to *minima* of the Gibbs (or chemical) potential.

There exist no experimental data as yet on the orientation of nuclei, with which the theory can be compared directly. After nucleation, nuclei grow into much larger crystallites. The final orientation distribution of crystallites depends on the initial distribution of nuclei, the growth law, and the time during which growth is allowed to proceed. The kinetic transformation of nucleus into a crystallite is not completely understood. Kamb (*1*, *4*; see also *5*) was able to predict that the preferred growth orientation corresponds to a *minimum* of the Gibbs potential, that is, it coincides with one of the most probable orientations of the nuclei. We can describe qualitatively the effect of growth on the initial nucleus distribution. If the Gibbs potential is minimum for only one orientation, the initial and final distributions remain similar. The only effect of growth is to sharpen the distribution (that is, the standard deviation decreases), since the most probable nucleus orientation is favored even further by growth. If the Gibbs potential has several minima, one minimum corresponding to the fastest growing orientation wil be selected at the expense of the others. (The process described above will then be repeated for that minimum.)

For instance, consider the case of α-quartz being recrystallized into β-quartz. For simplicity take $\overline{\sigma_{xx}} = \overline{\sigma_{yy}}$, $\overline{\sigma_{zz}}$ being slightly different, with $|\overline{\sigma_{zz}}| > |\overline{\sigma_{xx}}|$, and call $\overline{\sigma'_{zz}} = \overline{\sigma_{zz}} - \overline{\sigma_{xx}}$ (for this simple case, G and ρ are obviously independent of ϕ). It can be checked at once that β-quartz has a larger volume than α-quartz (*6*), hence we must take $\bar{\sigma} = \overline{\sigma_{xx}}$. Then Eq. 3 reduces to

$$2G = \overline{\sigma'^2_{zz}}\,[s_{ijkl}\, u_{iz}\, u_{jz}\, u_{kz}\, u_{lz}] \quad (7)$$

which for β-quartz becomes (*6*) (up to a constant which does not affect ρ)

$$2G = [.12 \sin^2\theta - .25 \sin^4\theta]\, \overline{\sigma'^2_{zz}}\, 10^{-12}\ \text{dyne/cm}^2 \quad (8)$$

We check at once that G is minimum for $\theta = 0$ and $\theta = \pi/2$ (notice that W is independent of ψ as well as ϕ). Taking plausible values for V and $\overline{\sigma'_{zz}}$ completes the determination of ρ. For instance, if $V \sim 10^9$ (angstroms)^3, $\overline{\sigma'_{zz}} \sim 100$ bars, we find standard deviations of a few degrees around the most probable orientations. Reliable results on β-quartz recrystallization have been reported by Green (*7*). Indeed, it is found that for moderate growth, crystallites are distributed around $\theta = 0$ and $\pi/2$ with standard deviations of a few degrees. As expected for moderate growth, the distribution of crystallites is close to the distribution of nuclei. It is also clear from the experiments (*7*) that growth favors the direction $\theta = 0$. In particular, if the specimen is annealed, the distribution of large crystallites around $\theta = 0$ is extremely sharp. Small crystallites, on the other hand, that are less affected by growth, are not so strongly oriented (*7*).

Preliminary data (*7*) on α-quartz can also be easily interpreted in the light of the theory. It can easily be checked that the distribution of nuclei in orientation for α-quartz happens to be essentially independent of the choice of $\bar{\sigma}$. Hence we need not concern ourselves with the sign of the change in volume during the recrystallization. For α-quartz (again up to an irrelevant constant and taking, for instance, $\bar{\sigma} = \overline{\sigma_{xx}}$), we have (*6*)

$$2G = [.86 \sin^3\theta \cos\theta \cos\psi\,(\cos^2\psi - 3\sin^2\psi) - .22 \sin^2\theta + .56 \sin^4\theta]\, \overline{\sigma'^2_{zz}}\, 10^{-12}\ \text{dyne/cm}^2 \quad (9)$$

