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Rate for Nucleotide Release from Tubulin*

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The lower limit for the first order rate constant for dissociation of GDP from the tubulin E-site has been determined to be 0.14 s^{-1}; this corresponds to a reaction with a half-life of 5 s. Using this rate constant and the previously determined equilibrium constant for GDP dissociation, equal to 6.1 × 10^{-8} M (Zeeberg, B., and Caplow, M. (1979) *Biochemistry* 18, 3880-3886), the calculated association rate constant is 2.2 × 10^{6} M^{-1} s^{-1}. The tubulin E-site is highly reactive and it is calculated that: the half-life is 5 s for quantitative displacement of E-site bound radioactive GDP, by added excess nonradioactive GDP; the half-life is about 260 ms for isotopic equilibration when a trace amount of radioactive GDP is added to 20 μM tubulin-GDP; the half-life is about 850 ms for re-establishing the equilibrium for GDP binding, when 20 μM tubulin is diluted 20-fold. Thus, tubulin-GDP nucleotide exchange is rapid, so that added radioactive guanine nucleotides can be used in studies of relatively rapid reactions involving the tubulin subunit.

Guanine nucleotide which is bound to the exchangeable nucleotide binding site of tubulin becomes nonexchangeable when the tubulin is incorporated into microtubules (1), or tubulin oligomers (rings)(2). This property has allowed the use of radioactive GTP, GDP, or GTP analogs for analysis of the dynamic properties of steady state microtubules (3-8) and of tubulin oligomers (4, 9). That is, the kinetics for the incorporation of radioactive nucleotide into these polymers corresponds to that for tubulin subunits. Verification of this point is indicated by the equality of the rates for incorporation of [^{3}H]GDP and a radioactive derivatized tubulin subunit, [^{3}H]ethyltubulin, into steady state microtubules (5), and the similarity of the individually determined rates for incorporation of *in vivo* labeled [^{35}S]tubulin, [^{3}H]ethyltubulin, and [^{3}H]GDP into rings (9).

We have recently studied the kinetics for radioactive subunit incorporation under conditions where [^{3}H]GTP is added to steady state microtubules, and the rate is analyzed after incubation periods of approximately 10 s. It was found[1] that the subunit incorporation rate could be accurately measured, despite the fact that the half-life for GDP dissociation from the tubulin E-site[2] is reported to be 1.0 min (10). Since the dissociation of nonradioactive GDP from the E-site is required for formation of [^{3}H]GTP-labeled subunits, our observation of very rapid ^{3}H-labeled subunit incorporation would require that GDP dissociates very much more rapidly than previously reported. We have, therefore, reinvestigated the rate for GDP dissociation from tubulin and find that the half-life is less than or equal to 5 s. The basis for the discrepancy with the earlier work (10) has been analyzed.

EXPERIMENTAL PROCEDURES

Materials—Yeast hexokinase and nucleoside diphosphate kinase, beef heart lactic dehydrogenase, phosphoenolpyruvate, and nucleotides were obtained from Sigma. Rabbit muscle pyruvate kinase (3 IU/μl in 3.0 M ammonium sulfate) was obtained from Calbiochem-Behring. Different batches of this enzyme preparation were found to contain 75-100% of the stated enzyme activity, when measured at 37 °C in 50 mM triethanolamine hydrochloride buffer, pH 7.6, with 10 mM $MgCl_2$, 80 mM KCl, 2.8 mM ADP, 3.3 × 10^{-4} M NADH, 2 mM phosphoenolpyruvate, and 0.34 IU/ml of lactic dehydrogenase. In reactions of Tu-[^{3}H]GDP in which the pyruvate kinase concentrations were equal to 3 IU/ml, the ammonium sulfate from the enzyme solution made the final reaction mixture 3.0 mM in this substance. When a very high pyruvate kinase concentration was used, 100 μl of the protein crystals (1.2 mg of protein), which were suspended in 3 M ammonium sulfate, were centrifuged for 2 min at 160,000 × *g* in a Beckman Airfuge and the protein was dissolved in 20 μl of reassembly buffer. This solution was dialyzed for 90 min at 4 °C (11), using a Millipore VSWP membrane. In control studies with a pyruvate kinase:[^{14}C]sucrose mixture, it was determined that the dialysis is about 85% complete under these conditions. The centrifugation, dialysis, and dilution with Tu-[^{3}H]GDP probably reduced the ammonium sulfate concentration in the final reaction mixture to about 3 mM. The microdialysis procedure also resulted in an approximately 40% loss of enzyme activity, and the amount of enzyme present in the reaction mixtures was corrected for this loss. Radioactive GDP was obtained from New England Nuclear and the ethanol and contaminating tritium oxide were removed by evaporation at 37 °C.

