

Fig. 3. Humidity profile derived from IRIS data near Brownsville, Texas, on 22 April 1969 at 1737 G.M.T. Humidity data from a radiosonde at 1800 G.M.T. are shown for comparison.

research tasks may be listed, but the list may easily be expanded.

How well can the humidity in the lowest atmospheric layers be determined in the tropics?

How well can tropospheric temperature inversions in the polar regions be recognized?

How well can one obtain the surface temperatures of the oceans from space?

Is it possible to see residual ray phenomena in desert areas where minerals are exposed? Can minerals be identified by this technique?

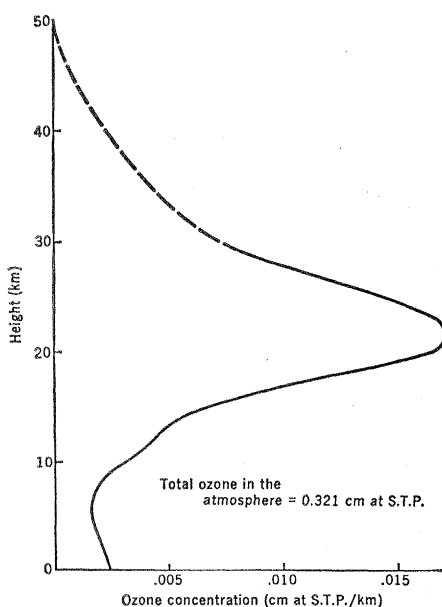


Fig. 4. Ozone profile [expressed in terms of centimeters at standard temperature and pressure (S.T.P.) per kilometer of altitude] derived from the same data as the temperature and humidity profiles (Figs. 2 and 3). The values above 30 km are extrapolated. The ozone profile was derived by C. Prabhakara.

Can air pollutants be detected?

These examples show the broad scope of scientific investigations made available by measurement of the thermal emission spectrum.

If present plans go well, calibrated spectra will be deposited in the National Space Science Data Center (1) in the near future. Details on the format of the magnetic tapes may be obtained from the Nimbus 3 User's Guide (2).

An improved IRIS experiment will be flown on the next Nimbus (D). The improvements include an extended spectral range toward longer wavelength, 200 to 1600 cm^{-1} , and a spectral resolution of 3 instead of 5 cm^{-1} . Increases in the signal-to-noise ratio and a decrease in the field of view to a cone of about 2.5° half angle will also be implemented.

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References and Notes

1. Interested researchers may obtain these data by writing to the National Space Science Data Center (Code 601), Goddard Space Flight Center, Greenbelt, Maryland 20771.
2. This guide was prepared as part of the Nimbus Project, R. R. Sabatini, editor.
3. Even though only a few people can be mentioned, many have contributed to the success of the experiment. L. Chaney (University of Michigan) helped in the early instrument development, which culminated in a 1966 balloon flight. B. Schlachman (Goddard Space Flight Center) and the group at Texas Instruments under J. Taylor and C. Prokesh contributed to the production of the flight instrument. C. Prabhakara, V. Kunde, M. Forman, A. Simmons, and G. Wolford helped in the analysis and data reduction. Credit goes to the Nimbus team for placing the instrument in a perfect orbit.

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Heterogeneity of Presumably Homogeneous Protein Preparations

Abstract. Some highly purified glycolytic enzymes have been subjected to isoelectric focusing and found to contain a number of enzymatically active species. Crystalline aldolase A and glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle were resolved into five components, crystalline aldolase from yeast was resolved into three components, pyruvate kinase from rabbit muscle yielded four components, and yeast enolase was resolved into two components. Rabbit muscle lactate dehydrogenase (M_4) gave one major peak of protein and enzymatic activity. The profiles of aldolase, glyceraldehyde-3-phosphate dehydrogenase, and yeast aldolases suggest random combinations of two closely related subunits into tetramers and dimers, respectively. The molecular heterogeneity of the other enzymes is not so easily related to subunit structure.

