cal. A major band at about 23 kD, which was present at 2 months, was retained by lenses from mice on the restriced diet at 11, 30, and even at 49 months, but was missing in the age-matched controls. There were no controls at 49 months because mice on the control diet do not live that long. Additional experiments were performed on lens supernatants from age-matched mice on restricted and control diets at ages 21, 24, 25, and 28 months and on the lenses from mice on restricted diets at ages 41, 44, and 49 months. In all cases, the HPLC profiles or the SDS-PAGE results, or both, showed that lenses from animals on the restricted diet retained larger quantities of soluble gamma crystallins (data not shown).

In rodents and many other species, there is a progressive decrease with age in the concentration of the soluble structural protein, gamma crystallin (*8, 9, 12*). The precise mechanism for this decline remains to be determined. Using Raman spectroscopy, Kuck *et al.* (*19*) monitored the total sulfydryl concentration in the intact lenses of rats and mice. They observed a rapid loss with age of SH groups concomitant with an increased insolubilization of gamma crystallins. Free SH groups are also lost with aging in other proteins, including nonhistone chromatin (*20*) and serum albumin (*21*). It would be interesting to determine whether diet restriction influences the free SH concentration in the lens. Such an effect might help to clarify the role of the high concentrations of free SH groups found in the lens. Our results indicate that animals on dietary restrictions can provide a useful experimental model for the study of aging processes in the eye.

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Enzymatic Catalysis in Organic Media at 100°C

Abstract. *Porcine pancreatic lipase catalyzes the transesterification reaction between tributyrin and various primary and secondary alcohols in a 99 percent organic medium. Upon further dehydration, the enzyme becomes extremely thermostable. Not only can the dry lipase withstand heating at 100°C for many hours, but it exhibits a high catalytic activity at that temperature. Reduction in water content also alters the substrate specificity of the lipase: in contrast to its wet counterpart, the dry enzyme does not react with bulky tertiary alcohols.*

Water has a dual effect in enzymatic systems: it is essential for acquisition and maintenance of the enzymes' catalytically active conformation (*1*), and it is required for most enzyme inactivation processes, in particular those of thermal inactivation (*2*). Several enzymes have been shown to function in lipophilic organic media in the presence of a few percent of water (*3*). We now report that, upon further dehydration, enzymes exhibit some new catalytic properties.

Porcine pancreatic lipase (*4*), a well-characterized enzyme (*5*), was used as a model in most of our work. We investigated the lipase-catalyzed transesterification reaction between tributyrin (tributyryl glycerol) and various alcohols and found that the reactions yielded a monoester of butyric acid and dibutyrin. The substrate mixture (usually a 2*M* solution of an alcohol in tributyrin) also served as the reaction medium. A powder of the enzyme was added to the reaction mixture, and the suspension was shaken vigorously at 20°C; samples were periodically withdrawn and analyzed by gas chromatography (*6*). The water concentration, measured by the optimized method of Fischer (*7*), was controlled (i) in the solvent by passing it through a molecular sieve column and subsequently adding a known amount of water and (ii) on the enzyme by evacuating the powder under vacuum.

The rate of the lipase-catalyzed reaction between tributyrin and heptanol was studied as a function of the concentration of water in the liquid phase in the range from 0 to 1.1 percent (Fig. 1) (*8*). The enzyme remained catalytically active at concentrations of water as low as 0.015 percent. Even when the amount of water adsorbed on the enzyme (0.48 percent) was included, the water content of the system sufficient for the lipase to act as a catalyst was still less than 0.02 percent. Virtually the same profile was observed for the enzyme preparation that contained 3.6 percent water (the "wet" lipase). We measured the water content of the lipase powders after they were incubated in the transesterification reaction mixture and found that the "dry" lipase (0.48 percent initial water content), when placed in the reaction mixture with 0.8 percent water, adsorbed water from the solvent; after 3 hours of incubation the powder contained 3.6 percent water. The wet lipase