Methods—Pig brain tubulin was prepared as previously described (12) and stored at −80 °C. Prior to use, the tubulin was carried through an additional polymerization cycle in potassium reassembly buffer (0.1 M 2-(*N*-morpholino)ethanesulfonic acid, 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid 1 mM $MgCl_2$, adjusted to pH 6.8 with KOH). After disassembly of the microtubules at 0 °C, aggregated protein was removed by centrifugation at 50,000 rpm, 4 °C, (Beckman Ti-50 rotor) for 10 min. In order to displace residual GTP from the E-site the protein was made 100 μM in GDP, after which the excess nucleotides and glycerol were removed by column chromatography on Sephadex G-25. The tubulin fractions were pooled and centrifuged at 250,000 × *g* for 25 min at 4 °C to remove nontubulin proteins. Analysis by gel electrophoresis revealed that tubulin constituted about 85% of the microtubular protein. Phosphocellulose purification of tubulin was carried out as described elsewhere (13), except that immediately after elution from the column, the protein solutions were adjusted to 1.0 mM in $MgCl_2$. This protein, which was about 90% pure tubulin, was stored frozen at −80 °C and centrifuged (250,000 × *g*, 10 min, 4 °C) immediately before use.

The isolation of Tu-[^{3}H]GDP, with tubulin preparations which had been depleted in nontubulin proteins by centrifugation, was accomplished by an approximately 5-s incubation (at 4 °C) of the protein with a trace amount of [^{3}H]GDP (6.1 Ci/mmol), followed by column centrifugation (14). Since the column centrifugation step quantitatively eliminates unbound nucleotide (15), and this procedure results in an insufficient dilution of the relatively concentrated protein solution to allow for significant GDP dissociation, it can be concluded

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[1] B. P. Brylawski and M. Caplow, unpublished results.

[2] The abbreviations used are: E-site, the exchangeable nucleotide binding site on tubulin; Tu-GDP, tubulin-containing GDP at the E-site; Tu, tubulin which is free of nucleotide at the E-site.

that virtually all of the isolated radioactive nucleotide is bound to the tubulin E-site.

Isolation of Tu-[^{3}H]GDP with phosphocellulose-purified tubulin presented a special problem. The E-site of the purified protein is 55% GTP[3] (see Fig. 1 in Ref. 16) and since this protein contains significant transphosphorylase activity (16), we found that after a brief incubation with [^{3}H]GDP the tubulin-guanine nucleotide complex which was isolated by column centrifugation was 55% Tu-[^{3}H]GTP, even when all steps were carried out at 4 °C. In order to isolate Tu-[^{3}H]GDP, the protein was incubated for 3 min at 37 °C with 0.1 mM glucose, 0.1 mM ADP, 0.26 IU of hexokinase/ml, and 3.2 IU of nucleoside diphosphate kinase/ml, to convert the GTP to GDP. This mixture was then incubated for 5 s with a trace amount of high specific activity [^{3}H]GDP and then subjected to column centrifugation so as to remove the unbound [^{3}H]GDP, glucose, and ADP. Using this procedure it was possible to isolate tubulin in which about 90% of the radioactive nucleotide at the E-site was GDP.

