes (*3*). It seems probable, therefore, that the reaction with a substrate lacking a second phosphate is limited by the inability of this substrate to bring about a conformational change through binding of a second phosphate and that the group that fails to participate in the acceleration of catalysis is the same group that is affected by carboxypeptidase treatment of aldolase.

The removal of three C-terminal tyrosine residues by carboxypeptidase (*3*) is not a specific treatment to alter the kinetic properties of aldolase. Subtilisins A and B (*15*) and chymotrypsin (Worthington) also modify aldolase by cleavage at as-yet unknown positions. With all of these enzymes, aldolase activity with FDP is reduced to a final constant value of 5 percent. The K_m and V_{max} values of the stable products

of these proteolytic enzymes are identical to those found with the carboxypeptidase altered aldolase. All of the degraded enzymes are equally depressed in the ability to catalyze the detritiation of labeled DHAP (*5*), consistent with the conclusion that a single step in the overall process has been affected.

Although several proteolytic enzymes with different specificities produce the same changes in the catalytic properties of aldolase, this enzyme should not be considered as a structure so delicately poised that every peptide bond is essential to maintain the most efficient catalyst. Aldolase-T, prepared by Szabolcsi and associates (*16*) by treatment of a *p*CMB derivative of aldolase with trypsin, has only slightly reduced activity in the usual assay and shows only a proportional loss in the detritiation reaction (*17*).

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References and Notes

1. O. Meyerhof, K. Lohmann, P. Schuster, *Biochem. Z.* **286**, 301, 319 (1936).
2. I. A. Rose and S. V. Reider, *J. Am. Chem. Soc.* **77**, 5764 (1955); B. Bloom and Y. J. Topper, *Science* **124**, 982 (1956).
3. E. R. Drechsler, P. D. Boyer, A. G. Kowalsky, *J. Biol. Chem.* **234**, 2627 (1959).
4. W. J. Rutter, O. C. Richards, B. M. Woodfin, *ibid.* **236**, 3193 (1961).
5. I. A. Rose, E. L. O'Connell, A. H. Mehler, *ibid.* **240**, 1758 (1965).
6. G. R. Bartlett, *ibid.* **234**, 449 (1959).
7. W. Mejbaum, *Z. Physiol. Chem.* **258**, 117 (1939).
8. Z. Dische, *Ann. N.Y. Acad. Sci.* **75**, 129 (1958).
9. A. H. Mehler, *J. Biol. Chem.* **238**, 100 (1963).
10. A. Ginsburg and A. H. Mehler, *Biochemistry* **5**, 2623 (1966).
11. D. E. Koshland, Jr., *Science* **142**, 1533 (1963).
12. W. P. Jencks, *Ann. Rev. Biochem.* **32**, 639 (1963).
13. F. C. Hartman and R. Barker, *Biochemistry* **4**, 1068 (1965).
14. I. A. Rose *Ann. Rev. Biochem.* **35**, 23 (1966).
15. Kindly given to us by Dr. Martin Ottesen of the Carlsberg Laboratories.
16. E. Biszku, L. Boross, G. Szabolcsi, *Acta Physiol. Acad. Sci. Hung.* **25**, 161 (1964).
17. Unpublished experiments of Dr. G. Szabolcsi, Institute of Biochemistry, Hungarian Academy of Science, while she was a visiting scientist in the laboratory of one of us (A.H.M.) at the National Institutes of Health in 1964.
18. Supported by NSF grant No. GB-4084. The technical assistance of Mrs. Carol Cramer is gratefully acknowledged.

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Fossil Alpha-Particle Recoil Tracks: A New Method of Age Determination

Abstract. *The discovery of a new type of fossil nuclear track in mica is reported. This track is produced by the recoil nucleus accompanying the α-particle decay of uranium and thorium impurities. The tracks are very short and can be seen with phase contrast microscopy. Measurement of fossil α-recoil track densities, coupled with determinations of the thorium and uranium contents, provides a new dating technique analogous to the previously discovered "fission-track method." The primary advantage of the α-recoil method is a several-thousand-fold increase in sensitivity over the fission-track technique. The α-recoil method should also prove useful in studying the problem of extinct isotopes in meteorites.*

It has been known for some time that fission fragments from the spontaneous fission of U^{238} produce stable-track latent images in a variety of materials (*1*, *2*). These latent images can be developed by a chemical etching technique to the point where they are easily visible in an ordinary optical microscope. Measurement of the density of ancient (fossil) tracks followed by a reactor irradiation and a subsequent track count forms the basis of the "fission-track dating method" that has been successfully applied to a variety of terrestrial samples ranging in age from 20 years to 1.5×10^9 years. In meteorites, excess fission tracks (defined as the number of tracks over and above the number expected from spontaneous fission of U^{238}), have been interpreted as arising (*3*) from the spontaneous fission of the now-extinct isotope Pu^{244}. Using phase contrast microscopy we have seen a new kind of fossil track. Figure 1A shows a normal fission track in etched mica observed with transmitted light. Figure 1B shows the same sample illuminated with a Zernicke phase-contrast system in a Leitz microscope. Numerous shallow etch pits, invisible in normal illumination, are apparent in the phase-contrast picture. These shallow pits are in reality very short etched tracks.

That the small tracks are produced by the heavy recoil nuclei accompanying α-particle emission of uranium and thorium impurities is shown by the following: (i) The ratio of small tracks to spontaneous fission tracks is approximately constant in samples with very different uranium concentrations; (ii) the depth of the shallow pits, or track lengths, inferred on the basis of the α-recoil hypothesis agrees with the experimental value obtained by electron microscopy; (iii) the measured length is also compatible with the theoretical range of α-recoils; and (iv) an α-emitter, Th^{228}, placed next to annealed mica produces new shallow pits with the proper frequency.

Fossil fission tracks are produced almost completely by U^{238}. On the other hand, α-recoil tracks are produced by both U and Th. The ratio of small tracks to fission tracks should thus be expected to vary for different samples depending on Th/U ratio. However, since the ratio of Th to U does not vary much in nature (*4*), one might expect to find a roughly constant ratio of α-recoil to fission tracks. To check this point we made track counts in five samples of muscovite mica etched for 2 hours at 20°C in 48 percent hydrofluoric acid, and in one sample of phlogopite mica etched for 60 seconds in the same solution. Although the fission-track density varied by a factor of 30, ranging from 5×10^2 cm^{-2} to 1.5×10^4 cm^{-2}, the ratio of small tracks to large tracks was approximately constant, varying from 2.3×10^3 to 4.5×10^3 with an average value of 3.5×10^3.

Only tracks that intersect a free surface can be revealed by the acid attack. The density of revealed tracks is thus proportional to the etchable track length. Specifically the density of fission tracks is given with reasonable precision by the following relation (*2*)

$$\rho_f = N_o C_U \lambda_f(U) T R_f \qquad (1)$$

where λ_f (U) is the decay constant for spontaneous fission, C_U is the concentration of uranium, R_f is the total etchable range of the two fragments emitted in a single fission, T is the time and N_0 is the number of atoms per cubic centimeter.

The corresponding relation for the density of α-recoil tracks is

$$\rho_\alpha = N_o C_U \lambda_\alpha(U)\, T\, R_\alpha + N_o C_{Th} \lambda_\alpha(Th) T R_\alpha \qquad (2)$$