may provide sufficiently different forces of natural selection to promote the genetic divergence necessary for speciation. Finally, the discovery that kinetically different enzyme homologs are indistinguishable when conventional electrophoretic techniques are used emphasizes again the importance of electrophoretically silent mutations in the protein divergence of species.

JOSEPH SIEBENALLER*
GEORGE N. SOMERO
*Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093*

### References and Notes

1. J. F. Grassle, H. L. Sanders, R. R. Hessler, G. T. Rowe, T. McLellan, *Deep-Sea Res.* **22**, 457 (1975); N. S. Jones and H. L. Sanders, *ibid.* **19**, 737 (1972); G. T. Rowe, *ibid.* **18**, 569 (1971); ——— and R. J. Menzies, *ibid.* **16**, 531 (1969); H. L. Sanders and R. R. Hessler, *Science* **163**, 1419 (1969); R. R. Hessler, *Bull. Scripps Inst. Oceanogr.* **15**, 1 (1970); M. A. Rex, in *Biology of Benthic Organisms*, B. F. Keegan, P. O. Ceidigh, P. J. S. Boaden, Eds. (Pergamon, Oxford, 1977), p. 521; R. J. Menzies, R. Y. George, G. T. Rowe, *Abyssal Environment and Ecology of the World Oceans* (Wiley-Interscience, New York, 1973).
2. A. G. Macdonald, *Physiological Aspects of Deep Sea Biology* (Cambridge Univ. Press, New York, 1975).
3. See papers in *Comp. Biochem. Physiol.* **52** [1B] (1975) and *Am. Zool.* **11** [3] (1971), entire issues.
4. H. G. Moser, *U.S. Natl. Mar. Fish. Ser. Fish. Bull.* **72**, 865 (1974).
5. M. Nei, *Am. Nat.* **106**, 283 (1972).
6. J. F. Siebenaller, in *Marine Organisms: Genetics, Ecology and Evolution*, J. A. Beardmore and B. Battaglia, Eds. (Plenum, New York, 1978), p. 95.
7. C. L. Hubbs, *Am. Mus. Novit.* **216**, 1 (1926); K. O. Emery, *The Sea Off Southern California* (Wiley, New York, 1960), p. 109.
8. K. J. Laidler, *Arch. Biochem.* **30**, 226 (1951); G. N. Somero and P. W. Hochachka, in *Adaptation to Environment*, R. C. Newell, Ed. (Butterworths, London, 1976), p. 480.
9. F. H. Johnson and H. Eyring, in *High Pressure Effects on Cellular Processes*, A. M. Zimmerman, Ed. (Academic Press, New York, 1970), p. 1.
10. P. W. Hochachka, C. Norberg, J. Baldwin, J. H. A. Fields, *Nature (London)* **260**, 648 (1976); G. N. Somero, *J. Exp. Zool.* **194**, 175 (1975); R. B. Merrit, *Am. Nat.* **106**, 173 (1972).
11. P. H. Yancey and G. N. Somero, *J. Comp. Physiol.*, in press.
12. D. E. Atkinson, *Biochem. Soc. Symp.* **41**, 205 (1976).
13. The assay mixture contained 80 m*M* tris-HCl buffer (*p*H 7.5; assay temperature, 10°C), 0.15 m*M* NADH, 100 m*M* KCl, and optimal sodium pyruvate (4 m*M* or 5 m*M*). The reaction was followed by monitoring the change in absorbance at 340 nm in a Varian Techtron Model 635 spectrophotometer equipped with a 5-ml high-pressure cell [G. N. Somero, M. Neubauer, P. S. Low, *Arch. Biochem. Biophys.* **181**, 438 (1977)].
14. The volume changes were calculated according to equation 27 of F. H. Johnson and H. Eyring, (*9*, p. 18).
15. Acrylamide gel sieving was done in the tris-glycine buffer (*p*H 8.3) of O. Gabriel [*Methods Enzymol.* **22**, 565 (1971).
16. G. B. Johnson, *Genetics* **83**, 149 (1976); *Biochem. Genet.* **15**, 665 (1977); J. A. Coyne, *Genetics* **84**, 593 (1976); R. S. Singh, R. C. Lewontin, A. A. Felton, *ibid.* p. 609.
17. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
18. R. C. Neuman, W. Kauzmann, A. Zipp, *J. Phys. Chem.* **77**, 2687 (1973).
19. We thank R. R. Hessler for advice and discussion and M. Neubauer for assistance in the laboratory. Supported by NSF grants PCM-09498 and PCM-7804321.

