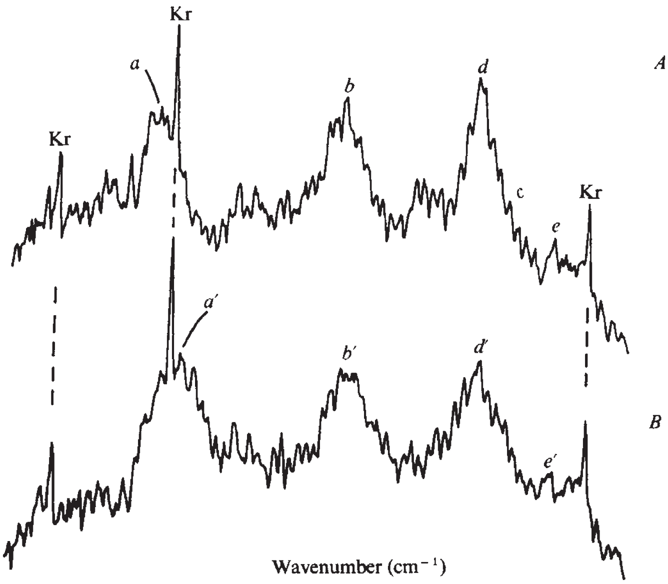

Fig. 1 Low-frequency (100–450 cm^{-1}) region of the resonance Raman spectrum of deoxyHb formed chemically by the addition of dithionite (*A*) and deoxyHb formed within 8 ns of the 442 nm photolysis of HbCO (*B*). Frequency scales of both spectra are calibrated by krypton lines which were optically superimposed. Frequency positions of resonance Raman bands are given in Table 1.

deoxyHb from the high oxygen affinity (R) state to the low oxygen affinity (T) state.

The low-frequency spectra allow the transient to be characterized in more detail. The low-frequency (100–450 cm^{-1}) TR^3 spectra obtained with pulsed (8 ns) excitation of the deoxyHb transient formed immediately after the removal of CO from HbCO is shown in Fig. 1 and the frequency positions of these bands are given in Table 1. The appearance of the 225 ± 2 cm^{-1} band in the TR^3 spectrum represents a shift of 11 cm^{-1} from the 214 ± 2 cm^{-1} band seen in the pulsed resonance Raman spectrum of chemically stabilized deoxyHb (Table 1). The positions of these bands correlate well with the frequencies observed for the R (221 cm^{-1}) and T (215 cm^{-1}) forms of chemically stabilized deoxyHb by Kitagawa and co-workers[6].

Other aspects of the low-frequency TR^3 spectrum caution against an exact identification of the transient species with chemically stabilized (R)-deoxyHb: (1) the 225 ± 2 cm^{-1} band lies about 4 cm^{-1} higher in frequency than the 221 cm^{-1} observed in chemically stabilized (R)-deoxyHb. (2) The 303 cm^{-1} band which has the same frequency in chemically stabilized samples of (R)- and (T)-deoxyHb appears at $306 \pm$ cm^{-1} in the TR^3 spectrum. (3) The 341 cm^{-1} band, although a weak feature in both the (R)- and (T)-deoxyHb spectrum, is not seen at all in the TR^3 spectrum. (4) A general broadening of the low-frequency TR^3 bands is observed.

These differences may be attributable to any one, or to a combination of three factors. First, the resonance Raman spectra of (R)- and (T)-deoxyHb have been recorded only for chemically stabilized forms, the preparation of which involves chemical modification of the protein. As a result, these chemically formed models of (R)- and (T)-deoxyHb may not be exact representations of the deoxyHb formed photolytically. Second, the photolytically generated (R)-deoxyHb may be a modified form of the chemically stabilized (R)-deoxyHb to which it is converted at times longer than 8 ns. TR^3 spectra over longer reaction times should measure such a conformational transformation. Third, the TR^3 spectrum may contain contributions from excited electronic states of deoxyHb. The TR^3 spectra of excited electronic states exhibit both frequency shifts and line broadening relative to the ground electronic state and have been observed in many different molecular systems in similar experimental conditions to those used here[9–16]. The importance of excited electronic states to transient absorption spectra measured in the HbCO system has been suggested previously[17].

Qualitatively, the spectra suggest that the transient resembles (R)-deoxyHb. The band in the 220 cm^{-1} region has been correlated with the Fe(II)–N_ε (His F8) bond[6] and the high frequency of this band in the spectrum of the transient is evidence for a compressed Fe(II)–N_ε (His F8) bond. This would arise from either an expansion of the Fe electron cloud and/or movement of the iron towards the histidine in times less than 8 ns, with movement of the histidine-containing protein chain occurring on a longer time scale.

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Table 1 Frequency positions (cm^{-1}) of resonance Raman bands in the pulsed resonance Raman (PRR), c.w. and time-resolved resonance Raman (TR^3) spectra of deoxyhaemoglobin

Band	Hb† PRR‡	(T)-Hb§ c.w.	Band*	HbCo‖ TR^3‡	(R)-Hb§ c.w.
a	214 ± 2	215	*a*′	225 ± 2	221
b	301 ± 2	302	*b*′	306 ± 2	302
c	340 ± 2	341	—	—	341
d	364 ± 1	366	*d*′	365 ± 1	366
e	403 ± 2	404	*e*′	403 ± 2	404

Deoxyhaemoglobin was prepared either in a chemically stable form or by photolysis of HbCO. The frequency of laser excitation (c.w. and pulsed) was 442 nm. Samples were between 0.05 mM (this work) and (1 mM (ref. 6)) in haem and were examined at room temperature.
*See Fig. 1.
†Chemically stabilized by addition of dithionite (see text and Fig. 1*a*).
‡This work.
§Chemically stabilized[6].
‖Prepared by N_2 purging and examined under an atmosphere of CO.

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Corrigenda

In the letter 'Stringency without ppGpp accumulation' by A. Spodaro *et al.*, *Nature* **291,** 256–258, line 5 in paragraph 6 should read 'present in TA1001 is caused by the *his*T mutation, although it is possible that strain TA1001 harbours one or more additional mutations'.

In the matters arising item 'Expression of herpesvirus-induced antigens in human cervical cancer' by C. C. Smith *et al.*, *Nature* **292,** 388–389, the authors in ref. 13 should read 'Smith, C. C., Aurelian, L., Cohen, G. H. & Eisenberg, R.'.

In the letter 'Thermoproteales—a third order of thermoacidophilic archaebacteria' by W. Zillig *et al.*, *Nature* **293,** 85–86, the journal cited in refs 17 and 20 should read '*Zentbl. Bakt. Hyg., I. Abt. Orig. Cl,*' and the journal in ref. 21 is *C2* of that series. The volume number for ref. 25 should read **41.**