Multiple forms of enzymes (isoenzymes) have been detected and isolated by a variety of procedures. Each isolated species may appear homogeneous by conventional criteria (sedimentation, electrophoretic, and immunological properties, as well as crystallizability); such behavior is compatible with, but does not prove, homogeneity. The degree of heterogeneity of an enzyme population is of considerable interest on chemical, physiological, and genetic grounds. For example, studies of the molecular and catalytic properties of enzymes as well as their regulatory characteristics usually require or assume a homogeneous population of molecules.

The technique of electrofocusing, which involves separation of proteins of differing isoelectric points by electrophoresis in a pH gradient, has extraordinary resolving capability (1). Using this technique, we showed that crystalline aldolase A (E.C. 4.1.2.7) from rabbit muscle, homogeneous by standard criteria, was composed of five catalytically active major components (2). This result suggested that the two subunits previously detected in this enzyme (2, 3) interacted randomly to form aldolase tetramers. This five-membered hybrid "subset" then seems analogous to the five-membered hybrid sets previously produced from binary combinations of aldolase A, B, and C subunits (4). This

supposition has been confirmed (2). Using the electrofocusing procedure, we now have evidence of significant heterogeneity in other enzyme preparations that appear homogeneous by conventional methods.

Figure 1 presents typical electrofocusing profiles for several enzymes. The recovery of the total activity was excellent; the specific activities of most of the recovered components were unchanged or higher than those of the starting material. The electrofocusing procedure, therefore, did not appear to have deleterious effects on the enzymes.

As described (2), five distinct peaks of protein and enzymatic activity were resolved on electrofocusing crystalline aldolase A (Fig. 1a). Experiments with aldolase C from rabbit brain have indicated that this enzyme also can be resolved into five active species.

Crystalline glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12)

from rabbit muscle was resolved into one minor and four major peaks of activity (Fig. 1b). This enzyme, like aldolase, is a tetrameric molecule (5). The profile is consistent with that predicted from random combination of two nonidentical subunits in a ratio of 2:1. Yeast aldolase (E.C. 4.1.2.7), unlike muscle aldolase, is a dimeric molecule (6). As shown in Fig. 1c, crystalline aldolase from yeast was resolved into three major components. This heterogeneity is consistent with a random combination of two different subunits into dimers.

Some other enzymes showed heterogeneity which was not easily related to their subunit compositions. A sample of highly purified yeast enolase (E.C. 4.2.1.11), which could be resolved by analytical polyacrylamide-gel electrophoresis into one minor and two major bands (7), was fractionated by isoelectric focusing into at least seven com-

ponents in two major groups with isoelectric points around pH 5.7 and 6.7. The principal electrophoretic component was isolated from this preparation by tetraethylaminoethyl cellulose chromatography and subjected to isoelectric focusing. It gave two major peaks at pH 6.7 and 6.8 with shoulders at pH 6.5 and 7.4. This group of peaks corresponds to the set detected in fractions 75 to 95 of Fig. 1d.

Rabbit muscle pyruvate kinase A (E.C. 2.7.1.40) contained four major peaks of protein and enzymatic activity. The specific activities for the two major components were 720 and 550, respectively—somewhat higher than the values reported (8)—while the minor peaks have specific activity of 370 and 480. We have also resolved pyruvate kinase B from rat liver into two components that have significantly different kinetic and regulatory properties.

