The close similarity between the proposed prebiotic pathway for the synthesis of uracil derivatives from orotic acid and the contemporary biotic pathway for the conversion of orotidine 5′-phosphate to uridine 5′-phosphate (*3*) provides further support to our proposed prebiotic synthesis. There would have been no major discontinuities in the chemical reactions involved in the prebiotic processes and the biotic processes that developed later. The transition from prebiology to biology would have been a smooth one; the only requirement would have been the evolution of enzymes to facilitate the prebiological chemical processes (*14, 15*). Such enzymes would have been essential if the orotate derivative were assimilated by a biological system living in a crevice shielded from ultraviolet radiation or when sufficient oxygen developed in the primitive atmosphere so that ozone was formed and effectively "turned off" the solar ultraviolet light required for photodecarboxylation.

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Aggregation Effects on Oxygen Binding of Sickle Cell Hemoglobin

Abstract. *Deoxygenation of concentrated solutions (0.33 gram per milliliter) of sickle cell hemoglobin show (i) a "crisis point" where the oxygen binding curve is unusually steep (Hill coefficient of 5 to 6), and (ii) a simultaneous increase in light scattering. Nearly identical oxygen binding curves are obtained upon oxygenation and deoxygenation of these solutions. The influence of aggregation is to shift the curve toward higher pressures.*

Nearly 30 years ago it was recognized (*1*) that sickling of erythrocytes from patients with sickle cell anemia is due to gelation of deoxygenated sickle cell hemoglobin (HbS). The same tendency toward aggregation is manifested in the much lower solubility of deoxygenated HbS compared to oxygenated HbS (*2*). Oxygen binding curves are sensitive to such aggregation and crystallization effects, as has been shown in general by Wyman and co-workers (*3, 4*) and considered in detail for HbS by Minton (*5, 6*). The study of such curves provides the basic information for understanding the oxygen-linked aggregation process and the physiological consequences. Solutions of HbS at nongelling concentrations show binding curves similar to normal hemoglobin (*7*). However, experimental difficulties, such as slow diffusion, arise in the study of gelling solutions. These difficulties have been circumvented by use of natural erythrocytes, but the presence of 2,3-diphosphoglycerate and the distribution of intracellular HbS concentrations complicates interpretation for these materials (*8*). Even cells with controlled concentrations of HbS have given scattered P_{50} values (*9, 10*).

Here we report "reversible" binding measurements on highly concentrated HbS solutions where a "crisis point" is detected upon deoxygenation and extraordinarily high cooperative oxygen binding is observed. Conditions were chosen (*p*H 7.1, 0.15*M* phosphate buffer, 25°C, 0.15 to 0.33 g of HbS per milliliter) to coincide with studies (*11, 12*) on critical gelation and kinetics of the aggregation process. We used a purified sample of HbS obtained from a homozygous source (*13*). The sample contained at least 98 percent HbS, the remainder being fetal hemoglobin (HbF). No more than 2 percent methemoglobin could be detected spectrally at the beginning of an experiment and no more than 5 percent formed in the course of the run.

