Primary Sequence Dependence of the Deamidation of Rabbit Muscle Aldolase

Abstract. *The first-order deamidation half-time of the peptide, Gly-Ser-Asn-His-Gly in phosphate buffer, pH 7.4, ionic strength at 0.2, 37.0°C, is 6.4 ± 0.5 days. This compares favorably with the in vivo deamidation half-time of 8 days for this sequence in rabbit muscle aldolase. This fact is discussed with respect to the general hypothesis that sequence-controlled deamidation of glutaminyl and asparaginyl residues is a mechanism by which molecular and organismic development and aging are timed.*

It is possible that sequence-controlled deamidation of glutaminyl and asparaginyl residues serves as a general molecular timer of protein turnover and organismic development and aging (*1*). The protein aldolase is of particular current interest because recent investigations (*2–9*) have implicated aldolase deamidation in all three processes: turnover, development, and aging.

Carboxypeptidase digestion of aldolase decreases the aldolase enzymatic activity during the initial stage of digestion (*2*). Early observations of aldolase heterogeneity were attributed to random combinations of subunits, while a variety of other explanations were offered for heterogeneity in other similar molecules (*3*).

Experiments in Horecker's laboratory showed an age-dependent heterogeneity in rabbit muscle aldolase and proved that this heterogeneity resulted from deamidation of the asparaginyl residue that is the fourth residue from the carboxyl end of the protein (*4, 5*). Subsequently, secondary forms of aldolase with decreased enzymatic activity have been observed in drosophila (*6*), and inactive aldolase accumulation with age has been observed in mouse liver (*7*) and nematodes (*8*).

Flatmark and Sletten (*10*) were the first to make in vivo measurements of deamidation rate. They showed that the deamidation half-time of rat kidney cytochrome c is 15.6 days, and this deamidation has been shown to be sequence controlled (*11*). Midelfort and Mehler (*9*) measured the in vivo deamidation rate for rabbit muscle aldolase. They applied a large correction factor for reuse of the isoleucine tracer (*12*) and estimated the deamidation half-time to be 8 days. This half-time was equated with the half-life in vivo of rabbit muscle aldolase (*9*).

We have synthesized the peptide, Gly-Ser-Asn-His-Gly (*13*) by the usual methods of Merrifield solid phase peptide synthesis (*14*) with the amino terminal Gly labeled with ^{14}C (*15*). The second, third, and fourth residues of this peptide are the same as those in the carboxyl peptide of rabbit muscle aldolase (*5*). The rate of deamidation of this peptide in phosphate buffer (*p*H 7.4, ionic strength = 0.2, 37.0°C) was measured as described (*1, 11, 16*). The deamidation half-time is 6.4 ± 0.5 days, and the first-order rate constant is $1.25 \pm 0.10 \times 10^{-6}$ sec^{-1}. The experimental results are shown in Fig. 1.

The close agreement between our value of 6.4 days and the in vivo value (*9*) of 8 days indicates that the initial deamidation of rabbit muscle aldolase in vivo is a nonenzymatic sequence-controlled event.

The rates of deamidation of more than 60 glutaminyl and asparaginyl pentapeptides of different sequences have been measured under the same solvent conditions as used here (*1, 11, 16*). This value of 6.4 days is the fastest deamidation rate that has been observed. It is possible that hydrogen-bond formation between the Ser side chain and the Asn side chain and also that intramolecular catalysis by the His side chain are responsible for this very fast deamidation rate.

There are many other asparaginyl and glutaminyl residues in aldolase, and many of them undoubtedly deamidate in vivo at biologically significant rates. These deamidations may be involved with aldolase turnover and organismic development and aging. We have shown that the first of these deamidations that has been followed in vivo is, in accordance with the general hypothesis (*1*), primarily a nonenzymatic, sequence-controlled event.

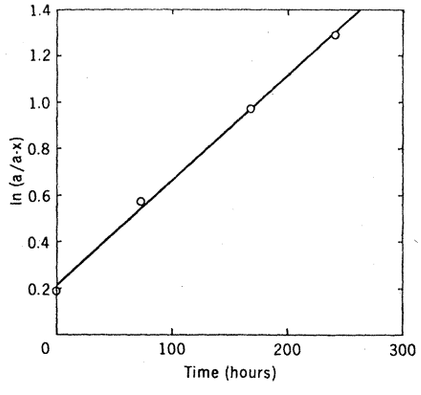

Fig. 1. Deamidation of a 0.001*M* solution of [Gly^1-^{14}C]Gly-Ser-Asn-His-Gly in phosphate buffer, *p*H 7.4; ionic strength at 0.2; 37.0°C. The sum of the number of ^{14}C counts in the amidated and deamidated bands is designated *a*; the sum of the number of ^{14}C counts in the deamidated band is designated *x*. The straight line is calculated by the method of least squares, and the calculation is based on the assumption that the deamidation reaction is first order in peptide concentration. The first-order rate constant, *k*, is $1.25 \pm 0.10 \times 10^{-6}$ sec^{-1}, and the deamidation half-time, $t_{\frac{1}{2}}$, is 6.4 ± 0.5 days. Errors were estimated with a 75 percent reliability limit for the mean, with a 75 percent reliability limit for the variance, and with the assumption of a normal distribution of error in *k*. The correlation coefficient of the straight line is 0.9989.

There are many other asparaginyl and glutaminyl residues in aldolase, and many of them undoubtedly deamidate in vivo at biologically significant rates. These deamidations may be involved with aldolase turnover and organismic development and aging. We have shown that the first of these deamidations that has been followed in vivo is, in accordance with the general hypothesis (*1*), primarily a nonenzymatic, sequence-controlled event.

JAMES H. MCKERROW
ARTHUR B. ROBINSON*
Bonner Laboratory of Biology and Chemistry, University of California, San Diego, La Jolla 92037

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* Present address: Institute of Molecular Medicine, 2700 Sand Hill Road, Menlo Park, California 94025. Reprint requests should be directed to this address.

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