Of the enzymes tested, only rabbit

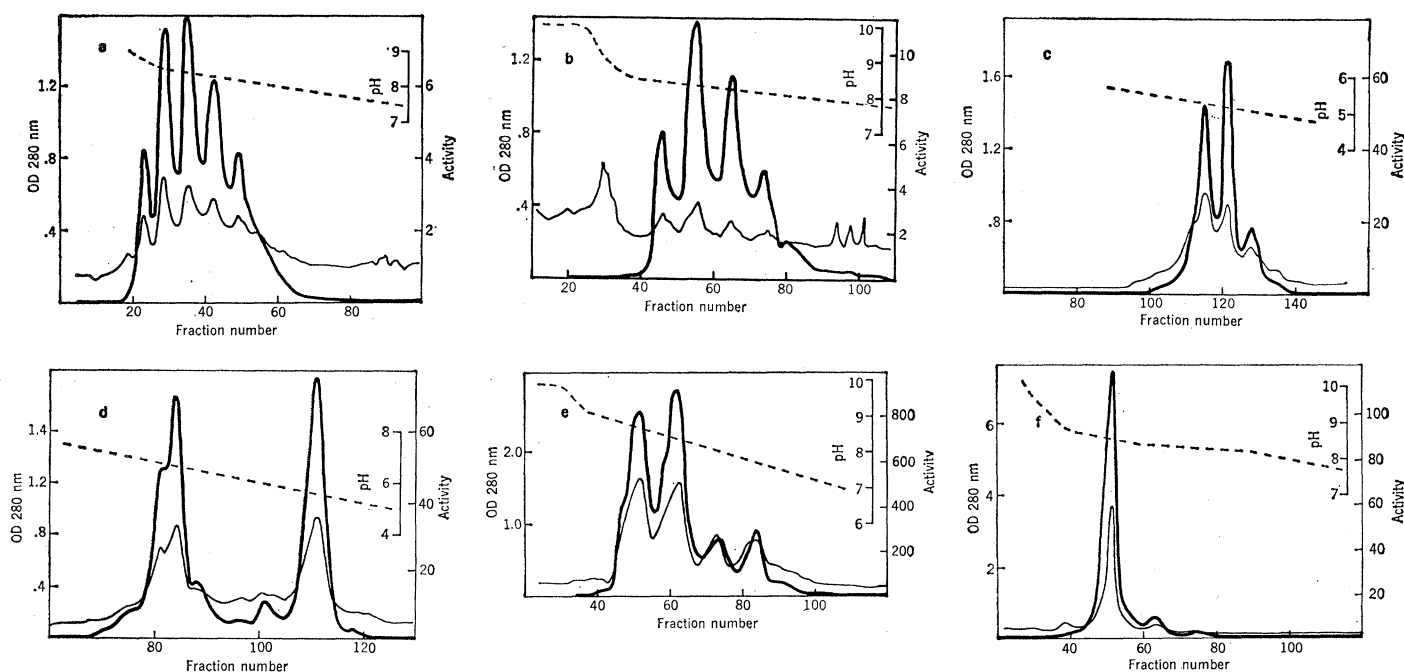


Fig. 1. Isoelectric resolution of (a) aldolase A, (b) glyceraldehyde-3-phosphate dehydrogenase, (c) yeast aldolase, (d) yeast enolase, (e) pyruvate kinase A, and (f) lactate dehydrogenase A. Isoelectric focusing was performed with synthetic ampholytes containing $10^{-2}M$ β -mercaptoethanol (10), with the cathode solution at the bottom of the 110-ml column. Activities of the enzymes are presented in micromoles of substrate reacted per milligram of protein (11), and specific activities are expressed in micromoles of substrate reacted per milligram of protein (12). (a) Crystalline, rabbit muscle aldolase A of specific activity 16 was electrofused on an ampholine gradient (pH 7 to 9). The specific activities of the peak fractions were: pH 8.2, 12.5; pH 8.3, 14.8; pH 8.4, 16.7; pH 8.5, 15.2; and pH 8.6, 15.0. (b) Crystalline, rabbit muscle glyceraldehyde-3-phosphate dehydrogenase of specific activity 50 was electrofused on a gradient at pH 7 to 9. The specific activities of the peak fractions were: pH 8.1, 71; pH 8.2, 96; pH 8.3, 86; pH 8.4, 59; and pH 8.5, 49. (c) Crystalline, yeast aldolase of specific activity 56 was electrofused on an ampholine gradient (pH 5 to 8). The specific activities of the peak fractions were pH 5.1, 38; pH 5.2, 64; and pH 5.3, 45. (d) Yeast enolase of specific activity 120 was electrofused on an ampholine gradient (pH 3 to 10). The specific activities of the peak fractions and shoulders were: pH 5.4, 33; pH 5.6, 99; pH 6.0, 46; pH 6.6, 65; pH 6.7, 103; pH 6.8, 92; and pH 7.1, 52. (e) Rabbit muscle pyruvate kinase of specific activity 300 was electrofused on an ampholine gradient (pH 3 to 10). The specific activities of the peak fractions were: pH 7.8, 480; pH 8.0, 370; pH 8.2, 720; and pH 8.6, 550. (f) Crystalline, rabbit muscle lactate dehydrogenase A (M_4) of specific activity 212 was electrofused on a gradient consisting of equal parts of ampholine of pH 3 to 10 and 7 to 9. The specific activities of the peak fractions were: pH 8.3, 145; pH 8.4, 161; and the major peak, pH 8.5, 260. Heavy lines, micromoles of substrate reacted per minute per milliliter; light lines, optical density at 280 nm; broken line, pH.