G now depends on ψ as well as θ. Experimentally only the distribution of c-axes is given (*7*). This corresponds to the marginal distribution in θ, obtained by integrating ρ over ψ. It is easier and qualitatively correct to replace ψ by the value that makes G minimum. Then

$$2G \simeq [-.86 \sin^3\theta\,|\cos\theta\,| - .22 \sin^2\theta + .56 \sin^4\theta]\, \overline{\sigma'^2_{zz}}\, 10^{-12}\ \text{dyne/cm}^2 \quad (10)$$

In the present case G, given by Eq. 10, is minimum for only *one* value, $\theta_0 \simeq 47°$ (the direction $\pi - \theta_0$ is, of course, essentially the same solution). The standard deviation is again of a few degrees (taking the same V and $\overline{\sigma'_{zz}}$ used earlier for β-quartz). Growth will make the distribution sharper but the orientation of the crystallites will remain centered around $\theta = \theta_0$ as checked by experiments (*7*).

In conclusion, the distribution of nuclei in orientation computed from the present theory is consistent with experimental results. Once a reliable growth law is known it will be possible to compute the final distribution of crystallites exactly by using the initial distribution of nuclei as determined here. The influence of the initial distribution is more important when growth is allowed to proceed only a short time. The initial distribution is centered around the *minima* of the Gibbs potential and is sharper for big nuclei, large deviatoric stress, and soft materials.

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Multiple Forms of Bacterial NADP-Specific Isocitrate Dehydrogenase

Abstract. *Electrophoretically distinct forms of nicotinamide adenine dinucleotide phosphate–specific isocritrate dehydrogenase have been observed in extracts of* Escherichia coli *grown under different culture conditions. In glucose-grown cells, two distinct bands of isocitrate dehydrogenase activity were observed on polyacrylamide gels and have been completely resolved by employing ion-exchange chromatography. These multiple forms of the enzyme have been studied and their possible metabolic role is discussed.*

Recent studies in our laboratory have demonstrated the existence of two electrophoretically distinct NADP-isocitrate dehydrogenases (*1*, *2*) in extracts obtained from *Escherichia coli*, strain E-26, grown aerobically in a simple mineral salts medium containing glucose as the sole carbon source (*3*). In this report, we describe the chromatographic separation of these two forms of the enzyme and present further evidence for the existence of multiple forms of bacterial NADP-specific isocitrate dehydrogenase.

Cell-free extracts were prepared as previously described (*4*). All subsequent steps were conducted in a cold room at 4°C. Ten milliliters of the crude extract, containing 90 mg of protein, were placed on a DEAE-Sephadex (A-50) column (17 by 250 mm) equilibrated with 0.1*M* potassium phosphate buffer, *p*H 7.5. The enzyme was eluted with a decreasing *p*H gradient. For this purpose, the mixing chamber contained 300 ml of 0.1*M* potassium phosphate buffer, *p*H 7.5; the reservoir contained 150 ml of 0.4*M* KH_2PO_4 and $10^{-4}M$ dithiothreitol. The enzyme was eluted as a single, sharp peak.

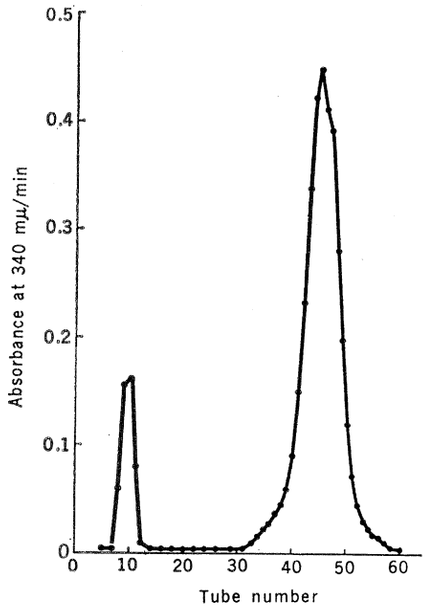

Fig. 1. Elution pattern of isocitrate dehydrogenase from CM-Sephadex. The assay mixture contained (in micromoles): potassium phosphate buffer, *p*H 7.5, 150; $MnCl_2$, 0.5; NADP, 0.5, and DL-isocitrate (K salt), 2.5. Two 0.1-ml samples were assayed, and the total volume of the reaction mixture was 1.0 ml.