* Present address: Department of Ecology and Evolution, State University of New York, Stony Brook 11794.

15 December 1977

## $L_{III}$-Edge Anomalous X-ray Scattering by Cesium Measured with Synchrotron Radiation

Abstract. *Diffraction of monochromatized synchrotron radiation by crystals of cesium hydrogen tartrate has been used to measure the magnitude and phase of x-ray scattering for cesium near the* $L_{III}$ *absorption edge. In this wavelength region the scattering amplitude of cesium is reduced by as much as 25 electrons per atom, compared to scattering of copper* K$\alpha$ *x-rays. This change, which varies as a function of wavelength, affects the diffraction intensities in a manner similar to isomorphous substitution, and it is large enough to have promise for phase determination in the study of macromolecular structures. This experiment also demonstrates that accurate diffractometer measurements are possible with synchrotron radiation produced by an electron storage ring.*

We report here x-ray diffraction experiments carried out with an automatic diffractometer and a focused monochromatic x-ray beam of synchrotron radiation from the SPEAR storage ring at the Stanford Synchrotron Radiation Laboratory (*1*). Diffraction measurements with crystals of cesium hydrogen (+)-tartrate have shown that this equipment can give reproducible results at wavelengths chosen from the continuous spectrum of the synchrotron radiation. We have derived from these data the anomalous scattering terms for cesium near the $L_{III}$ absorption edge and find that the scattering power of cesium is reduced by as much as 25 electrons. To our knowledge this is the largest such effect yet observed in an x-ray diffraction experiment. This reduction in scattering power, which is approximately equivalent to removing a rubidium atom from the structure, could be used as a substitute for or a complement to isomorphous replacement in solving the phase problem for macromolecular structures. This technique has the advantage that the crystal structure is exactly the same as that studied at another wavelength, thus avoiding the imperfect isomorphism that generally occurs when atoms are added to or replaced in the crystal.

An atom scatters x-rays with an amplitude and phase that can be represented by the complex number $f = f_0 + f' + if''$, where $f_0$ is the value appropriate for very short wavelengths (*2*). The anomalous scattering terms $f'$ and $f''$, which are functions of the wavelength, describe the in-phase and out-of-phase components of the change due to finite binding energies of the electrons in the atom. While $f_0$ decreases as the scattering angle increases, $f'$ and $f''$ are nearly independent of angle.