The dissociation of [^{3}H]GDP from Tu-[^{3}H]GDP and the subsequent pyruvate kinase-mediated conversions to [^{3}H]GTP was measured by analysis (16) of the nucleotide composition of perchloric acid-quenched aliquots of a reaction mixture. Control experiments, using [^{3}H]GTP which had been purified by thin layer chromatography, indicated that 10% of the [^{3}H]GTP is hydrolyzed during the charcoal absorption, evaporation, and thin layer chromatography steps and the nucleotide composition was corrected for this factor. Reactions were initiated by addition of pyruvate kinase to a Tu-[^{3}H]GDP:phosphoenolpyruvate (0.53 mM) mixture, except in the case where the effect of dilution of the Tu-[^{3}H]GDP on the rate was studied; in this case the reaction was started by adding a small aliquot of a concentrated solution of Tu-[^{3}H]GDP to a large volume of buffer containing pyruvate kinase and phosphoenolpyruvate.

RESULTS AND DISCUSSION

An earlier study of the rate for guanine nucleotide dissociation from the tubulin E-site (10) utilized a coupled enzyme system in which GDP (or GTP) release was followed by spectrophotometric analysis of the conversion of NADH to NAD. This method is relatively insensitive, since the molar extinction coefficient of NADH is only 6220, so that with a usable NADH concentration (0.2 mM) and a relatively high tubulin concentration (0.01 mM), there is only a 5% absorbance change associated with the reaction. Although a high Tu-GDP concentration is required for the spectrophotometric assay, from consideration of the nucleotide dissociation (k_{-1}) and coupling reaction (k_2)

$$\text{Tu-GDP} \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} \text{Tu} + \text{GDP} \xrightarrow[\text{PEP}]{k_2\ \text{PK}} \text{GTP} + \text{Pyr} \xrightarrow[\text{NADH}]{\text{LDH}} \text{NAD} + \text{lactate} \quad (1)$$

where PK is pyruvate kinase, PEP is phosphoenolpyruvate, and LDH is lactic dehydrogenase, it is apparent that it is desirable to use low concentrations of Tu-GDP. This is the case since under these conditions the GDP which is released in the dissociation step (k_{-1}) is less likely to be recaptured by the equilibrium concentration of free tubulin via the k_1 step, rather than react with pyruvate kinase in the k_2 reaction. Recombination of the released GDP with tubulin would make it appear as if nucleotide dissociation were slow. Since the spectrophotometric assay is limited to Tu-GDP concentrations of 10^{-5} M or greater, we chose to change the assay method to one in which no such limitation holds. This is the case when pyruvate kinase is used to convert the [^{3}H]GDP which dissociates from Tu-[^{3}H]GDP into [^{3}H]GTP, and the reaction is followed by nucleotide analysis of quenched reaction mixtures.

Kinetic Properties of Pyruvate Kinase—Rabbit muscle pyruvate kinase is able to function with GDP, although V_{max} with this substrate is only about 60% of that for the normal substrate, ADP (17). We have measured the activity of pyruvate kinase with GDP in microtubule reassembly buffer containing 0.53 mM phosphoenolpyruvate and 1 mM $MgCl_2$ and find that V_{max} is 1.25×10^3 mol of NADH/mol of enzyme/s and K_m for GDP is 0.6 mM; with 3 mM $MgCl_2$ V_{max} is 2.0×10^3 mol of NADH/mol of enzyme/s and K_m is 0.77 mM. Since the equilibrium concentration of GDP with 10^{-5} M Tu-GDP is only 0.75 μM (15), the pyruvate kinase will be functioning in the coupling reaction (Equation 1) under nonsaturating conditions. With the pyruvate kinase V_{max}/K_m equal to 2.1×10^6 M^{-1} s^{-1} (1 mM $MgCl_2$) or 2.6×10^6 M^{-1} s^{-1} (3 mM $MgCl_2$), the calculated half-life for the first order conversion of GDP to GTP under nonsaturating conditions with 3 units of pyruvate kinase/ml (2.56×10^{-8} M) is about 10 s.