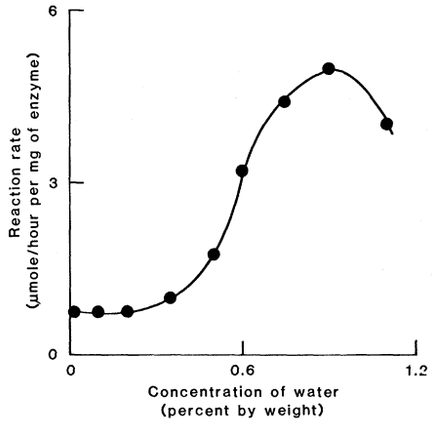

Fig. 1. Dependence of the rate of the transesterification reaction between tributyrin and *n*-heptanol catalyzed by porcine pancreatic lipase on the concentration of water in the substrate mixture. A 2*M* solution of heptanol in tributyrin was dehydrated by passing through a column packed with a 3-Å molecular sieve adsorbent, and a known amount of distilled water was added. To 10 ml of the reaction mixture, 100 mg of dried lipase containing 0.48 percent water was added, and the suspension was shaken vigorously at 20°C.

lost some of its water when placed in the mixture with 0.015 percent water; the final water content of this powder was 1.9 percent. These data indicate that the water in the system equilibrates between the enzyme and the organic medium.

An increase in the water content of dry enzymes is thought to result in loosening of the protein molecules (*9*). Since conformational mobility is necessary for partial unfolding, which in turn is the first step of the thermoinactivation process (*2, 10*), dry enzymes might be expected to be more thermostable than wet ones. We have verified this hypothesis by experiments with the dry porcine pancreatic lipase in various environments. In water or 0.1*M* phosphate buffer (*p*H 8.0) at 100°C, the enzyme lost its activity almost instantaneously (Fig. 2A). When the lipase powder was placed in the tributyrin-heptanol mixture containing 0.8 percent water, its thermoresistance increased greatly (Fig. 2A). The dry enzyme in a drier organic medium (0.015 percent water) was highly stable (Fig. 2A): its half-life at 100°C was more than 12 hours. Similar results were obtained with another enzyme, lipase from *Candida cylindracea*: whereas the half-life of the enzyme powder (3 percent water content) in the wet tributyrin-heptanol mixture at 100°C was less than 2 minutes, it was almost 1.5 hours in the dry reaction mixture.

Thermal stability of both wet and dry porcine pancreatic lipase decreased as the water content of the tributyrin-heptanol mixture increased (Fig. 2B). However, at water concentrations below approximately 0.3 percent, the stability of the dry lipase at 100°C remained constant upon further dehydration of the system (Fig. 2B), apparently indicating that the rate-limiting step in the irreversible thermoinactivation of the enzyme no longer involves water. The thermostability in nearly anhydrous organic media depended on the nature of the solvent: the half-lives of lipase at 100°C in heptanol, decanol, toluene, hexadecane, and tributyrin were 5, 12, 6, 10, and 26 hours, respectively. Exclusion of molecular oxygen from tributyrin had no appreciable effect on thermostability, ruling out the possibility of direct O_2 oxidation of the enzyme's functional groups.

Table 1. The initial rates of the transesterification reactions between tributyrin and various alcohols catalyzed by dry and wet porcine pancreatic lipase.

Alcohol	Initial rate* (μmole/hour per 100 mg of lipase)	
	Dry lipase†	Wet lipase‡
n-Butanol	60	200
n-Heptanol	75	420
n-Decanol	80	340
n-Hexadecanol	75	330
Geraniol	62	280
S(+)-2-Octanol	75	150
R(−)-2-Octanol	70	130
trans-2-Methylcyclopentanol	15	40
cis-2-Methylcyclopentanol	15	30
tert-Butanol	0	22
3-Methyl-3-hexanol	0	20

*Conditions: 100 mg of lipase powder (*4*) of a given moisture content were added to 10 ml of a 1*M* alcohol in tributyrin containing either 0.015 percent or 0.7 percent water. The mixture was shaken (250 rev/min) at 20°C and periodically assayed (*6*). †Water content: 0.015 percent in the medium, 0.48 percent on the enzyme. ‡Water content: 0.7 percent in the medium, 3.6 percent on the enzyme.