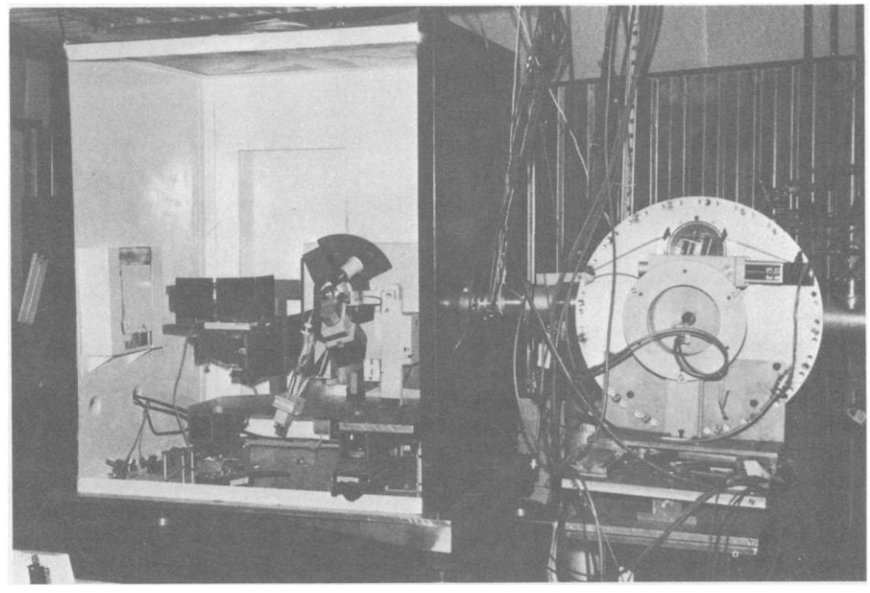

Fig. 1. View of the experimental apparatus. The tank housing the two-crystal monochromator is seen on the right. The monochromatized x-ray beam enters the hutch (which has one side removed to show the inside) and passes into the entrance collimator of the diffractometer. The diffractometer is supported by the computer-controlled carriage, seen in the lower part of the hutch. The $2\theta$ arm holding the detector can be seen below the median plane, and the film carousel (which is removed when the detector is being used) is to the left.


0036-8075/78/0721-0257$00.50/0 

The $f''$ term results in the breaking of Friedel's law. With an appreciable $f''$ contribution the $hkl$ and $\bar{h}\bar{k}\bar{l}$ reflections for noncentrosymmetric structures are no longer equal. This effect has long been used to determine absolute polarity (*3*), absolute configuration (*4*), and to provide protein phase information (*5*). There has been little use of $f'$, as one needs to collect data at different wavelengths for it to have a useful effect on the diffraction pattern.

Near an absorption edge of an atom, where the x-ray energy is close to the binding energy of an inner shell electron, both $f'$ and $f''$ vary rapidly with wavelength; $f''$ jumps rapidly up to a maximum while $f'$ dips and then rises again as the x-ray energy goes from below to above the edge. Absorption edges are thus useful for phasing, as $f''$ can be maximized for maximum Friedel pair differences and a large change in $f'$ can be obtained with a small change in incident beam energy. The recent availability of synchrotron radiation as a tunable source of x-rays made it possible to use these effects to obtain phase information from diffraction patterns from macromolecules (*6*).

We measure $f'$ and $f''$ by adjusting calculated structure factors to fit observed values, using the method of least squares, with these two quantities and a scale factor as the only independent variables. Cesium hydrogen (+)-tartrate, which crystallizes in space group $P2_12_12_1$, was chosen for its desirable physical properties and favorable symmetry. Its lattice parameters, atomic coordinates, and thermal parameters are known very accurately from an experiment with Mo $K\alpha$ radiation and conventional methods (*7*). The values of $f'$ and $f''$ for the light atoms can be estimated with sufficient accuracy from calculations at other wavelengths (*8*); they are small and insensitive to wavelength in the region of interest here.

The synchrotron radiation used in this study is focused by a doubly curved mirror and monochromatized by a rapidly tunable two-crystal germanium (111) monochromator (*9*). An Enraf-Nonius CAD4 diffractometer is mounted behind the monochromator on a motorized carriage, which is used to align the diffractometer to the x-ray beam (see Fig. 1). A PDP 11/34 computer controls the diffractometer, the monochromator, and the alignment carriage. To align the diffractometer we developed a program to move the carriage until the beam passing through the collimator into the detector (at $2\theta = 0$; $\theta$ is the Bragg angle) is maximized in intensity. The diffractometer is mounted with the $2\theta$ arm swinging in the vertical plane because the polarization vector of the synchrotron radiation is almost completely horizontal.