Rate of GDP Release from Tubulin—To assure that the [^{3}H]GDP associated with the tubulin is bound at the E-site, the tubulin which had been incubated with [^{3}H]GDP was purified by a rapid column centrifugation step (14) immediately before use. Studies were carried out with tubulin which had been depleted of microtubule-associated proteins by high speed centrifugation, or by phosphocellulose chromatography. We have found that the former material is more active for microtubule assembly;[1] phosphocellulose-purified tubulin was used in a previous study (10). Rates were measured (and found to be indistinguishable) with 1.0 and 3.0 mM $MgCl_2$; the higher concentration was used in an earlier study (10).

For the reaction sequence described in Equation 1, under conditions where the equilibrium favors GDP binding, the initial rate for formation of [^{3}H]GTP is:

$$\text{rate} = \frac{k_2 k_{-1}\,(\text{Tu-GDP})(\text{PK})}{k_1\,(\text{Tu}) + k_2\,(\text{PK})} \quad (2)$$

where PK is pyruvate kinase. According to Equation 2: (*a*) the rate will be faster at progressively lower tubulin concentrations under conditions where k_1 (Tu) > k_2 (pyruvate kinase); and (*b*) a limiting rate constant equal to k_{-1} will be observed at sufficiently high pyruvate kinase concentrations; a relatively lower pyruvate kinase concentration is required to attain this limiting rate when the Tu-GDP (and consequently the Tu) concentration is low.

The rate for [^{3}H]GTP formation was measured using a fixed pyruvate kinase concentration of 3 IU/ml, with Tu-[^{3}H]GDP concentrations equal to 20, 2, and 7.3×10^{-3} μM (Table I). Decreasing the Tu-GDP concentration from 20 to 2 μM decreases the free tubulin concentration 3.3-fold (Table I), so that if k_1 (Tu) > k_2 (pyruvate kinase) in Equation 2, a 3.3-fold rate increase is expected by the dilution. A 2-fold rate increase is seen. Based upon the fact that an increased rate is observed with a higher pyruvate kinase concentrations (Reaction 3), it is concluded that the pyruvate kinase reaction is at least partially rate-limiting (*i.e.* k_1 Tu ≥ k_2 (pyruvate kinase)) with pyruvate kinase concentration equal to 3 IU/ml.

The rate with 8×10^{-3} μM Tu-[^{3}H]GDP and 3 IU of pyruvate kinase/ml (Reaction 4) is substantially greater than that seen with higher Tu-GDP concentrations (Reactions 1 and 2). In fact, at this very low Tu-[^{3}H]GDP concentration the observed rate is about equal to that measured with a nonsaturating [^{3}H]GDP concentration in the absence of tubulin (Reaction 5). A rate constant equal to 0.054 s^{-1} is calculated for this rate from the V_{max}/K_m equal to 2.1×10^6

[3] The high percentage of the E-site which is GTP results from the higher affinity for GTP, as compared to GDP (15), and the fact that excess GTP which is associated with the protein elutes from phosphocellulose more rapidly than does GDP (B. P. Brylawski and M. Caplow, unpublished observations).

TABLE I

Rate of GDP dissociation from tubulin

Reaction no.	Tubulin total	Pyruvate kinase	Tubulin free[a]	k[b]
	μM	*IU/ml*	μM	s^{-1}
1	20[c]	3	1.04	0.005
2	2[c]	3	0.32	0.01
3	9[c]	790	0.71	0.14
4	7.3×10^{-3}[c]	3	6.6×10^{-3}	0.032
5	0[d]	3		0.037
6	23[e]	3	1.15	0.012
7	22[e]	730	1.15	0.14

[a] Calculated from the dissociation constant from GDP equal to 6.1×10^{-8} M (15).

[b] First-order rate constant, calculated from the initial rate of [^{3}H]GTP formation.

[c] Tubulin dimer purified free of microtubule-associated proteins by centrifugation.

[d] This reaction was run with 5×10^{-8} M [^{3}H]GDP, without added tubulin.