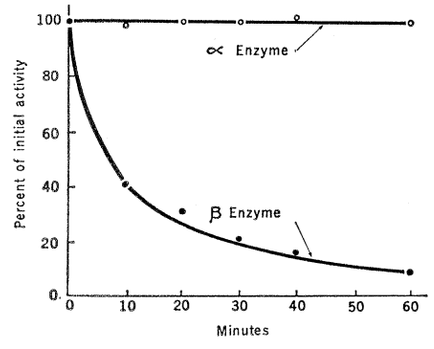

Fig. 2. Stability of α- and β-isocitrate dehydrogenase in 2.0*M* urea at 0°C. Assay conditions were the same as described in Fig. 1.

The tubes containing enzyme activity were combined, made 85 percent saturated with respect to $(NH_4)_2SO_4$, and the protein precipitate was recovered by centrifugation. The protein was dissolved in 5 ml of sodium acetate buffer, *p*H 5.4, containing 0.15*M* KCl and was placed on a CM-Sephadex (C-50) column (28 by 350 mm) equilibrated with the same buffer. The isocitrate dehydrogenase activity was eluted with a KCl gradient. The mixing chamber contained 200 ml of the acetate buffer used to prepare the Sephadex and contained, in addition, $10^{-4}M$ dithiothreitol. The reservoir contained 300 ml of the same buffer with the KCl concentration increased to 0.35*M*.

As shown in Fig. 1, the NADP-specific isocitrate dehydrogenase of *E. coli* is clearly resolved into two distinct peaks of enzymatic activity. The first enzyme eluted from the CM-Sephadex column, under these experimental conditions, we have designated as α-isocitrate dehydrogenase, and the second, β-isocitrate dehydrogenase. The highest specific activity (micromoles of NADPH formed per minute, per milligram of protein) of the α form was 3.31, and that in the β form was 9.35. Both forms of the enzyme are specific for NADP, have an absolute requirement for a divalent metal which can be satisfied with either Mg^{++} or Mn^{++}, have a *p*H optimum slightly above neutrality, and appear to catalyze the reverse reaction. This is, in the presence of both $NaHCO_3$ and α-ketoglutarate the enzyme catalyzes the oxidation of NADPH.

The β form of the enzyme is extremely labile in the presence of 2.0*M* urea at 0°C, while the α form is very stable under these conditions. An aliquot of the α- and of the β-isocitrate dehydrogenase was mixed with an equal volume of freshly prepared 4.0*M* urea and placed in an ice bath at 0°C. Initially, and after various time intervals, the activity of each enzyme was determined. The results are shown in Fig. 2. It can be seen that the α form retained 100 percent of the initial catalytic activity after 1 hour under these conditions. In contrast, the β form lost approximately 50 percent of the initial activity after only 5 minutes and nearly 90 percent after 60 minutes.

In contrast to mammalian systems, yeast, and many plant tissues which contain both an NAD and an NADP-specific isocitrate dehydrogenase, *E. coli* and most other bacterial systems contain only the NADP-specific form of the enzyme (*5–7*). Isocitrate dehydrogenase occupies an important position in the tricarboxylic acid cycle of terminal oxidation; it is also important both in biosynthetic pathways and as a mechanism for providing the cell with reduced pyridine trinucleotides. In systems where both the NAD and NADP-specific forms of the enzyme occur, the activity of the former appears to be regulated by the intracellular levels of adenosine monophosphate (AMP) (*5, 6*). There is a paucity of information concerning the regulation of the NADP-specific isocitrate dehydrogenase (see *8*).

Evidence has been presented that in *E. coli* multiple forms of NADP-specific isocitrate dehydrogenase occur. Reeves *et al.* (*3*) have reported that electrophoretically distinct forms of the enzyme occur in *E. coli* grown under different culture conditions. For example, extracts from glucose-grown cells contain two electrophoretically distinct isocitrate dehydrogenases, while cells grown on acetate contain only one enzyme with an electrophoretic mobility different from either of the two observed in glucose-grown cells. Thus, in the absence of an AMP-regulated NAD-specific isocitrate dehydrogenase, the metabolism of isocitric acid may be regulated by the specific synthesis of different forms of the NADP-specific isocitrate dehydrogenase.

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