The x-ray beam intensity is proportional to the electron beam current and drops as the stored beam slowly decays. We monitored this intensity variation by using an ion chamber placed in the beam and normalized the diffraction intensities accordingly. To calibrate the wavelength of the monochromator we measured the absorption of a sample of cesium chloride as a function of the monochromator setting. A large jump in the intensity ratio of the incident to the transmitted beam identifies the exact position of the absorption edge of cesium.

We collected diffraction data from two crystals of cesium hydrogen tartrate—a needle with 15 sharply defined faces and dimensions of about 0.093 by 0.110 by 0.40 mm, and a nearly spherical crystal with a mean diameter of 0.28 mm. With the needle we measured 16 or 17 independent reflections in the $\theta$ range 18° to 40° for each of three wavelengths. With the sphere, 15 to 17 independent reflections in the $\theta$ range 40° to 48° were measured in each experiment. In each case, most of the measurements were duplicated by repetition and with equivalent reflections. With absorption parameters in the range 300 to 720 $cm^{-1}$, correction for absorption is critical, with factors ranging up to 49 for the needle. With the sphere the absorption effect is more nearly the same for all reflections, and the values of the corrections are less important.

The results of the least-squares calculations are listed in Table 1. The agreement between data from the two crystals gives us confidence that absorption is not a serious source of bias in the results.

The values of $f''$ at the longest wavelength are similar to the value 3.565 calculated by Cromer and Liberman (*8*) for La at Cr $K\alpha$, a reasonably similar case. The other $f''$ values are also plausible. We know of no previously measured or calculated $f'$ values as negative as those listed here. At the $K$ edge of copper,

Table 1. Anomalous scattering terms for cesium.

| λ, (Å) | Crystal | $R$* | $f'$ | $f''$ |
|---|---|---|---|---|
| 2.470 | Needle | 0.086 | −16.1 ± 2.6 | 11.1 ± 2.2 |
| 2.473† | Needle | 0.067 | −24.5 ± 1.2 | 5.9 ± 0.7 |
| | Sphere | 0.083 | −27.1 ± 0.9 | 4.9 ± 0.6 |
| 2.477 | Needle | 0.060 | −19.9 ± 0.9 | 3.5 ± 0.5 |
| | Sphere‡ | 0.049 | −21.4 ± 0.6 | 3.8 ± 0.3 |
| | Sphere‡ | 0.034 | −20.8 ± 0.5 | 4.1 ± 0.3 |

*The discrepancy factor $R = \Sigma|\Delta F|/\Sigma|F_o|$, where the $F$'s are structure factors. †Inflection point of $L_{III}$ edge, measured with CsCl. ‡Two different experiments, with different storage ring conditions and different mechanical adjustment of the monochromator.