[e] Tubulin dimer purified free of microtubule-associated proteins by phosphocellulose chromatography.

M^{-1} s^{-1}. The higher rate in Reaction 4, as compared to Reactions 1 and 2, indicates that in the latter reactions a fraction of the GDP which formed from Tu-GDP reacts with tubulin rather than with pyruvate kinase.

The observed rate constant for dissociation of GDP from 23 μM phosphocellulose-purified tubulin in the presence of 3 IU of pyruvate kinase/ml is 0.012 s^{-1} (Fig. 1 and Table I), which is in good agreement with a value equal to 0.0113 s^{-1}, previously measured (10) under identical conditions. GDP dissociation appears to be about twice as fast with phosphocellulose-purified protein, as with tubulin which has been depleted of microtubule-associated proteins by centrifugation (compare Reactions 1 and 6). We believe that this difference reflects the experimental error in preparing identical reaction mixtures, the error in the rate measurements involving nucleotide product analysis, and the fact that the phosphocellulose chromatography results in a loss in nucleotide binding by a fraction of the tubulin.[1] This last factor would make the k_1 (Tu)(GDP) rate slower and k_{obs} larger for Reaction 6, as compared to Reaction 1 (see Equation 2).

Equation 2 indicates that when k_{obs} is independent of the pyruvate kinase concentration, the observed rate correctly reflects the dissociation (k_{-1}) process in Equation 1. It has been found that k_{obs} is equal to 0.14 s^{-1}, in a reaction using 730 IU of pyruvate kinase/ml (Fig. 1 and Table I). It is not technically feasible to further increase the pyruvate kinase concentration. However, based upon the fact that the rate with 730 IU of pyruvate kinase/ml is not linearly dependent upon the pyruvate kinase concentration (the 243-fold increase in pyruvate kinase in Reaction 7, as compared to Reaction 6, results in only an 11.7-fold rate increase), it is concluded that pyruvate kinase (k_2) > k_1 in Equation. 2. Therefore, a lower limit for k_{-1} is 0.14 s^{-1}, which corresponds to a half-life of about 5 s. From the previously determined GDP dissociation constant equal to 6.1×10^{-8} M (15) and the dissociation rate constant of 0.14 s^{-1}, a second order rate constant equal 2.2×10^6 M^{-1} s^{-1} is calculated for the association rate constant for GDP with tubulin.

It appears that the previous determination of the GDP dissociation rate (10) was carried out with an insufficient amount of pyruvate kinase (3.0 IU/ml) to trap the released GDP. As a result, the observed rates were too low. It should be noted that the substrate dissociation process (k_{-1}) may be rate-limiting even when the observed rate for a coupled enzyme assay (Table I, Reaction 1) is slower than that observed when the substrate is directly added to the coupling assay reaction mixture (Table I, Reaction 5). The point is that it is the relative magnitudes of the k_1 (Tu) (GDP) and the k_2 (pyruvate kinase) (GDP) steps in Equation 1 that determine which step is rate-limiting; the relative magnitudes of the k_{-1} (Tu-GDP) and the k_2 (pyruvate kinase) (GDP) steps have no bearing in determining which step is rate-limiting.

It has previously been reported that [^{32}P]GTP, which is formed by a transphosphorylase-mediated reaction of [^{32}P]UTP and tubulin-derived GDP, is more rapidly bound to the tubulin E-site than in added [^{3}H]GTP (see Fig. 10 in Ref. 18). This result was accounted for by a mechanism in which Tu-GDP dissociation is relatively slow and the substrate for the transphosphorylase is undissociated Tu-GDP. We do not believe that our conclusion that GDP dissociation from the tubulin E-site is rapid is contradicted by these results. The earlier results (18) could arise if, in the analysis of the relative rates for [^{3}H]GTP and [^{32}P]GTP binding, no account was taken of the fact that the specific activity of the added [^{3}H]GTP is diluted (as a result of the transphorylase reaction) by the approximately 100 μM E-site GDP. There is no equivalent dilution in the specific activity at the γ-phosphate moiety of the added [^{32}P]UTP or the synthesized [^{32}P]GTP, by E-site GDP. Thus, it might appear as if more moles of synthesized [^{32}P]GTP were bound than of added [^{3}H]GTP, if this fact was not taken into account. It is likely that this was not taken into account, since the (incorrect) assumption that GDP dissociation is slow would preclude the need to consider any dilution of the [^{3}H]GTP specific activity by the E-site GDP.