muscle lactate dehydrogenase A (E.C. 1.1.1.27) appeared to have a low degree of heterogeneity. The preparation exhibited a single major component (isoelectric point, pH 8.55) containing more than 80 percent of the protein and 85 percent of the activity. In addition, two minor activities and two inactive components were detected. The ultraviolet absorbing material at pH 9.9 is largely the result of an accumulation of electrolysis products from the cathode and was found in all electrofocusing experiments.

All of the enzymes thus far subjected to isoelectric focusing have exhibited some degree of heterogeneity, even though most of them were crystalline proteins and homogeneous by commonly accepted criteria. Some of the heterogeneity detected here is probably the result of degradation or modification of the proteins during isolation. We believe, however, that many of the multiple forms occur intracellularly. The molecular heterogeneity of glyceraldehyde-3-phosphate dehydrogenase and yeast aldolase revealed by electrofocusing can be explained by the random formation of tetrameric and dimeric molecules, respectively, from two similar, but nonidentical subunits, as for aldolase A. The heterogeneity observed in the other enzyme preparations is not so obviously related to the subunit composition. Molecular heterogeneity may arise by selective modification, as for ribonucleases A and B (9), or by combinative or conformational variation. Alternatively, these variants could be the products of separate nonallelic genes or simply distinct allelomorphous products derived from a heterozygote. The latter would be more easily reconciled with their apparently strong structural and catalytic similarity and the lack of an obvious physiological function. We consider translational infidelity or ambiguity an unlikely mechanism because of the restricted number of species detected.

Regardless of the origin or function of this molecular heterogeneity, it should be given careful consideration when studying enzyme mechanisms (kinetics) or structure, since interpretations of such studies often depend on homogeneous molecular populations.