Oxygen saturation was determined by

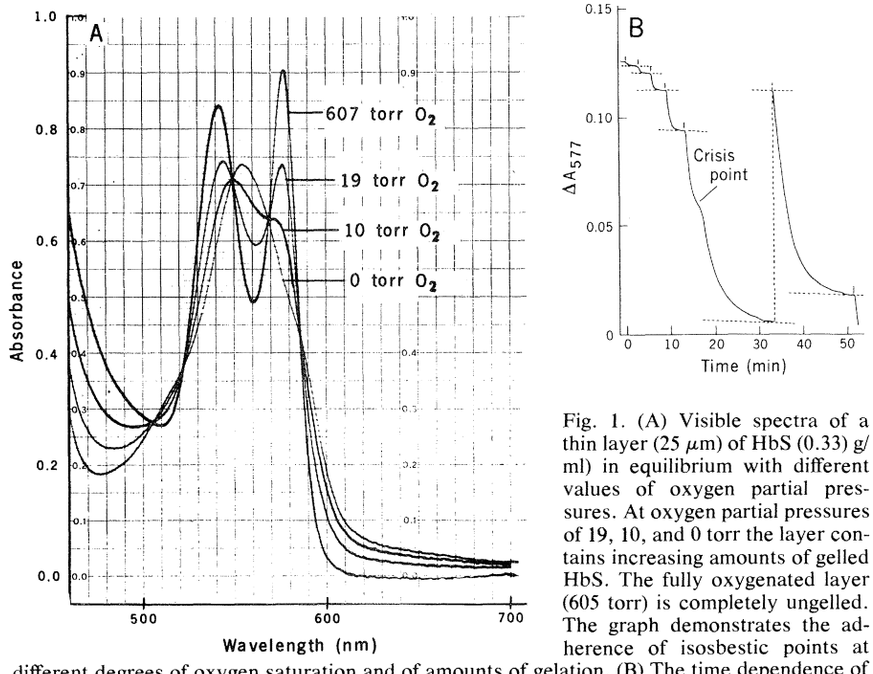

Fig. 1. (A) Visible spectra of a thin layer (25 μm) of HbS (0.33) g/ml) in equilibrium with different values of oxygen partial pressures. At oxygen partial pressures of 19, 10, and 0 torr the layer contains increasing amounts of gelled HbS. The fully oxygenated layer (605 torr) is completely ungelled. The graph demonstrates the adherence of isosbestic points at different degrees of oxygen saturation and of amounts of gelation. (B) The time dependence of absorbance at 577 nm of a thin layer of HbS (0.33 g/ml) as oxygen partial pressure is decreased in precise steps. The total absorbance at full oxygenation was 0.8. Maximum instrument sensitivity (0.1 absorbance, full scale) was used. The inflection point in the fifth step is defined as the crisis point. This point corresponds to a fraction of oxygen saturation, $\theta = 0.78$.

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means of optical absorption of a thin layer of HbS (25 μm thick). One side of the layer was defined by a stretched oxygen permeable film (General Electric MEM213) that was in contact with a gas phase whose oxygen partial pressure could be varied in precise steps. The optical absorption of the thermostatically controlled layer was measured by a Cary 17 spectrophotometer. The general operation of an earlier version of the present apparatus has been described (*14*). Figure 1A shows visible spectra for a layer of HbS (0.33 g/ml) at various degrees of oxygenation. In this sample isosbestic points are preserved for lower degrees of saturation where a significant fraction of the sample is aggregated. This indicates that a change in absorbance at a wavelength such as 577 nm directly measures the change in degree of oxygen saturation and that light-scattering effects from the thin layer are insignificant.

Figure 1B shows a typical trace of the absorbance at 577 nm for several deoxygenation steps. The relaxation time to achieve a new equilibrium state is dependent on the extent of deoxygenation for a particular step, and a simple exponential decay is closely followed. However, the time course of one step is notably different. It is characterized by a point of inflection shown by the relaxation curve which we define as the "crisis point." It is determined by the degree of oxygen saturation where the point of inflection occurs. The occurrence of the crisis point is concentration-dependent; at low concentrations it does not appear and at high concentrations it is moved to higher degrees of oxygenation. We attribute this point to the initiation of aggregate formation, the aggregates formed having a low oxygen affinity. Although this point is undoubtedly sensitive to the rate of oxygen removal from the layer and to the kinetics of the aggregation process (*12*) it is a point that may be useful in testing the efficacy of antisickling drugs. The location of the crisis point is reproduced in repeat runs on deoxygenation of the same sample. In separate experiments with a light-scattering technique a sudden increase in scattering occurs at the same point as determined by the oxygen pressure.

Figure 2 gives the results of experiments on samples of two concentrations where aggregation does and does not occur. The data are presented in the form of a Hill plot. The curve for the lower concentration of HbS (0.15 g/ml) shows normal hemoglobin behavior with a well-defined lower asymptote, with a slope approaching 3.0 at P_{50} = 16 torr, and a roughly defined high asymptote. The