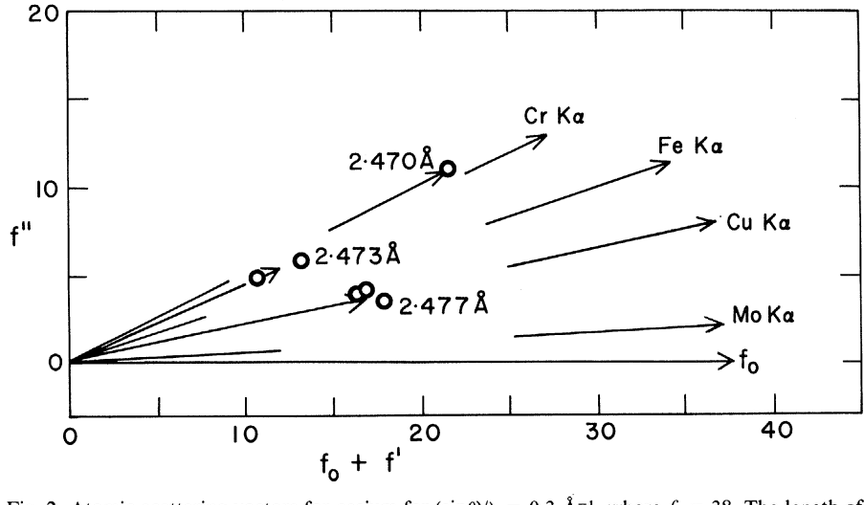


Fig. 2. Atomic scattering vectors for cesium for $(\sin\theta)/\lambda = 0.3$ Å$^{-1}$, where $f_0 = 38$. The length of each vector represents the amplitude of the wave scattered by the atom, and the angle from the horizontal represents the phase shift relative to scattering by a free electron. The circles represent our experimental points. The other vectors are calculated (*8*) for some wavelengths available from conventional x-ray tubes.

where fewer electrons are involved, values lower than −8 have been reported (*10*). For gallium at the *K* edge −10 has been observed (*11*). In the latter case the maximum negative value of $f'$ occurs close to the inflection point of the rising wave of $f''$. Our extreme value of $f'$ occurred at the inflection point of the rising absorption curve of cesium.

Our results are compared in Fig. 2 with values for some wavelengths available from conventional x-ray sources, to show how much the scattered wave amplitude is reduced at this *L* absorption edge.

The resolution of the monochromator (*9*) is approximately $\Delta\lambda/\lambda = 10^{-3}$, where $\lambda$ is the x-ray wavelength. Thus we are measuring $f'$ averaged over this wavelength range. It is possible that with a more monochromatic beam an even larger negative value of $f'$ could be observed. This method is general, and similar experiments can be carried out with other elements at either the *K* or the *L* edge.

JAMES C. PHILLIPS
*Department of Chemistry, Stanford University, Stanford, California 94305*
DAVID H. TEMPLETON
LIESELOTTE K. TEMPLETON
*Department of Chemistry* and *Lawrence Berkeley Laboratory, University of California, Berkeley 94720*
KEITH O. HODGSON
*Department of Chemistry, Stanford University*

### References and Notes

1. Production and properties of synchrotron radiation are described in R. E. Watson and M. L. Perlman, *Science* **199**, 1295 (1978).
2. S. Ramaseshan and S. C. Abrahams, Eds., *Anomalous Scattering* (Munksgaard, Copenhagen, 1975).
3. D. Coster, K. S. Knol, J. A. Prins, *Z. Phys.* **63**, 345 (1930).
4. A. F. Peerdeman, A. J. van Bommel, J. M. Bijvoet, *Proc. K. Ned. Akad. Wet. Ser. B* **54**, 16 (1951).
5. D. M. Blow and M. G. Rossmann, *Acta Crystallogr.* **14**, 1195 (1961).
6. J. C. Phillips, A. Wlodawer, M. M. Yevitz, K. O. Hodgson, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 128 (1976); J. C. Phillips, A. Wlodawer, J. M. Goodfellow, K. D. Watenpaugh, L. C. Sieker, L. H. Jensen, K. O. Hodgson, *Acta Crystallogr. Sect. A* **33**, 445 (1977).
7. L. K. Templeton and D. H. Templeton, *Acta Crystallogr. Sect. A* **34**, 368 (1978).
8. D. T. Cromer and D. Liberman, *J. Chem. Phys.* **53**, 1891 (1970).
9. J. Hastings, P. Eisenberger, B. Kincaid, *Nucl. Instrum. Methods*, in press.
10. A. Freund, in (*2*), p. 69.
11. T. Fukamachi and S. Hosoya, *Acta Crystallogr. Sect. A* **31**, 215 (1975).
12. Supported in part by a grant from the National Institutes of Health (CA16748, to K.O.H.) and by the Division of Basic Energy Sciences of the U.S. Department of Energy. K.O.H. is a fellow of the Alfred P. Sloan Foundation. Synchrotron radiation time and equipment were provided by the Stanford Synchrotron Radiation Laboratory, supported by the National Science Foundation under grant DMR 77-27489 in cooperation with the Stanford Linear Accelerator Center and the U.S. Department of Energy.