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- The electrofocusing apparatus and ampholytes were obtained from LKB Instruments, Rockville, Maryland. The column was cooled with circulating water at 4°C. Protein solutions were mixed with a small volume of ampholine-sucrose solution and placed at the center of the column. Voltage was increased to 800 volts over a period of 16 hours and allowed to equilibrate an additional 24 to 30 hours. Approximately 200 0.5-ml fractions were collected for assay.
- Aldolase A was purified from rabbit muscle by the method of B. M. Pogell [*Biochem. Biophys. Res. Commun.* **7**, 225 (1962)], as modified by E. Penhoet, M. Kochman, W. J. Rutter (*Biochemistry*, in press). Glyceraldehyde-3-phosphate dehydrogenase was prepared from rabbit muscle according to M. Kochman and W. J. Rutter [*Biochemistry* **7**, 1671 (1968)]. Yeast aldolase was purified by Dr. R. Kobes from baker's yeast by a modification (6) of the method of W. J. Rutter and J. R. Hunsley [*Methods Enzymol.* **9**, 480 (1966)]. Lactate dehydrogenase A was a gift from Drs. V. Hoagland and D. Teller and was prepared by the method of G. T. Cori, W. W. Slein, C. F. Cori [*J. Biol. Chem.* **173**, 605 (1948)]. Twice recrystallized enzyme was passed through DEAE-Sephadex [A. H. Gelderman, H. V. Gelboin, A. C. Peacock, *J. Lab. Clin. Invest.* **65**, 132 (1965)], and the muscle (M_4) enzyme was isolated. Yeast enolase, a gift from P. A. Hargrave and Dr. F. Wold, was prepared from baker's yeast by the methods of E. W. Westhead [*Methods Enzymol.* **9**, 670 (1966)] and E. W. Westhead and G. McLain [*J. Biol. Chem.* **239**, 2464 (1964)]. Pyruvate kinase A (M) was prepared from rabbit muscle by the method of T. Tanaka, Y. Harano, F. Sue, H. Morimura [*J. Biochem.* **62**, 71 (1967)]. Enzymatic activities were determined at 25°C on a recording spectrophotometer by the following procedures: for aldolase A, R. Blostein and W. J. Rutter [*J. Biol. Chem.* **238**, 3280 (1963)]; for glyceraldehyde-3-phosphate dehydrogenase, S. F. Velick [*Methods Enzymol.* **1**, 401 (1955)]; for yeast aldolase, O. C. Richards and W. J. Rutter [*J. Biol. Chem.* **236**, 3177 (1961)]; for lactate dehydrogenase, A. Kornberg [*Methods Enzymol.* **1**, 441 (1955)]; for yeast enolase, E. W. Westhead [*ibid.* **9**, 670 (1966)]; and for pyruvate kinase, T. Bucher and G. Pfleiderer [*ibid.* **1**, 435 (1955)].
- Protein concentrations were determined by absorbance at 280 nm, assuming $E_{1\%}^{1\text{cm}} = 10$. Since the ampholine carrier compounds absorb at 280 nm, proteins from electrofocusing columns were estimated by subtracting the average baseline absorbance of the ampholine.
- Supported in part by PHS grant HD-02126.
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Diphosphonates Inhibit Hydroxyapatite Dissolution in vitro and Bone Resorption in Tissue Culture and in vivo

Abstract. Two diphosphonates containing the P-C-P bond, $Cl_2C(PO_3HNa)_2$ and $H_2C(PO_3HNa)_2$ retard the rate of dissolution of apatite crystals in vitro. They inhibit bone resorption induced by parathyroid extract in mouse calvaria in tissue culture and in thyroparathyroidectomized rats in vivo.

Inorganic pyrophosphate inhibits the precipitation (1, 2) and the dissolution (3) of hydroxyapatite crystals in vitro. Because pyrophosphate is present in plasma (4), teeth (5), and bone (6), we have suggested (2) that it might regulate both the formation and destruction of mineralized tissues in vivo. Although pyrophosphate and longer-chain condensed phosphates can inhibit the deposition of calcium phosphate in chick embryo femurs in tissue culture (7) and in the aorta (8) and skin (9) of the rat, it has not yet been possible to demonstrate an effect on the resorption of living bone. This failure to influence bone resorption may be due to hydrolysis of the P-O-P bond locally in the bone by pyrophosphatase before it can reach its site of action.

Substances were therefore sought that would be related in structure to pyrophosphate but resistant to chemical and enzymatic hydrolysis. Various compounds containing the P-C-P bond have been synthesized and found to have an effect similar to that of condensed phosphates on the precipitation of hydroxyapatite in vitro and on calcification in vivo (10). In addition, they were also active when administered orally (10). We now describe the effect of two such compounds containing a P-C-P bond, namely, sodium dichloromethylenediphosphonate [$Cl_2C(PO_3HNa)_2$] and sodium methylenediphosphonate [$H_2C(PO_3HNa)_2$], on the dissolution of apatite crystals in vitro and on bone resorption induced by parathyroid extract in tissue culture and