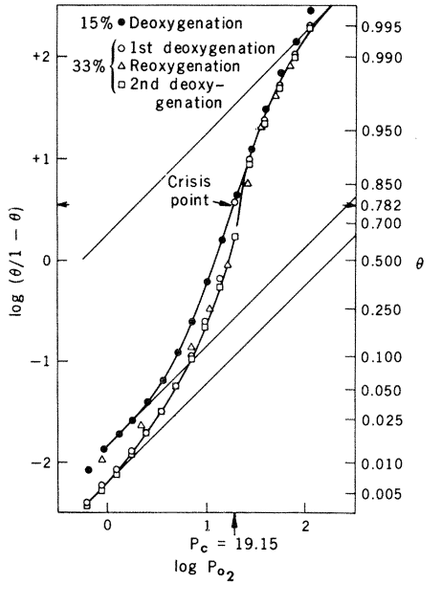

Fig. 2. Hill plots of oxygen binding to HbS at concentrations of 0.15 and 0.33 g/ml. Stepwise deoxygenation of the HbS at 0.15 g/ml showed no crisis point, P_{50} = 12 torr, and Hill plot slope equal to 3.0. Three successive runs made on the same layer of 0.33 g/ml HbS show crisis points at θ = 0.78 upon deoxygenation (first and third runs) and close reproducibility of points with the reoxygenation run (second run): the average value of P_{50} was 16 torr with a Hill plot slope determined near the crisis point of 5.5.

curve for the higher concentration of HbS (0.33 g/ml) is defined by three sequential experiments: first, stepwise deoxygenation; second, stepwise oxygenation; and third, stepwise deoxygenation. The points from these three experiments fall on each other within our experimental error, thus indicating that within the time frame of several hours and for the points obtained the system is reversible. (We have observed some hysteresis in the region of the crisis point as a result of refining the technique of adding oxygen to the system. Hysteresis is most noticeable at the 38 percent concentration where the viscosity is very high.)

These results on the Hill plot show behavior similar to the sample of lower concentration at high degrees of oxygenation, that is, above the crisis point, but deviate markedly after this point with a slope approaching five and a lower asymptote falling below that of the non-aggregating solution. The P_{50} value is higher than the value at the low concentration, as observed in studies of whole cells (*8, 9*). The large value of the slope on the Hill plot is consistent with the ideas of effects of ligand-linked association (*3, 4*). The shape of the binding curve under conditions of sickling is not by itself physiologically unfavorable, rather it is the reverse owing to its increased steepness. The damage results from the distortion and resulting destruction of the cells caused by the formation of the gel fibers. The formation of the fibers is associated with the performance of mechanical work and is an example of the way a macromolecular system can act as a chemomechanical transducer. This is analogous to the simple case of ligand binding being associated with polymerization in a one-phase system as in the case of lamprey hemoglobin (*3*).

An analysis of these results can be based on the application of the oxygen-linked solubility equation of Wyman (*3*) to the two-phase model proposed by Minton (*5*). The combination of activity relations for concentrated hemoglobin solutions (*15*), solubility data (*11*), and specific volume data of the aggregate phase (*11*) indicates that the binding curves can be fitted to a model where the solution phase adheres to stepwise Adair constants and the gel is totally deoxygenated. A full discussion of the analysis of the binding curves will be published elsewhere (*16*). Our purpose here is to draw attention to the observation of a crisis point where the rate of deoxygenation of concentrated HbS suddenly increases and to note that near reversible binding curves with high slopes shown on Hill plots can be observed for aggregating HbS.

Note added in proof: Continued study of this sample has shown that it contained 0.2 mole of diphosphoglycerate to 1.0 mole of HbS tetramer. Similar observations to those reported here have recently been made with stripped HbS. In the case of stripped samples the primary effect is that the binding curves are shifted to somewhat lower values of oxygen pressure.

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Agglutination of Plant Protoplasts by Fungal Cell Wall Glucans

Abstract. *Glucans, called elicitors, isolated from cell walls of* Phytophthora infestans, *caused rapid agglutination and death of protoplasts isolated from potato leaf tissue. Cells incubated with high concentrations of elicitor were rapidly killed, but did not agglutinate. Agglutination and cell death did not occur with any of several commercial polysaccharides including laminarin, but laminarin did inhibit elicitor-mediated agglutination. The results are consistent with the existence of specific elicitor receptor sites on the outer surface of potato leaf plasma membranes.*