The dry lipase, when placed in the dry organic environment, withstood heating at 100°C for many hours (as determined by assay at 20°C) (Fig. 2A). To determine whether the enzyme could also act as a catalyst under such harsh conditions, we measured the lipase-catalyzed transesterification between tributyrin and heptanol at 100°C (no reaction took place in the absence of the enzyme). We found that not only is the dry lipase catalytically active in the reaction mixture containing 0.015 percent water at 100°C, but its activity exceeds that at 20°C in the same system by a factor of 5. This proved to be a general phenomenon: heptanol was replaced by a number of other primary and secondary alcohols (Table 1), and in all cases the lipase-catalyzed transesterification in this substrate mixture was faster at 100°C than at 20°C.

Comparison of Figs. 1 and 2 reveals that water had the opposite effects on lipase in the organic medium at 20° and 100°C. Whereas water activated the enzyme at 20°C as expected (*11*) (Fig. 1), at high temperatures it inactivated lipase. The latter was shown by an experiment in which addition of even a small amount of water to the vigorously functioning, dry lipase in the tributyrin-heptanol mix-

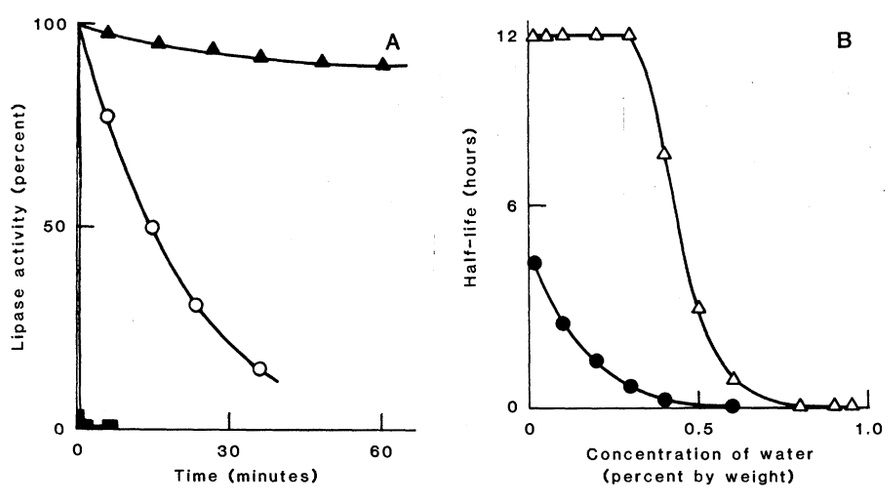

Fig. 2. (A) The time course of thermal inactivation at 100°C of the dried powder of porcine pancreatic lipase placed in water or 0.1*M* phosphate buffer (*p*H 8.0) (■) and in 2*M* solution of heptanol in tributyrin containing 0.8 percent water (○) or 0.015 percent water (▲). (B) The half-life at 100°C of wet ●) and dried (△) porcine pancreatic lipase placed in 2*M* solution of heptanol in tributyrin at different concentrations of water. To 1 ml of the substrate mixture, 20 mg of the lipase was added, and the suspension was stirred in a glycerol bath at 100°C. Samples were periodically withdrawn and analyzed potentiometrically at 37°C with 1*M* aqueous solution of tributyrin (*p*H 8.0) as a substrate in the hydrolysis reaction (identical results were obtained when samples were assayed by gas chromatography for the disappearance of heptanol and accumulation of heptyl butyrate in the transesterification reaction).

ture containing 0.3 percent water at 100°C led to deceleration and cessation of the enzymatic transesterification [because of the water-induced destabilization of the biocatalyst (Fig. 2B)].