Kinetics for Equilibration of Tu-GDP—There are three likely sets of circumstances where one might be concerned about the rate of equilibration of E-site bound and unbound GDP.

1) For the reaction:

$$\text{Tu-GDP} \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} \text{Tu} + \text{GDP} \qquad (3)$$

in which the free and bound GDP are initially radioactive, following the addition of a large excess of nonradioactive GDP, the isotope will be lost from the protein with an observed rate constant equal to k_{-1}; the half-time will be about 5 s. The process appears to be irreversible and the k_1 reaction does not influence the observed rate.

2) For a reaction in which a trace amount of [^{3}H]GDP is

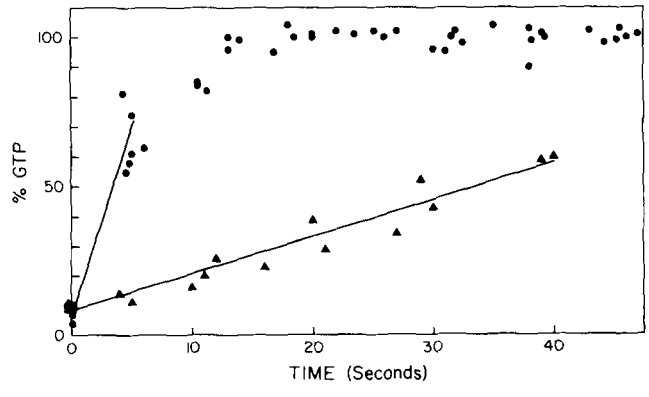

FIG. 1. **Rate of [^{3}H]GTP formation from Tu-[^{3}H]GDP.** The points (▲) and (●) are for Reactions 6 and 7, respectively (Table I). Results shown are from two separate reactions at each pyruvate kinase concentration. The rate constant for Reaction 6 was calculated from the initial rate. The rate constant for Reaction 7 was calculated using the measured extent of reaction in early time points in a first order rate equation; the line drawn for this reaction is an arbitrary one.

added to a solution containing an equilibrium mixture of Tu-GDP and GDP, the observed rate constant for incorporation of label into the protein is described by Equation 4 (19)[4]:

$$k_{obs} = k_{-1}\left[\frac{\text{Tu-GDP}_{Eq} + \text{GDP}_{Eq}}{\text{GDP}_{Eq}}\right] \quad (4)$$

The observed rate is first order, with a rate constant which reflects the equilibrium distribution of the GDP. Under conditions where the tubulin concentration is 20 μM, the equilibrium concentrations are 18.96 μM for [Tu-GDP$_{Eq}$] and 1.04 μM for [GDP$_{Eq}$] (15). Therefore, the observed rate constant is equal to 19.2 times k_{-1}; this corresponds to a half-life of 260 ms. Thus, equilibration of the E-site with a trace amount of added radioactive GDP will be very rapid.

3) If an equilibrium mixture containing Tu-GDP and GDP is diluted, so that GDP dissociation must occur so as to reestablish equilibrium, the time dependence of the Tu-GDP concentration is calculable from Equation 5 (see "Appendix"):

$$\text{(Tu-GDP)} = [b + \sqrt{-q}]\exp[-\sqrt{-q}\,\Delta t] - [b - \sqrt{-q}]\left[\frac{2cx_0 + b + \sqrt{-q}}{2cx_0 + b - \sqrt{-q}}\right]\Big/2C\left[\frac{2cx_0 + b + \sqrt{-q}}{2cx_0 + b - \sqrt{-q}}\right] - \exp[-\sqrt{-q}\,\Delta t] \quad (5)$$