1 March 1978

# Enzyme-Linked Immunosorbent Assay for Identification of Rotaviruses from Different Animal Species

Abstract. *Rotaviruses cause gastroenteritis in man and a wide variety of animal species. They cross-react in many immunologic tests and have a similar appearance by electron microscopy, making differentiation among them difficult. Rotaviruses derived from different host species were distinguished by postinfection serum blocking virus activity in an enzyme-linked immunosorbent assay (ELISA). Thirty-three rotavirus isolates from children living in three different parts of the world could not be differentiated by this technique, but they were distinct from four strains recovered from calves, and a series of strains isolated from piglets, foals, monkeys, and infant mice. The four bovine strains were similar, but they could be differentiated from the other animal strains, each of which exhibited a distinct pattern when tested by the ELISA blocking technique.*

Rotavirus is an important cause of gastroenteritis in infants and young children (*1*, *2*). Rotavirus infections have been documented in newborn calves, piglets, lambs, nonhuman primates, and other animals (*3*). Different members of the rotavirus group are similar in appearance by standard electron microscopic techniques, and they cross-react in a variety of immunological tests (*4*, *5*).

Several species of newborn animals are susceptible to infection with human rotavirus; however, the role of animal rotaviruses in human disease and the exchange of rotaviruses among species is not known (*6*, *7*). Elucidation of this area has been hampered by the lack of a simple method for distinguishing the different rotaviruses. This report describes the use of an enzyme-linked immunosorbent assay (ELISA) to distinguish members of the rotavirus group.

Human rotavirus was obtained from children with symptomatic diarrheal illness living in Washington, D.C. (strains USA 1 to 17), Santa Maria Cauque, Guatemala (Guat 1 to 10), and Dacca, Bangladesh (Bang 1 to 6). The specimens were tested either as a 2 percent stool suspension or a 2 percent stool filtrate. The animal viruses were obtained as described in Table 1.

Serums that contained antibodies to human rotavirus were obtained from children convalescing from rotavirus infection. Six serums were available from children living in the United States (infected with strains USA 1 to 6) and three were from children living in Bangladesh (infected with strains Bang 1 to 3). Antiserums to human rotavirus were also obtained from gnotobiotic calves and piglets (experimental infection serums) following infection of these animals with strains of human rotavirus (*6*, *7*). Antiserums to the UK and NCDV bovine viruses and to horse rotavirus were obtained from animals infected with the

Table 1. Viruses for ELISA blocking study.

| Virus | Host | Country | Year (original isolate) | Source | Passage |
|---|---|---|---|---|---|
| USA 1 to 17 | Human | United States | 1974–1977 | Human d.s.* | Original |
| Bang 1 to 6 | Human | Bangladesh | 1976 | Human d.s. | Original |
| Guat 1 to 10 | Human | Guatemala | 1964–1967 | Human d.s. | Original |
| NCDV 1 | Calf | United States | 1967 | Bovine d.s. | Three times in gnotobiotic calves |
| NCDV 2 to 3 | Calf | United States | 1972 | Bovine d.s. | Original |
| UK-1 | Calf | United Kingdom | 1973 | Bovine d.s. | Original |
| P-1 | Pig | United States | 1974 | Piglet d.s. | Five to six times in piglets |
| H-1 | Horse | United Kingdom | 1975 | Foal d.s. | Original |
| EDIM | Mouse | United States | 1957 | Pooled mouse d.s. | Multiple times in mice |
| SA-11 | Nonhuman primate | South Africa | 1958 | Simian rectal swab | Multiple times in African Green monkey kidney cells (AGMK) |
| "O" | Unknown | South Africa | 1965 | Intestinal wash cattle and sheep (offal) | Multiple times in AGMK |

*Abbreviation: d.s., diarrhea stool.


0036-8075/78/0721-0259$00.50/0 