On the basis of these data, the structure of the dry lipase, although similar to that of its wet counterpart (*12*), appears to be more rigid. If so, then even though the wet porcine pancreatic lipase [an enzyme known for its wide substrate specificity (*5*)] will accept nearly any alcohol as a nucleophile in the transesterification reaction, the dry lipase will perhaps be unreactive toward bulky alcohols because it lacks the conformational mobility needed to accommodate them in the active center. To test this prediction, we examined the reaction of tributyrin with various alcohols (Table 1) catalyzed by dry and wet lipase. Upon increase in size or transition from primary to secondary alcohol, the reactivities of the wet and dry enzyme remained comparable. However, with tertiary alcohols the dry lipase, in contrast to the wet, was completely inactive (Table 1). Thus, dehydration of the enzyme not only enhances its thermal stability but also changes its substrate specificity (*13*). This phenomenon, should it prove to be a general one, may form a basis for a new approach to the improvement of catalytic properties of enzymes (*14*).

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Satellite Observations of the 1982–1983 El Niño Along the U.S. Pacific Coast

Abstract. *Satellite infrared temperature images illustrate several effects of the 1982–1983 El Niño: warm sea-surface temperatures with the greatest anomalies near the coast, weakened coastal upwelling, and changes in surface circulation patterns. Phytoplankton pigment images from the Coastal Zone Color Scanner indicate reduced productivity during El Niño, apparently related to the weakened coastal upwelling. The satellite images provide direct evidence of mesoscale changes associated with the oceanwide El Niño event.*

The El Niño event that has caused major physical and biological changes in the equatorial Pacific since early 1982 began to affect the waters off California by December. Changes in surface and subsurface temperature, sea level, surface currents, and plankton have been noted (*1*, *2*). Oceanographic data obtained fortuitously on previously scheduled cruises, at shore stations, or from sampling programs initiated in haste after the event had been recognized are limited in spatial and temporal coverage. Global sea-surface temperature (SST) data analyzed routinely by the National Oceanic and Atmospheric Administration (NOAA) (*3*) provide good coverage, but only after averaging over large areas has removed energetic mesoscale features.

Fortunately, systematic sampling of the ocean surface has been carried out with a variety of sensors aboard orbiting satellites during the entire course of the

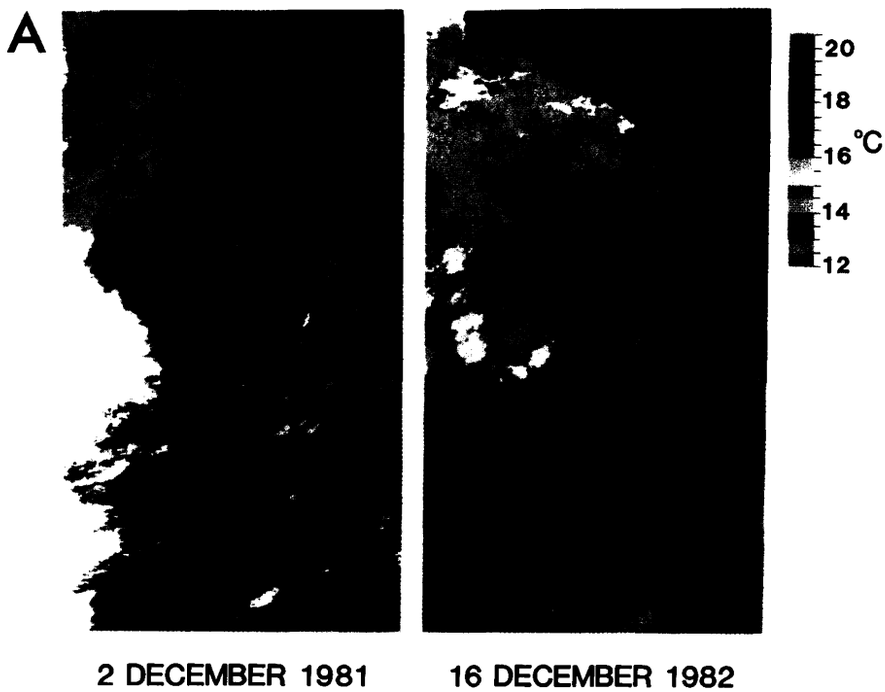

Fig. 1A. Advanced Very High Resolution Radiometer sea-surface temperature image off southern California.