where $b = -k_1$ (tubulin$_{total}$), $c = -k_1$, $q = -k_1^2 - 4k_1k_{-1}$ (tubulin$_{total}$), x_0 = the Tu-GDP concentration immediately after dilution, and Δt is the time which has elapsed in the relaxation to the new equilibrium position. The complexity of Equation 5 comes from the fact that the reaction shown in Equation 3 is first order in one direction and second order in the other direction. Based upon the values for k_1 and k_{-1} described here, and an equilibrium constant for GDP dissociation equal to 6.1×10^{-8} M (15) (required to calculate x_0), it is calculated that if a solution containing 20 μM Tu-GDP were diluted 20-fold, the establishment of new equilibrium would be 50% complete in 850 ms.

Acknowledgment—We are pleased to acknowledge Dr. Barry Zeeberg's assistance in this work.

APPENDIX

For the reaction scheme:

$$\text{Tu-GDP} \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} \text{Tu} + \text{GDP}$$

the rate equation for Tu-GDP is:

$$\frac{-d\,\text{(Tu-GDP)}}{dt} = k_{-1}\,\text{(Tu-GDP)} - k_1\,\text{(Tu)(GDP)}$$

and the equations for conservation of mass are:

$$\text{Tu}_T = \text{(Tu-GDP)} + \text{(Tu)}$$

$$\text{GDP}_T = \text{(Tu-GDP)} + \text{(GDP)}$$

$$\text{Tu}_T = \text{GDP}_T$$

From these the following substitutions are made:

$$-\frac{d\text{(Tu-GDP)}}{dt} = k_{-1}\,\text{(Tu-GDP)} - k_1\,(\text{Tu}_T - \text{Tu-GDP})(\text{GDP}_T - \text{Tu-GDP})$$

$$-\frac{d\text{(Tu-GDP)}}{dt} = k_{-1}\text{(Tu-GDP)} - k_1\,\text{Tu}_T\text{GDP}_T + k_1\,\text{(Tu-GDP)}(\text{Tu}_T + \text{GDP}_T) - k_1\,\text{(Tu-GDP)}^2$$

$$-\frac{d\text{(Tu-GDP)}}{dt} = k_{-1}\,\text{(Tu-GDP)} - k_1\,(\text{Tu}_T)^2 + k_1\,\text{(Tu-GDP)}(2\,\text{Tu}_T) - k_1\,\text{(Tu-GDP)}^2$$

$$\frac{+d\text{(Tu-GDP)}}{k_{-1}\,\text{(Tu-GDP)} - k_1\,(\text{Tu}_T)^2 + k_1\,\text{(Tu-GDP)}(2\,\text{Tu}_T) - k_1\,\text{(Tu-GDP)}^2} = -dt$$

The integral has the form:

$$\int\frac{dx}{X} = \frac{1}{\sqrt{-q}}\ln\frac{2\,cx + b - \sqrt{-q}}{2\,cx + b + \sqrt{-q}}$$

where $X = a + bx + cx^2$; $a = -k_1\,(\text{Tu}_T)^2$; $b = k_{-1} + k_1\,(2\,\text{Tu}_T)$; $c = -k_1$; and $q = 4\,ac - b^2 = -k_{-1}^2 - 4\,k_1k_{-1}\,\text{Tu}_T$.

Integration with the initial conditions: (Tu-GDP) = Tu$_T$ at time = 0, yields Equation 5.

Note Added in Proof—It has very recently been reported that the rate constants for GDP and GTP dissociation from tubulin are approximately 0.15 s^{-1} (Engelborghs, Y., and Eccleston, J. (1982) *FEBS Lett.* **141,** 78–81).

[4] There is a typographical error (k_1 replaces k_{-1}) in Equations 3, 4, and 7–10 in Ref. 19. Our Equation 4 is a truncated version of Equation 9 in Ref. 19, in which we assume that at the low unbound GDP concentrations present in our reaction mixtures, there is no nucleotide dissociation via a bimolecular displacement reaction.

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