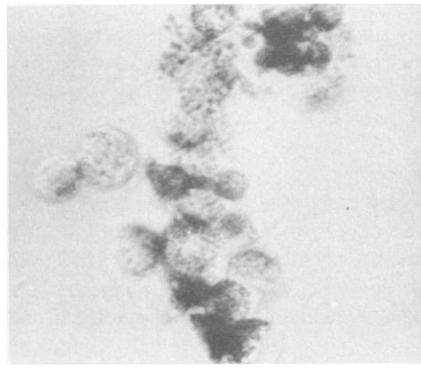
Elicitors are polysaccharides or glycoproteins of fungal origin that are toxic to a wide range of higher plants (*1*). The name of these chemicals comes from the fact that they elicit phytoalexin synthesis when applied to various plant tissues (*2*). Phytoalexins are fungistatic compounds that are synthesized by a plant in response to invasion by a microorganism. The accumulation of these chemicals is suspected to be a mechanism of disease resistance (*3*). Elicitors may thus be initiators of plant disease resistance. Mancarella (*4*) and Kota and Stelzig (*5*) have obtained evidence based on the electrophysiology of elicitor-treated potato petiole tissue that elicitor-mediated responses may be initiated at the outer surface of plant plasma membranes.

Our research was based on two premises: (i) that elicitor receptors exist on the outer surface of potato leaf plasma membranes and (ii) that elicitors are multivalent and therefore should be able to agglutinate isolated potato leaf protoplasts.

Cell walls of *Phytophthora infestans* (Mont.) de Bary were isolated by the method of Ayers *et al.* (*6*) and homogenized with laminaranase, a commercial preparation of β-1,3-glucanase (*7*). The homogenate was dialyzed (Spectrapor, 3500-MW cutoff) at 37°C against 6 liters of the homogenization buffer. The dialysis tubing was changed at 24-hour intervals, and after 72 hours the dialyzate was subjected to sequential cation and anion exchange or Bio-Gel P-2 chromatography (*8*).

Kennebec potato plants were grown (*9*) and protoplasts were isolated from well-expanded leaflets (*10*).

In initial experiments, 1 ml of protoplasts containing 2×10^5 cells were mixed with 1 ml of osmotically balanced salt solution or a similar solution containing elicitor isolated by ion exchange or a commercial polysaccharide. The protoplasts incubated with elicitor agglutinated within 10 minutes, whereas there was no agglutination with salt solution or the commercial polysaccharides even after 60 minutes (Table 1). The protoplasts incubated with elicitor agglutinated in about 3 minutes, and by 10 minutes debris had accumulated in the mass of agglutinated cells (Fig. 1). The nature of this debris is not known, but it is pos-



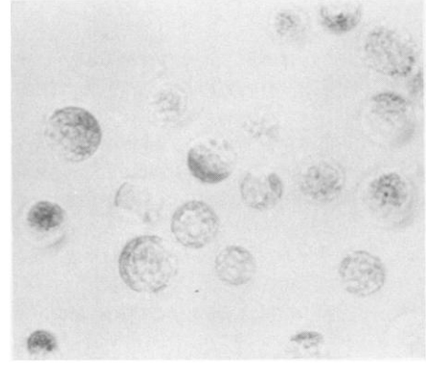


Fig. 1 (left). Potato leaf protoplasts that were incubated for 10 minutes with an elicitor from *P. infestans* at a final concentration of 250 μg of D-glucose equivalents per milliliter (× 800). Fig. 2 (right). Potato leaf protoplasts that were incubated for 1 hour with laminarin at a final concentration of 1000 μg/ml (× 800).

Table 1. Laminarin inhibition of elicitor-mediated agglutination of potato protoplasts.

Laminarin (μg/ml)	Agglutination	
	10 minutes	1 hour
0	+ + + +	+ + + + +
8	+ + + +	+ + + + +
16	+ +	+ + + +
31	+ +	+ + + +
62	+	+ + + +
125	+	+ + +
250	±	+ + +
500	±	+ +
1000	±	+ +
2000	±	+ +
4000	±	+

Table 2. Chemicals tested for their ability to agglutinate potato protoplasts.

Compound	Final concentration (μg/ml)	Agglutination	
		10 minutes	1 hour
Elicitor	250	+	+
Laminarin	8 to 1000	−	−
Soluble carboxymethylcellulose (high viscosity)	250 to 1000	−	−
Soluble carboxymethylcellulose (low viscosity)	250	−	−
Glycogen	250 to 1000	−	−
Gum arabic	250 to 1000	−	−
Inulin	250 to 500	−	−
Pectin (four preparations)	250 to 1000	−	−
Soluble starch	250 to 1000	−	−
Isotonic salt		−	−

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